

# ***PROCEEDINGS***

of the American Academy of Forensic Sciences

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ATLANTA 2012

# SPECIAL SESSIONS



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## S1 Interdisciplinary Symposium - International and Interdisciplinary Symposium ... Now Boarding

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**Educational Objective:** After attending this presentation, attendees will be introduced to a diversity of views, a variety of topics and a wealth of knowledge in the field of forensic science that can be used to improve our methodologies as well as promote further research, collaboration, and understanding.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the practice, elevating the standards, and advancing forensic sciences worldwide.

Worldwide forensic research in action will come to you! Preeminent forensic research scientists from ten countries will amaze you. It's a once in a lifetime opportunity to hear from:

- International Committee of the Red Cross, Switzerland**
- International Commission on Missing Persons, Bosnia/Herzegovina**
- International Criminal Investigative Training Assistance Program, Worldwide**
- National Institute for Legal Medicine, Portugal**
- Panel Host: President, Douglas Ubelaker, Smithsonian Institution, Washington, DC**

The American Academy of Forensic Sciences (AAFS) serves a distinguished and diverse membership of over 6,200 members, divided into eleven forensic sections representing all 50 United States, Canada, and 62 other countries worldwide. What is our "Forensic Science Edge?" Our advantage or "Edge" is access to preeminent forensic experts worldwide. This symposium encourages international interdisciplinary collegial collaboration that professionally advances us by reinforcing, challenging and/or transforming our thinking.

This symposium is the stuff of which legends are made. See forensic research around the world in action. Toxicology in Sweden! Questioned Documents in Canada! Psychiatry & Behavioral Science in Germany! Physical Anthropology in Korea! Odontology in Belgium! Digital & Multimedia Sciences in the Netherlands! Pathology/Biology in Portugal!

General in Switzerland! Jurisprudence in Bosnia/Herzegovina! Criminalistics from ICITAP! And, Engineering from the United States!  
**International Forensic Science, Victim Identification, Accident Reconstruction**

## S2 Contemporary Topics in the Forensic Science Community

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**Educational Objective:** After attending this presentation, attendees will have a better understanding of the scope of the different fields of forensic science. Participants will learn how the different fields of forensic science work together to each play a significant role in casework. Both casework and research will be presented by the speakers. These presentations will show how casework and research are intertwined and how they both contribute to advancing forensic science. In addition, attendees will learn about each section represented by the AAFS and about the benefits of membership in the Academy. Participants will learn about various cases and research being done by their peers at the posters and slides sessions, and they will learn valuable skills needed to secure a job within the forensic science field at the breakfast session.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing encouragement, tools, resources, and support needed to give new and future professionals the ability to positively contribute to the forensic science field.

For more than a decade, the Young Forensic Scientists Forum (YFSF) has provided a program for new and young forensic scientists ranging from students in both undergraduate and graduate programs to professionals new to their career in forensic science with five years experience or less. YFSF is designed to attract both members and non-members of the American Academy of Forensic Sciences (AAFS). The continuing goal is to provide this audience with topics relevant to their education, training, and skill levels. The program is also designed to provide a comfortable environment for students and new professionals to present to their peers as well as an opportunity to effectively and efficiently network with experienced members and Fellows of the AAFS. The opportunities to present range from presenting at the YFSF Bring Your Own Slides (BYOS) session or the YFSF Bring Your Own Posters (BYOP) session and to showcase emerging forensic scientists. The Emerging Forensic Scientist Award winner is always invited to present his/her award winning paper.

For the AAFS 64th Annual Scientific Meeting in Atlanta, Georgia, the YFSF Special Session will present the theme: "Contemporary Topics in the Forensic Science Community." The special session to be held on Tuesday, February 21, will include speakers who will discuss trends currently observed among various forensic disciplines. With the rise of different types and variations of evidence being submitted to various laboratories, as well as new technologies in evidence examination, the focus of the 2012 forum will be to highlight and educate young forensic scientists on these trends. Lunch is provided to both attendees and speakers who are registered for the special session.

The annual YFSF BYOS, scheduled for Wednesday evening, includes presentations from students and new forensic scientists. The program will continue Thursday morning with the annual YFSF breakfast session with the theme: "Creating the Resume That Will Get Your Foot in The Door." The breakfast session is included in the registration for the special session held on Tuesday. YFSF does not require presenters of BYOS or BYOP to be members of AAFS and does not require they attend the special session but we do encourage them to do so.

One of the goals of the YFSF is to foster relationships between the participants of the session with their 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.

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**Forensics, Evidence Submission, Current Trends**

## ES1 The Casey Anthony Trial — From the Defense, Medical, and Scientific Viewpoint

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**Educational Objective:** After attending this presentation attendees will understand that experts at trial must limit their opinions and conclusions to those which are scientifically supportable despite pressures to tailor testimony to popular community and media sentiment.

**Impact on the Forensic Science Community:** This presentation will seek to educate all as to the limits of the areas of forensic science testified to by the defense experts in the Casey Anthony trial.

The biggest media case, arguably larger than O.J. Simpson, was the 2011 trial of Casey Marie Anthony for first-degree death penalty murder. This was the first trial that not only engulfed the media but also occurred in the climate of burgeoning social networking, such as Facebook® and twitter®. As a result, everyone worldwide became an armchair juror or lawyer, influenced by rating-conscious talking heads with little understanding of the scientific evidence. Despite the recent pronouncement by the National Academy of Sciences (NAS) Report, the court allowed forensic testimony that had not been validated. The pressure on experts to put forth supportable medical and scientific testimony, despite the unpopularity of the defendant in this case, was great; however, the opinions of scientific experts must be independent of the desires of those who call upon them - unlike the attorneys, they are not advocates for either side.

In 2011 in Orlando, Florida, Casey Anthony was tried for the murder of her two-year-old child Caylee Marie Anthony. Jurors who had not been subject to pretrial publicity were picked by both sides from a different county and sequestered in Orlando, hearing about six weeks of evidence. The result is widely known – that Casey Anthony was acquitted of death penalty murder and all lesser included charges related to the death of her daughter. In order to bolster the emotional argument that the prosecution presented against Ms. Anthony, various forensic disciplines were embraced for the trial. These included not only medical and pathology testimony but also anthropological, entomological, chemistry, toxicology, botanical, and DNA opinions.

The defense claimed that much of the medical and scientific testimony was novel and had exceeded the boundaries of validated forensic science and should not have been allowed in by the gatekeeper. The judge, who did not accept the NAS Report as authoritative, allowed the jury to hear from experts whose opinions and conclusions had never been utilized in court, had not been subject to rigorous error rates, failed to have peer review protocols or quality control, and appeared to fall short of United States Supreme Court standards.

The defense prepared their own scientific conclusions beginning with a second autopsy and anthropological examination. It is important for all forensic experts to understand that at trial the role of the expert and

the jury's acceptance of expert testimony have substantially changed in the past decade.

The controversial nature of some of the testimony allowed to be presented to the jury in this matter, along with the actual systematic assistance provided by the experts for the defense, will be explored so that future forensic experts testifying in any trial, especially a high profile trial, will better understand why jurors accept or reject their conclusions.

This presentation will be a constructive examination of rapidly developing scientific technologies and the National Academy of Sciences concerns as to courtroom admissibility.

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**Casey Anthony Trial, NAS Report, Unvalidated Forensic Science**

## **ES2 National Missing and Unidentified Persons System (NamUs) Best Practices for System Use**

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**Educational Objectives:** After attending this presentation, attendees will learn best practices for using the NamUs system including case entry, case enhancement, and utilization of NamUs forensic services. At the conclusion of the session, attendees will be able to efficiently navigate, operate, and utilize the NamUs system to assist their agency in applying NamUs to resolve missing and unidentified person's cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how increased awareness and use of NamUs offers the promise of reducing backlog of unidentified remains cases in the United States and streamlining the investigation of missing persons by rapidly matching cases across state lines.

The National Missing and Unidentified Persons System, NamUs, combines two databases to assist law enforcement, medical examiners, coroners, and family members in the search for missing persons and the identification of unidentified human remains. The system, to date, has helped resolve over 150 cases of missing persons and unidentified remains. The information presented in the session offers agencies proven best practices for using NamUs to successfully resolve these cases.

This presentation, developed in conjunction with the National Association of Medical Examiners, Georgia Bureau of Investigation, and the Florida Department of Law Enforcement, will outline the benefits and best practices for using NamUs. Death investigators and law enforcement will see firsthand how the NamUs system benefits practitioners in their field. This presentation will be given by registered users from the medical examiner, coroner, and law enforcement fields and will prove vital to the attendees' agencies success with the NamUs program.

First, attendees will be walked through the registration process for becoming a secure user such as law enforcement or a medical examiner or coroner. Once through the registration process, basic and advanced case entry and searching will be demonstrated. Attendees will learn how to enter the best possible case information and use advanced searching techniques based on forensic identifiers such as dental records and tattoos.

Next, the secure user dashboard will be presented, illustrating the benefits of NamUs and its ease of use for users and agencies. The exclusions process and exclusions menu, a significant benefit of the system, will be explained and demonstrated. Under this menu, law enforcement, medical examiners, and coroners can track potential case matches that have been excluded based on forensic examination. The cases are archived so that users can actively monitor these cases for any changes or future comparison.

NamUs rates cases on a star system, from one to five stars, based on the amount of identifiers present in the case record. Five star cases have a higher likelihood of reaching resolution through the system. Identifiers found in five star cases include DNA, fingerprints, and dental records. This presentation will demonstrate how to enter a five star case, or enhance an existing case to five star status. Attendees will also learn how to import all agency cases that reside in the FBI's NCIC database into NamUs electronically, saving them time and money by avoiding manual case entry.

Through NamUs, the National Institute of Justice also offers a wide variety of free forensic service assistance including dental and anthropological services, fingerprint examination, and DNA sampling. This presentation will address how these services are provided, protocols for obtaining the services, and methods for case enhancement using forensic services.

The final topic of the presentation will present system successes. Cases resolved with the assistance of NamUs will be highlighted, demonstrating how the system helps family members, medical examiners/coroners, and law enforcement work together to resolve agency cold cases, bring closure to families and give people their identities back.

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**NamUs, Identification, Missing Persons**



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# BREAKFAST SEMINARS



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## BS1 Postmortem Examination and Personal Identification of Victims of the Great East Japan Earthquake

*Yasuhiro Aoki, MD, PhD\*, Department Forensic Medicine, Nagoya City University School of Medicine, 1 Kawasumi Mizuho-cho Mizuho-k, Nagoya, 467-8601, JAPAN*

**Educational Objective:** The goal of this presentation is to familiarize attendees with an outline of the forensic investigation of victims of the Great East Japan Earthquake Disaster which was carried out with the assistance of members of the Japanese Society of Legal Medicine (JSLM).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing foundational knowledge on the features of the Japanese medico-legal system and mass disaster management, and highlighting the role of forensic personnel in the response to overwhelming natural disasters.

A massive earthquake of magnitude 9.0 struck eastern Japan at 2:46 p.m. on March 11, 2011. The epicenter was approximately 130 km off the Pacific Coast of northeast Japan. Strong tremors were observed across a wide area. However, both human casualties and property damage were concentrated on three prefectures (government jurisdictions in Japan), Iwate, Miyagi, and Fukushima, located along the Pacific Coast of the northeastern part of the main island of Japan (Honshu). This was primarily due to the huge tsunami triggered by the earthquake, over 15 m in amplitude and 40 m in run-up height, which engulfed the coastal areas of those prefectures.

Faced with the devastation, the JSLM established the *ad hoc* Disaster Response Headquarters on March 12, 2011, and dispatched member pathologists, physicians, and dentists to the three prefectures in cooperation with the National Police Agency. This was the first time such a headquarters had been established since the society issued a guideline in 1997 for an integrated support system of mass disaster management based on the experience of the 1995 great Hanshin-Awaji (Kobe) earthquake. The first response team consisting of three pathologists and three dentists departed Tokyo at 10:00 p.m. on the same day, traveling in police vehicles due to paralysis of the public transportation network. Examination of victims in Rikuzentakata, Iwate began the following afternoon. Most of the remains were immersed in water and covered with mud, and some victims had suffered extensive burns. Hypothermia was also the cause of death in some cases. The Headquarters successively organized and dispatched JSLM members through July 6, 2011. Some 122 pathologists and physicians contributed a total of 1,090 person-days of work, and 31 dentists performed a total of 298 person-days of work at the disaster sites. Aside from local physicians and dentists associations, the Japan Dental Association and Japan Self Defense Force also sent support teams to the affected areas.

As of July 29, 2011, the remains of 15,645 victims, including 27 non-Japanese, had been recovered, and another 4,984 people were still listed as missing. Approximately 90% of the victims were positively identified, in most cases from personal belongings and body features including dentition. In some cases, identity was established by DNA profiling, and more extensive identification attempts using a computer-assisted dental comparison system and kinship analysis of DNA profiles, which will be important for identification of as yet unidentified victims and yet to be discovered victims, are now in process. The latter is being conducted by the National Research Institute of Police Science and scientific criminal investigation laboratories of regional police headquarters. Fingernails and blood are the first choice source of DNA.

No fewer than 25 countries, regions, and international organizations have sent rescue teams and other specialists to the disaster areas; however, partly because of the language barrier, direct overseas assistance in the examination and identification of bodies has not been possible thus far.

Recently, the Japanese police departments have been increasing the number of prefectural police officers, especially those in charge of the investigation of death scenes and victims. This was effective in handling the large number of remains through wide-range mobilization from outside of the devastated areas. On the other hand, the medico-legal investigation system, such as the medical examiner system, is immature and remains an issue to be addressed from the standpoint of mass disaster management.

**Natural Disaster, Personal Identification, Japan**

## BS2 The Cleveland Cyanide Murder Case: A Multidisciplinary Approach to Crime Investigation Including Chemical Identification, Cause of Death, Capture, and Court Proceedings

*Douglas E. Rohde, MS\*, Lake County Crime Laboratory, 235 Fairgrounds Road, Painesville, OH 44077; Amanda J. Jenkins, PhD\*, University Massachusetts Memorial Medical Center, Department of Hospital Labs, 365 Plantation Street, Biotech 1, Worcester, MA 01605; Elizabeth K. Balraj, MD\*, 32795 Ledgehill Drive, Solon, OH 44139; and Gary McKee, BA\*, Highland Heights Police Department, 5827 Highland Road, Highland Heights, OH 44143*

**Educational Objective:** The goal of the presentation, is to offer attendees insight into the investigation of a high profile homicide and the cooperation between various governmental agencies and forensic disciplines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the complexities of a murder case and a successful team approach to investigation.

On February 24, 2005, a married, 38-year-old mother of two, driving to meet her sister at a movie theater, called a friend to inform her she was feeling nauseous. The female said that she had taken a calcium pill that her husband, a Cuyahoga County physician, gave her just before she left their house. Shortly after the female's cell phone conversation with her friend, she was involved in a low speed automobile accident and was rushed to a local hospital.

When the female arrived at the hospital, she exhibited shallow breathing, an erratic heartbeat, and was unresponsive; however, she had no major signs of trauma. After 30 to 40 minutes of attempting to revive her, the attending physician saw no signs of improvement and pronounced her dead. At the time of autopsy, there was no indication of external injury, and an internal examination showed no evidence of trauma. As a result, the coroner was unable to determine cause of death and awaited toxicology results. Routine toxicology testing, which included volatile, OTC, prescription, and illicit drug screens, did not reveal any unusual substances in the decedent's system.

On March 17, 2005, the husband voluntarily met with the investigating detective and provided a statement concerning his wife's death. In the course of the interview, the husband stated that his wife had been taking prenatal vitamins and calcium supplements. Later that evening, the detective retrieved these items from the home. After this meeting, the husband fled the United States, leaving his children in the care of the decedent's family.

On March 22, 2005, the calcium capsules that were retrieved by the detective were examined by a local county crime laboratory. The chemical examination revealed that nine of the 56 capsules submitted contained cyanide. On April 21, 2005, additional toxicology testing performed on the decedent's specimens revealed 9.1 mg/L of cyanide in her blood. On April 22, 2005, the coroner concluded that the cause and manner of death was homicide by acute cyanide intoxication.

On October 6, 2006, authorities in Cyprus arrested the husband while he was traveling under a false Lebanese passport. He fought extradition in Cyprus until December 12, 2008, when the Cypriot Minister of Justice issued an official surrender warrant. The husband was returned to the United States on January 9, 2009, and arraigned on January 14, 2009.

A jury trial commenced on January 19, 2010, and lasted until March 8, 2010, when the jury returned a verdict of guilty of aggravated murder, as charged in the indictment. The husband appealed his conviction. On May 26, 2011, after a review of the record and pertinent law, the Court of Appeals of Ohio affirmed the appellant's conviction for the aggravated murder of his wife.

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### Cyanide, Homicide, Multidisciplinary Approach

## BS3 Forensic Science and Cultural Heritage – Examination of the Gettysburg Address

*Henry Swofford, BS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and Fenella France, PhD\*, United States Library of Congress, 101 Independence Avenue SE, Washington DC, DC 20540*

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**Educational Objective:** The goal of this presentation, is to provide attendees with an understanding of a new application of hyperspectral imaging as a non-destructive technique to detect latent fingerprints, specifically on an historical document.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through a discussion of the detection of latent fingerprints on the Nicolay Copy of the Gettysburg Address – one of our nation's most precious documents – using hyperspectral imaging. Through a collaborative effort between the Preservation Research and Testing Division (PRTD) of the United States Library of Congress, and the Latent Print Branch of the United States Army Criminal Investigation Laboratory, progress is being made in the recovery of additional artifacts and information of historical significance through forensic examinations using hyperspectral imaging techniques.

Hyperspectral imaging involves a spectroscopic analysis of materials to distinguish certain materials, one from another, as a function of their differential reflectance and absorption properties across a multitude of wavelengths in the electromagnetic spectrum. The particular wavelengths at which materials reflect and absorb will differ based on the chemical composition of the material giving it a characteristic reflectance spectrum. It is this characteristic reflectance spectrum which distinguishes certain materials. The normal human eye can distinguish the difference in materials based on their color. The color of a material is a descriptive property corresponding to the particular wavelength of electromagnetic energy that is reflected back towards the eye. The brain then detects and defines this reflected energy as a particular color depending on the wavelength of the reflected energy. The human eye is sensitive to distinguishing electromagnetic energy between 390nm and 750nm – commonly referred to as the “visible spectrum.” The visible spectrum represents only a very small portion of the entire electromagnetic spectrum which is infinite and continuous. Hyperspectral imaging relies on hundreds of narrow and contiguous wavelengths across the electromagnetic spectrum resulting in characteristic reflectance spectra for each pixel in a digital image allowing the viewer to see the image as a function of the differential reflectance spectra. The hyperspectral imaging technique is undergoing rapid improvements with continuous developments of more

powerful imagery sensors and image processing algorithms expanding the technique to be used for a multitude of additional applications.

In 2008, the United States Library of Congress PRTD began utilizing spectral imaging to examine a number of documents having historical interest to our nation including original drafts of the Declaration of Independence, the Gettysburg Address, and the Waldseemüller 1507 World Map, the first map to refer to the continent as America. The application and development of hyperspectral imaging for the preservation of cultural heritage materials and analysis of historic documents provides a powerful non-invasive technique for assessing documents. This technique utilizes an imaging system that captures the spectral response of materials from the ultraviolet, visible and near infrared regions of the spectrum (UV-VIS-NIR) and also reveals obscured or hidden information. The Library imaging system comprises a MegaVision 39 Megapixel monochrome camera (7216 x 5412) E6 back, and APO-Digitar 5, 6/120 lens, integrated through customized software with light emitting diode (LED) illumination panels that span the spectral range of 365nm to 1000nm for reflected, transmitted and raking (side-lighting) imaging modes. Recent advances include the development of a lens that had increased sensitivity in the UV region.

Non-destructive spectral imaging can be used to characterize historic documents by capturing the unique chemical spectral response of composite materials including substrates (paper, parchment, photographic materials) and media (inks, pigments, colorants). Capturing UV, VIS, and NIR spectral data in various illumination orientations minimizes handling of fragile items and allows greater capacity for materials analysis and post-acquisition processing to uncover hidden and obscured text and information. All images are accurately registered, enabling almost unlimited combinations of spectral wavebands for further processing. The integrated system uses low heat and reduced light exposure on the document, ensuring preservation of original materials.

During the examination of these historic documents, multiple latent fingerprints were developed on the Nicolay and Hay copies of the Gettysburg Address. The United States Library of Congress and the United States Army Criminal Investigation Laboratory began a collaborative effort to obtain additional items of historical significance pertaining to President Lincoln for non-destructive forensic examinations with the intent of developing additional latent fingerprints to compare with those on the Gettysburg Address. This presentation will give the attendees a chance to become familiar with the application of hyperspectral imaging for latent print development, view the latent fingerprints which were developed on the Gettysburg Address, and discuss the imaging and processing techniques used to capture and non-invasively develop these prints.

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### Hyperspectral Imaging, Forensic Documents, Latent Prints

## BS4 Theater of the Absurd – Ethics and the Truth Versus the Fiction of the Courtroom

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**Educational Objective:** The objective of this presentation is to introduce the contrasting fictional portrayal of the court in media versus the reality of actual trial practices with the goal of allowing forensic witnesses to benefit from lessons learned—both good and bad—by the presenters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing the attendee to better understand unrealistic portrayals and expectations placed on the forensic witness by attorneys, juries, and others based on a comparison with media presentations.

The forensic practitioner is challenged with expectation placed on him/her by many interests. These often come to light most directly in the courtroom where counsel for both parties have a vested interest in

substantiating their version of the facts. This adversarial system draws in the neutral practitioner who is faced with expectations of performance from the judge, law enforcement, jury, and others. In the modern era, the media has periodically gone through apparent feeding frenzies of interest in high-profile “case of the century” vignettes with ever-increasing frequency. The particulars of day-to-day forensic work have been glamorized on television in the form of fictional crime dramas and reality television, leaving many viewers expecting investigative miracles from the forensic practitioner. The “CSI effect” is an all too real phenomenon.

Using a compare and contrast case study basis with use of video clips from dramatic courtroom events and actual sworn testimony, the dilemmas for the forensic witness will be presented. The ethical dilemmas for the witness in how to properly convey complex scientific testimony to a lay jury while attempting to stay neutral and objective is a continuing challenge for the witness – even if well-experienced. Trying to ensure that the jury has a complete picture of the science underlying the testimony, while not reading in real or perceived shortcomings, is a challenge. At times, counsel might not ask the appropriate questions or the jury might believe that certain evidence “should be present” when it is not. These are all too familiar scenarios to those who have been in the witness box.

The 2009 National Academy of Sciences Report stressed the need for reinforcing ethics in forensic practice. Trial testimony is often where one’s moral code can be put to the test by not only what one says but what is not said and how these ends are achieved. The impartial scientist should hold fast to the purity of the science and, while understanding the possible motives of parties involved, not become an advocate. The witness’s use of logic should remain that and not become mere rationalization, intended to raise doubt. To this end, the practitioner must be able to convey the difference between “hard” and “soft” science, between reality and illusion.

Areas of specific inclusion would relate to the strength of opinion (that is overstating or understating testimony), misrepresenting facts or underlying principles, and the ethics of being a private versus governmental employee. While any ethical lapses may end with the net result that the ideal of “the truth, the whole truth, and nothing but the truth” is not fully conveyed to the jury, the difference between outright lies, deception, and misleading testimony is an important consideration. Media versions may allow some blurring of an ethical line that must remain clear if the foundation of the science is to remain sound.

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#### **Testimony, Courtroom, Ethics**

### **BS5 The Battle of Gettysburg: How Today’s Technology Connected Jennie Wade to Abraham Lincoln After 145 Years**

*Rod Englert, BS\*, Englert Forensic Consultants, PO Box 605, West Linn, OR 97068; and John Sotos, MD\*, 1788 Oak Creek Drive, Palo Alto, CA 94304*

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**Educational Objective:** During this presentation, attendees will learn of historical events being affected by today’s technology involving blood patterns, luminol, DNA, bullet trajectory, and clues to President Lincoln’s medical condition.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by relating historical events to modern-day scientific technology.

Can state-of-the-art forensic technology shed new light on mysteries that have remained unsolved for more than a century? This presentation reopens two intriguing historic events—the deaths of President Abraham Lincoln and Jennie Wade, the Battle of Gettysburg’s only civilian casualty—and puts them under the lens of modern science, subjecting 19th-century evidence to 21st-century technology including DNA analysis and luminescent chemical testing as well as advanced medical diagnostics and bullet trajectory analysis.

The first case discussed will be the shooting of Mary Virginia “Jennie” Wade during the Civil War in 1863. A team of crime scene reconstruction and DNA experts conducted a detailed examination of the Jennie Wade House in Gettysburg and discovered convincing evidence that historic accounts had the facts wrong. Attendees will be walked through significant points in this historic sniper shooting scene, revealing how trajectory analysis can be applied to bullet holes in the original wooden doors to determine that the bullet responsible for killing 20-year-old Wade came from a different angle and locale than originally believed. This presentation will include a reenactment of the crime as it unfolded. The forensic tests performed on the floorboards and the bread trough Wade was said to be leaning over when she was shot to death will also be discussed. Use of Luminol revealed chemical reactions suggesting the presence of bloodstains. Core samples were submitted for advanced DNA testing, which may ultimately determine after 148 years not only whether the blood is human but whether it belonged to Wade.

Equally fascinating from a forensic standpoint are other bloodstained artifacts from the Civil War era, including a lock of hair found in a private safe at the Wade House, which was allegedly preserved from President Abraham Lincoln’s autopsy. Medical historian and obscure-diagnosis expert John Sotos of the University of North Carolina School of Medicine has conducted exhaustive research on Lincoln and concluded that, based on his features and symptoms, the president suffered from a rare genetic cancer syndrome that would likely have killed him within months had he not been assassinated. By examining bloodstained Lincoln artifacts, this presentation may be able to expand science’s understanding of the sixteenth president’s medical condition.

In his presentation, Dr. Sotos will explain how a master diagnostician can analyze clues and pinpoint telltale details in photographic and other historical evidence to determine that Lincoln and three of his four sons had multiple endocrine neoplasia type 2B (MEN2B). The hallmarks of the disease and how they are recognizable in such distinctive characteristics as Lincoln’s unusual height and long limbs, sagging face and bumpy lips, fatigue and headaches, perpetually cold hands and feet, as well as the death of three of Lincoln’s sons before age 20 and his mother’s death at 34, will also be discussed.

The discussion will delve into the applications—and potential breakthroughs—that scientific technology offers to solve crimes, broaden our understanding of history, expand medical knowledge, and provide answers to questions that have eluded experts for generations.

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#### **Luminol, Trajectory, Lincoln**

### **BS6 Tables Turned: An Unsuccessful Case of a Newborn Kidnapping by Caesarian Section**

*Robert J. Morton, MS\*, Federal Bureau of Investigation, National Center for Analysis of Violent Crimes, Critical Incident Response Group, 2501 Investigation Parkway, Quantico, VA 22135*

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**Educational Objective:** After attending this presentation, attendees will understand the unique dynamics involved in cases of offenders who attack pregnant women to steal their fetuses. This case will highlight the need for forensic sciences to assist in unraveling complex homicide crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the complexities involved in homicide scenes and the need for forensic sciences to assist law enforcement in ascertaining the sequence of events involved in equivocal death investigations.

This presentation is designed to discuss a very rare and bizarre type of homicide where an offender attacks a pregnant woman and forcibly performs a crude caesarian section, removing the victim’s fetus, usually resulting in the death of the mother. A case will be discussed where a victim was stalked and attacked by a woman who was intending to steal her fetus

by caesarian section. In this instance, the victim was able to successfully fight off the offender, killing her. Additionally, this presentation will explore the history of newborn kidnapping by caesarian section, as well as the research that has been conducted on this unusual type of crime.

The FBI's National Center for the Analysis of Violent Crime (NCAVC) is routinely consulted by federal, state, local, international law enforcement, and criminal justice authorities in a variety of cases of unusual, bizarre, and repetitive violent crimes, especially homicides. NCAVC assistance was requested by local authorities in regards to a case of a pregnant woman who was attacked by an offender attempting to steal her fetus.

The victim did not previously know the offender. The offender concocted a ruse where a store had mistakenly delivered a package to her residence that was addressed to the victim. The offender was able to engage the victim in conversation regarding the victim's pregnancy learning that the victim was due to deliver in the next couple days.

When the victim went to pick up a second package, she became suspicious and attempted to leave the offender's residence. The offender attacked the victim with a knife, but the victim managed to disarm her, stabbing the offender three times. The victim fled the apartment with the knife and the offender's cordless telephone. The victim was able to contact the police and the offender was transported to the hospital where she died. The victim suffered minor defensive injuries and subsequently delivered a healthy baby.

The police conducted an equivocal death investigation into the case and discovered the offender had previously claimed to have been pregnant four other times. According to the offender, she lost the pregnancies due to miscarriage or stillborn deaths. No one in the offender's family ever visited her in the hospital or attended any funerals for the babies. Further, there were no medical records that substantiated the pregnancies. The offender had gone to great lengths to convince her family she was pregnant. The offender purchased furniture, baby supplies, diapers, and had set up a nursery in her residence. She even wore maternity clothing that was padded to resemble a pregnancy. The offender had a "delivery" kit containing surgical gloves, hemostats, surgical scissors, absorbent gauze, and a plastic clip to secure an umbilical cord.

The investigation revealed the offender was sexually and emotionally abused while growing up. Both of the offender's parents were alcoholics and the offender became the main caregiver to her other siblings. The offender had claimed her father had sexually abused her from ages 7 to 12 years of age, and he was subsequently convicted of molestation and sentenced to 20 years.

This case highlights the unusual dynamics involved in newborn kidnapping cases by caesarian section. It reinforces the role of forensic science, as well as the extensive investigative effort required in equivocal death investigations.

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**Forensic Science, Fetus Theft, Equivocal Death**

## **BS7 The Tale of the Black Cow, Granny, and How Animal DNA and Branding Experts Exonerated a Man From Wikieup, AZ**

*Jason D. Ricke, JD, LLM\*, Mohave County Public Defender's Office, PO Box 7000, Kingman, AZ 86402*

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**Educational Objective:** After attending this presentation, attendees will understand the impact of animal DNA in a criminal case and how jurors may ignore what we, as insiders, perceive as the strongest piece of scientific evidence in a case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing unique topics of cattle DNA and branding experts. It is imperative to recognize that as our understanding of DNA and comparison science evolves, it reaches into topics previously foreign to these types of scientific analysis.

This presentation will highlight a case arising out of events that took place in Wikieup, AZ, a small ranch town of 150 residents in Northwest Arizona. Everybody sees cattle when driving down country roads, and nearly everyone buys beef in the local grocery store, but how does that meat get from point A to point B? How do we decide who owns livestock that graze out on the open range? What happens when someone is accused of cattle rustling and then lies about it under oath?

When a lawyer takes on a unique area of law, they themselves must become experts in that field. This story highlights one attorney's journey into unfamiliar territory and how experts eventually led to a not guilty verdict at trial.

The defendant was caught with two cattle on his ranch that the State says were not his, one of which had an altered brand. The State asserted that the defendant altered or obliterated the brand on the animal, and then at a cattle ownership hearing in the Kingman/Cerbat Justice Court, lied under oath about when he branded the animal. At the outset of the case, the State had bovine DNA testing done to prove paternity of the two cattle involved, and was going to call as witnesses an animal DNA expert, a branding expert, as well as a veterinary cattle expert. The case looked bad for the defense.

Everything changed when a package from California arrived at the doorstep of the Public Defender's Office containing tissue and teeth of what the defendant claimed to be the mother of the cow with the altered brand, thereby proving these were his cows. In the end, the defense used the same animal DNA expert originally called by the State as well as their own retired branding expert to deal with the evidence against him which resulted in a not guilty verdict.

The greatest surprise of all was not the verdict at trial, but how the jurors perceived the scientific evidence that was presented. The State focused their closing argument on how the defense story was a bunch of bull. The defense waived their hand the other direction and said look at the DNA. Once the verdict was returned, some of the jurors explained that their entire decision was based on something that neither side saw coming.

Attendees may walk away reevaluating their own cases based on the story of the black cow, granny, and a small time ranch hand from Wikieup, AZ.

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**Animal DNA, Cattle Branding, Comparison Science**

## **BS8 Thomas Krauss Memorial Bitemark Breakfast – Fantasy of Forensics: How Junk Science Failed to Persuade the Jury in the Casey Anthony Case**

*Jose A. Baez, JD\*, 522 Simpson Road, Kissimmee, FL 34744*

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**Educational Objective:** During this presentation, attendees will learn firsthand how several attempts to introduce junk science in the courtroom in this mega high-profile case were rejected by the jury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for sound forensic science and the use of the judicial system as a gatekeeper.

What was dubbed "The Trial of the New Century," the *State of Florida vs. Casey Marie Anthony* posed several problems to law enforcement and state forensic experts. The daily barrage of news coverage in all forms of media resulted in unprecedented coverage. This was the first high profile case that involved social media such as blogs, Facebook®, and twitter®. Instant access was a must. This coverage placed undue pressure on state experts to render several opinions, which were at times questionable, to opinions that fell outside of their area of expertise. Expert witnesses felt this pressure and delivered for the prosecution in the media but delivered more so for the defense during trial.

In July 2008, the worldwide media began to focus on the case of a missing child under what many believed were mysterious circumstances. Caylee Marie Anthony (born August 9, 2005) was reported missing by her

grandmother, Cindy Anthony, on July 15, 2008. Casey Anthony was indicted on charges of first-degree murder and pled not guilty on October 14, 2008. Caylee's skeletal remains were discovered in a wooded area near the family home on December 11, 2008. The prosecution sought the death penalty and the trial lasted for six weeks. On July 5, 2011, the jury found Casey Anthony not guilty of murder, aggravated child abuse, and aggravated manslaughter of a child, but guilty of four misdemeanor counts of providing false information to a law enforcement officer.

In the center of it all was the defendant's vehicle that law enforcement said had the "smell of death." The remains of Caylee Marie Anthony were found less than a quarter mile from the Anthony home. What resulted in trial was a classic battle of the experts with a cutting edge twist. Flying in the face of the recent report issued by the National Academy of Sciences, novel and unproven science was allowed in the courtroom, which resulted in a battle involving every single discipline of forensic science. The prosecution asked the jury to engage in a "fantasy of forensics," which they ultimately rejected. They asked the jury to consider a phantom heart shaped sticker, a phantom stain of decomposition fluid, unverified dog alerts, false computer reports, novel science of air samples purporting to contain the odor of human decomposition, and even a video superimposition of the death of the child where no cause of death was determined. Despite this free-for-all of forensics, and despite the overwhelming inflammatory coverage of this case, the jury saw right through it and rejected the State of Florida's fantasy of forensics. Jose Baez, the defense attorney for Casey Anthony, explores this fascinating case examining not only the use of forensic science within the case but also the role of the media – with the case now being cited as an example of the unfairness of prejudicial pretrial publicity – and the impact that this could have on the rights of defendants.

**Forensic, Judicial, Defense**



ATLANTA 2012

# LUNCHEON SEMINARS



ATLANTA 2012

## L1 An Analysis of a Mine Incident That Led to Deaths Due to an Unknown Confined Space Hazard

*Gerald A. MacIntyre, DVM\*, 2700 1st Avenue South, Cranbrook, BC VIC 6Y3, CANADA*

**Educational Objectives:** After attending this presentation, attendees will understand how we are all at risk when dealing with inadequate and erroneous communications. This incident provides attendees with the knowledge that what you are told is not necessarily the facts in scene attendance. We are reliant on exact information that we are given. The information given may result in your own death from complacency.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting cases in which what you are told may kill you. This is a multidisciplinary death investigation of environmental concerns within a mine reclamation.

On December 21, 2001, the Sullivan lead, silver, and zinc mine production was discontinued after 100 years of production. The mine, which is located in Kimberley, British Columbia, Canada, had the dubious reputation of being one of the longest producing mines in the world.

Following production, a reclamation process began and the staff gradually diminished over the following years to a small skeleton staff for maintenance.

The reclamation of the mine site included covering tailings dumps with glacial till. Water drains were placed at the toe of this dump for water quality testing within the dump. A contractor was hired to monitor water quality.

On May 15, 2006, the contractor responsible for monitoring water quality failed to return to his home that evening. On May 17, 2010, the management at the mine site was alerted to the contractor's disappearance. Two employees were dispatched to search for Mr. Ericksen in separate vehicles. Mr. Bob Newcome found Mr. Erickson's truck at the water sampling shed at the toe of number one waste dump. He observed Mr. Ericksen collapsed in the water sampling shed and lying in the shallow water. He called 911 and reported the finding of an unconscious male lying in water. He entered the shed attempting to assess the physical state of Mr. Ericksen. Mr. Newcome also contacted his fellow employee to meet the ambulance at the main entrance gate. When the ambulance arrived, the attendants entered the shed separately. The senior attendant collapsed as she climbed down to assess Mr. Newcome and Mr. Ericksen. The second mine employee stated to the second ambulance attendant that his partner had collapsed. The attendant, in an effort to rescue his partner, fell into unconsciousness.

A Coroner's Inquest was held in July 2009 to investigate the deaths of Robert Newcome, Kimberley Weitzel, Shawn Currier, and Doug Ericksen. Several recommendations were submitted by the inquest jury to prevent a similar occurrence. The intention of this presentation is to discuss the cause of this incident and enlighten the membership of the various communication difficulties that occurred during this tragedy.

The investigation revealed that all four individuals died from an extremely anoxic environment. The cause of the anoxia was determined to include variable environmental and geochemical factors. The glacial till became compacted over the years and did not allow the dump to breathe. The chemical reactions created by acidophilic bacteria required oxygen to complete the oxidation of iron within the dump and consequently, oxygen within the sampling shed was utilized and drawn into the dump through the water sampling pipe. The oxygen void within the sampling shed appeared to be replaced with nitrogen from within the dump. There was no indication

of anoxia to any of the workers because no odor is associated with this phenomenon. The physiological sequence of events associated with anoxia resulted in hypercapnia and death.

The initial call to the ambulance did, in retrospect, contain all the relevant information regarding a confined space scenario but unfortunately a drowning scenario was used to dispatch the ambulance attendants. They were not prepared mentally to assess an anoxic hazard when they attended the scene and unfortunately died in an attempt to rescue two men that were down upon their arrival.

**Environmental Asphyxia, Anoxia, Environmental Anoxia**

## L2 Odor Mortis: What Is It Anyway?

*Jeffrey L. Ashton, JD\*, 4068 Tenita Drive, Winter Park, FL 32801; Bernard A. Raum, JD\*, Levin College of Law University of Florida, PO Box 1149, Newberry, FL 32669; Arpad A. Vass, PhD\*, Oak Ridge National Laboratory, 1 Bethel Valley Road, PO Box 2008 MS6120, Oak Ridge, TN 37831-6120; Jan C. Garavaglia, MD\*, District 9 Medical Examiner, 2350 East Michigan Avenue, Orlando, FL 32806; and Bruce A. Goldberger, PhD\*, University of Florida, College of Medicine, Department of Pathology, 4800 Southwest 35th Drive, Gainesville, FL 32608*

**Educational Objectives:** After attending this presentation, attendees will learn the significance of the use of *Odor Mortis* to identify sites where human decomposition has occurred, how this evidence is gathered, analyzed in the laboratory, and how this forms as a basis for expert opinion. The attendees will learn applicable rules of circumstantial evidence which govern the use of *Odor Mortis* in court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the emergence of *Odor Mortis* capture and analysis will establish a scientific basis for qualified witness at a crime scene or in a laboratory to specifically identify the "smell of death" as they recognize it from their experience and have that identification confirmed by laboratory analysis.

The presentation of a case based upon circumstantial evidence is all about putting the pieces of the puzzle together. Where that case is based largely on scientific evidence, one is faced with an additional challenge. Before the pieces can be arranged in such a way as to show the picture of the defendant's guilt the jury must first understand the evidence. The attorney presenting the evidence must know enough about the science to understand it themselves without allowing their presentation to become so immersed in the science that they lose the jury. This is the true challenge in the presentation of a complex scientific circumstantial evidence case. It is meeting this challenge in general and in particular the use of the concept of *Odor Mortis* in the case of *State of Florida v. Casey Marie Anthony* that will be discussed. Additional discussion will concentrate on the admissibility of the underlying scientific basis of *Odor Mortis* analysis, the expert testimony based upon that analysis, and the use of an expert's sense of smell to identify *Odor Mortis*.

In addition, specific discussion of scientific evidence in this presentation will focus on the development of the decompositional odor analysis database and will include collection methodologies, experimental design, controls, and ramifications of air collection protocols at crime scenes. *Odor Mortis* will be discussed and how the chemical signature of death changes over time. This discussion will end with a summary of how these analytical procedures were used as circumstantial evidence in the Casey Anthony case to confirm the presence of human remains in the trunk of the defendant's car.

For those individuals who smell decomposing bodies on an almost daily basis, they know it is a pungent, unique, and for many, an objectionable odor. How unique is the odor? Most experienced people can say it smells nothing like rotting food, small animals, or fish; however, is that partly because of the quantity of the rotting material we smell? Medical examiners are routinely confronted by these issues. For example, two scenarios involving a medical examiner will be discussed where large quantities of rotting material were associated with a case. The first is where a closed thermal cooler was found by the side of a road with decomposed, liquidized tissue of approximately a foot deep in which bones had sunk to the bottom. Is the smell of the inside of this cooler enough to enable a medical examiner to tell whether this was a rotting deer and not a human? The second scenario involves a driver of a dump truck filled with mildly rotting cow entrails from a slaughterhouse that was in a collision with a freight train on a hot summer day in Texas. Both the driver and the train engine became covered with the entrails that continued to rot in the summer heat while investigators worked the train crash. The smell was pungent and objectionable, but was it unique based on human smell alone? It might be difficult to make an *Odor Mortis* determination in either situation without some sort of scientific determination.

Lastly, toxicological analysis of decomposed remains will be discussed. Such toxicological analysis is routinely performed by forensic toxicology laboratories as an essential component of the medicolegal death investigation process. The presence of drugs and drug metabolites can be used to support a toxicological cause of death. A wide range of specimens are typically available, but the type and condition of the specimen is dependent on the decedent's state of decomposition. The usual specimens include decomposition fluid, solid organ tissue, cranial wash, bone, bone marrow, hair and nails. Toxicological analysis of specimens can also be used to identify products of decomposition. While these findings are not typically reported to the medical examiner, the results may prove useful in investigation and be used to support an *Odor Mortis* finding.

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**Odor Mortis, Human Decomposition, Circumstantial Evidence**



ATLANTA 2012

# WORKSHOPS



ATLANTA 2012

## W1 High-Profile Cases: The Los Angeles Experience

*Christopher B. Rogers, MD\*, Lakshmanan Sathyavagiswaran, MD\*, and Edward Winter, BA\*, Los Angeles County Department of Coroner, 1104 North Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will be familiar with techniques used in handling high-profile cases including media relations, office security, and preparation of the autopsy report.

This presentation will impact the forensic science community by providing practical techniques for handling high-profile cases.

Most medical examiners periodically handle high-profile cases. These cases can be especially challenging because of increased media attention and the need to maintain the security of the medical examiner's office. A thoughtfully prepared autopsy report may help to avoid unanswered questions in the future.

Los Angeles County has seen a number of prominent people become coroner's cases, as well as other cases that have attracted public attention such as officer-involved shootings, deaths in custody, and serial murders. Taking examples from these cases, this presentation will demonstrate methods of handling cases that generate high public interest.

Members of the media need accurate and current information about medical examiner's cases. In order to prepare for high-profile cases, the medical examiner should develop a media relations plan. In larger offices, there should be a few people responsible for media calls and public statements. Smaller offices may specify in advance the resources necessary to handle a large number of media calls. At times the number of calls is so great that it is a full-time job to deal with them. There should be an advance agreement about which section of government will be responsible for media releases in high-profile cases. This will avoid the confusion of having several officials announce different information.

Although there may be pressure to announce results, press releases and other documents should never be prepared in haste. Part of the media plan should give sample press releases for various situations. There is an increasing need to monitor social media, as internet sites can spread misinformation rapidly. The medical examiner's office may need to disseminate accurate information, starting as soon as possible, through social media outlets.

A media relations plan should include provisions for office security. In some cases a large number of news vehicles, grieving fans, or demonstrators block access for employees and others. The decedent must be protected from photographers and curious members of the staff and public. The medical examiner's office must, however, allow access to employees and those whose business is unrelated to the high-profile case. In the past, the police have assisted with security by limiting access to the parking lots.

During the autopsy, as in many forensic cases, details that seem small at first can assume great significance later. The autopsy surgeon should re-read and carefully follow office procedures. Any extraneous photographs (by surveillance cameras, cell phone cameras, etc.) should be prohibited. Photographs, x-rays, documents, and computer files should be secured. Toxicologists and other consultants need to be aware of questions that may come up concerning the case. The typed autopsy report should be proofread several times and checked against primary sources of data. It is possible to find flaws in even the most carefully prepared autopsy report, but they should be minimized to the fullest extent possible.

### High-Profile Case, Media Relations, Security

## W2 Postmortem Monocular Indirect Ophthalmoscopy Workshop

*Patrick E. Lantz, MD\*, Wake Forest University School of Medicine, Department of Pathology, Medical Center Boulevard, Winston-Salem, NC 27157-1072; Lezah P. McCarthy, MD\*, Coastal Pathology Associates, Onslow Memorial Hospital, Western Boulevard, Jacksonville, NC 28546; Candace H. Schoppe, MD\*, and Christina J. Tatum, MD\*, Wake Forest University, Baptist Medical Center, Medical Center Boulevard, Winston-Salem, NC 27157; Stephen Lenfest, MD\*, Wake Forest University School of Medicine, Medical Center Boulevard, Department of Pathology, Winston-Salem, NC 27157; and Vivian M. Hathuc, DO\*, Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston-Salem, NC 27157*

After attending this presentation, attendees will: (1) differentiate between direct and indirect ophthalmoscopy noting advantages and limitations of each technique for the postmortem detection of fundal hemorrhages; (2) discuss the fundal location of retinal hemorrhages relative to their projected aerial image during monocular indirect ophthalmoscopy; and, (3) on a fundal diagram, accurately draw retinal abnormalities observed during monocular indirect ophthalmoscopy with a simple ocular model.

This presentation will impact the forensic community by providing an overview of postmortem monocular indirect ophthalmoscopy, facilitating skill acquisition, and evaluating practical training.

Postmortem examination of the retina has relied on ocular evisceration. In most medical examiner/coroner jurisdictions, ocular enucleation is not a standard autopsy procedure unless child abuse is suspected, thus creating observational bias when citing the prevalence of postmortem fundal findings such as retinal hemorrhages (preretinal, flame-shaped or splinter, and dot/blot), perimacular retinal folds, retinoschisis, and postmortem artifactual retinal folds. Postmortem Monocular Indirect Ophthalmoscopy (PMIO) permits examination of the decedent's posterior fundus and portions of the peripheral retina. The required equipment necessary for PMIO is relatively inexpensive and when compared to direct ophthalmoscopy. This technique is less affected by corneal clouding, lens opacity, or vitreous hemorrhage. PMIO uses a focal light source and an aspheric, convex condensing lens. An excellent source of coaxial illumination is a halogen, xenon surgical, or procedural headlamp. This light source creates a collimated beam of light and permits the examiner to stabilize the condensing lens with both hands. Current aspheric lenses range from +14 to +40 diopters and come in different diameters permitting a field of view of 35°-55°. Postmortem corneal opacity may cause the fundus to appear hazy; however, by gently removing the epithelial layer of the cornea, the emergent image is usually of adequate quality to readily detect lesions such as fundal hemorrhages and retinal folds.

Learning how to perform and become proficient at PMIO can be perplexing and intimidating. Most pathology residents and forensic pathology fellows have limited exposure to indirect ophthalmoscopy. Because the projected aerial image is inverted and laterally reversed, precise descriptions or recording of fundal abnormalities can be challenging. Unlike binocular indirect ophthalmoscopy with a teaching mirror attachment, an instructor and the fellow or resident cannot view the projected aerial image simultaneously during PMIO. To address these learning obstacles, it is necessary to develop tools and models to facilitate skill acquisition. An hour or two with an inexpensive ocular model can shift the learning curve of the resident, fellow, or forensic pathologist substantially to the right demonstrating how to correctly position the light source and hold the indirect lens.

This workshop consists of three sessions. An initial discussion reviews the technique of PMIO, highlighting the optics, the equipment, and examples of abnormal fundal findings found at autopsy by PMIO. Next, attendees will have a realistic learning experience by practical hands-on training with a procedural headlamp, an aspheric indirect lens, and a simple ocular model containing a variety of retinal abnormalities observed at autopsy. The ocular models have variably sized “pupillary” openings and some will have clear acetate over the openings to simulate corneal glare. Facilitators will assist attendees in positioning the procedural headlamp, holding the indirect lens, viewing the projected aerial image, and accurately recording the retinal abnormalities. Following practice visualizing and diagramming numerous fundal images, attendees will be evaluated with a series of unknowns. Self-assessment of technical skill training and review of the unknown retinal findings concludes the workshop.

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**Forensic Science, Postmortem Monocular Indirect Ophthalmoscopy, Retinal Hemorrhages**

### **W3 Advanced DNA Mixture Interpretation and Statistical Approaches**

*Debra E. Glidewell, MS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297-5205; Debra A. Figarelli, BS\*, 7881 North 114th Avenue, North, Largo, FL 33773; Timothy S. Kalafut, PhD\*, and Joel D. Sutton, MSFS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297-5205; and Robert I. O'Brien, BS\*, 7881 114th Avenue, North, Largo, FL 33773*

After attending this presentation, attendees will be able to: (1) describe the importance and use of statistics for DNA mixtures; (2) describe all mixture statistical formulas and mixture interpretation approaches outlined in the SWGDAM DNA Mixture Interpretation Guidelines (approved 1/14/10); (3) use multiple approaches to DNA mixture deconvolution and statistical applications; and, (4) be familiar with one software tool for DNA mixture deconvolution.

This presentation will impact the forensic science community by providing attendees with a better understanding of complex mixture deconvolution techniques, the United States Army Criminal Investigation Laboratory (USACIL) software tool, and the application of statistical formulae outlined in the SWGDAM DNA Mixture Interpretation Guidelines.

DNA mixture deconvolution and the application of statistical methods is one of the most challenging aspects of forensic DNA analysis. This mixture workshop will be a hands-on workshop using both qualitative and quantitative approaches to mixture deconvolution. This workshop will review the SWGDAM Guidelines to include the use of analytical and stochastic thresholds, heterozygous peak balance, percent contribution to assess mixture proportions, and stutter considerations.

The NFSTC DNA Services team has trained over 100 forensic DNA analysts in DNA mixture interpretation and statistical applications using the 2010 DNA Mixture Interpretation Guidelines. NFSTC will provide devices for computerized interactive participation by attendees which will allow attendee opinions to be recorded anonymously and seen on a screen. This on screen anonymous feedback allows attendees to see how other laboratories are approaching DNA mixture deconvolution. This information can then be taken back to their laboratories to share with the analytical staff.

Due to the nature of the cases routinely received, the USACIL has years of experience in complex mixture interpretation. USACIL presenters will discuss their methods and demonstrate a software program used by USACIL staff. ArmedXpert™ is a commercial DNA data analysis program developed by the U.S. Army Criminal Investigation Laboratory and licensed to NicheVision Forensics, LLC. The program uses data tables generated from data analysis tools. The user is able to quickly compare samples from multiple tables to one another in a case, between cases, to a

laboratory staff database, QC databases, and create tables or cmf files for CODIS entry. In addition, the software uses a proportional allele method to deconvolute two and three contributor mixtures.

USACIL will provide real case examples of complex mixture data that will be worked through as interactive group activities. The panel of instructors will discuss and show how to apply all interpretation and statistical approaches outlined in the SWGDAM Guidelines for each case example. These case examples will then be deconvoluted using the software (ArmedXpert™) employed by USACIL in order to compare the manual versus automated deconvolution results.

When drafting the 2010 SWGDAM DNA Mixture Interpretation Guidelines, one of the goals of SWGDAM was to provide information that would enable greater consistency and accuracy among analysts within a laboratory. This workshop is designed to provide attendees with hands on applications of scientifically acceptable methods for mixture deconvolution. The interactive component is intended to encourage analysts and technical leaders to review their standard operating procedures and validation data in light of the training on these guidelines and to update their procedures as needed.

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**DNA, Mixture, Workshop**

### **W4 Sex-Related Homicide and Death Investigation: Practical and Clinical Perspectives—Significance of Pornography, Sexual Deviance, Autoerotic Fatalities, Signature and MO, Serial Murder Investigation, as Well as the Increase in African American Serial Killers Involved in These Events**

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After attending this presentation, attendees will: (1) understand the role of fantasy in sex-related death; (2) collect and preserve physical and psychological evidence in sex-related incidents; (3) determine the MO and signature characteristics in crime scenes; (4) understand investigative and behavioral analysis in criminal profiling; and (5) understand serial murder trends.

This presentation will impact the forensic science community by informing the attendees of the dynamics and proper procedures in the investigation of sex-related homicides and death investigations.

Sex-related homicides and deaths occurring during sexual events have drastically increased over the years and claim victims from all walks-of-life (men or women, lovers or strangers, elders, or children). These fatalities may occur from recreational misadventures or sex-related crimes, which are perpetrated by sex offenders and represent the most horrific crimes imaginable. The internet has certainly provided society with technological advances but has also resulted in the proliferation of pornography and easy access to sex-related materials to anyone with a computer or computer access. It is significant that the sex industry, which consists of commercial enterprises providing adult entertainment, earns over \$13 billion a year in the United States and how that may influence the increase in sex-related events.

Attendees of this presentation will better understand the significance of sexual deviancy, fantasy, and pornography in sex-related events as well as the investigative and behavioral analysis applied to these types of incidents. Attendees will understand the importance of the collection and preservation of evidence in sex-related events and the attendees will appreciate the impact of Signature characteristics and *modus operandi* as applied to sex-related homicides.

## W5 Paper Fundamentals for Forensic Document Examiners

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In the first segment, the presenters will provide examples of sexual deviance as well as the paraphiliac considerations in these type death investigations. There are over 35 Paraphilias described in the literature and a number of Paraphilias are cross-associated with sexual homicides. These will be explored at length in the presentation. The presenters will also explore the connection between Pedophilia and the sexual homicide of children with appropriate case studies provided for illustration. The attendees will then be apprised of the current knowledge regarding autoerotic fatalities.

Attendees will be apprised of the current knowledge regarding autoerotic fatalities including definitions, incidence, crime scene characteristics, typical and atypical methods, and victims. It will be demonstrated that the widely-cited incidence of 500 to 1,000 autoerotic deaths-per-year in the United States is no longer accurate. It will be explained that an incidence of 0.2 to 0.5 cases per million inhabitants per year is a better estimate of the incidence of autoerotic deaths. New epidemiological data have demonstrated that this incidence is higher in big cities compared to rural areas. There is no clear evidence of a preferential time-of-day for these deaths, but there appears to be slightly more incidences of autoerotic deaths during summer. Recently, the typical and atypical methods of autoerotic deaths have also been revisited based on the new standardized classification of asphyxia. New data on the crime scene characteristics will also be presented, along with a discussion of four types of atypical victims: non-white female, children, adolescent, and the elderly. This presentation will provide a comprehensive and practical illustration of specific examples and cases with a discussion of the investigative and clinical considerations, which will include pathology, pathophysiology, as well as the investigative and behavioral and forensic aspects of these events.

The second segment of the workshop will focus on the dynamics of sex-related homicides, which includes rape, lust murder, and serial killing. Using a case history format, the importance of “signature” and “*modus operandi*” will be demonstrated to illustrate the application of Investigative and Behavioral Analysis to the crime scene examination.

Current research regarding the frequency of sexual posing in homicide crime scenes and the phenomena of African-American sexual serial killers in the United States will be included. The myth that there are no African-American serial killers has been perpetuated based on media coverage on television and/or Hollywood movies such as: *The Red Dragon*, *Silence of the Lambs*, and *Hannibal* which exclusively focus on the white serial killers. However, there has been a drastic increase in the number of African-American serial killers since the 1980's. Only recently, with the arrest and conviction of Anthony Sowell in Cleveland, Ohio and the identification through the use of familial DNA of Lonnie Franklin, Jr. “The Grim Sleeper” that the media has acknowledged the fact that there are African-American serial killers reported on these cases. The current research indicates over 150 African-American sexual serial killers as of July, 2011.

The overall goal will be to provide comprehensive and practical information which will serve an investigative guide in sex-related homicide and death inquiries.

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### Sex-Related Homicide, Autoerotic Deaths, Serial Murder Trends

After attending this presentation, attendees will have a better understanding of ASTM training requirements. Individuals with little previous exposure to the subject area will have a comprehensive introduction, those with moderate exposure will have an updated refresher, and those with significant exposure will have had an opportunity to share their knowledge and case experiences with the other participants.

This presentation will impact the forensic science community by establishing and implementing standards for training and practice, and providing a more predictable skillset. The “questioned document” profession has established minimum training requirements which are documented as an ASTM standard; this includes training regarding paper. As both a supplemental initial training and continuing education opportunity, this workshop provides access to technical expertise that geographically dispersed individuals and groups could not realize.

Forensic document examiners are qualified through an apprenticeship process. The types of training required were determined by the profession and are described in the ASTM Standard *E2388-05 Standard Guide for Minimum Training Requirements for Forensic Document Examiners*.

In order to ensure that examiners are up-to-date regarding paper, the all-day workshop will be divided into four sessions: the first will include the history of paper and the paper manufacturing process. The second will cover techniques and casework that involve paper. The third will introduce security papers. The final session will include techniques for the examination of security papers, and casework examples in which security paper played a role.

**History:** Using extensive artwork and historical images, the “Paper History Timeline” will be shared with workshop participants. In Asia, the Middle East, and Europe, cultures were striving to have something on which to write! Fraud was soon to follow.

**Manufacturing Process:** Using extensive video, images, and marginally technical explanations, speakers will ensure the workshop participants comprehend the technical complexities that have radically changed the simple process that is papermaking.

**Paper Properties and Basic Examinations:** Various characteristics are inherent in a single piece of paper given the materials and process used. Knowing what they are, identifying differences, identifying reasonable variations, and deciding what they mean are the fundamental basis of paper examinations. In this session, how the characteristics are affected when paper is acted upon, and methods for assessing the condition of those characteristics will be described. Indented handwriting, fracture match, and paper fiber impressions will be discussed. Participants will be invited to share case summaries.

**Security Papers:** As the value of a paper document increases, the need to protect that document also increases. Over time, many elements have been changed or added to make documents more difficult to alter or counterfeit. These elements may be printed material, chemical additives, or other security materials (such as fibers or planchettes.) In this session, the entire range of security papers will be described.

**Security Papers – Examination Techniques and Case Examples:** Counterfeiters have only one job: to fool the person they need to fool. Typically, the person receiving a counterfeit item is not a forensic examiner and the counterfeiter knows this. Many efforts to duplicate security papers are amusing to the skilled document examiner; however, they are sufficient to be accepted as genuine. In this session, technical information regarding

examination techniques and equipment will be provided, as well as examples of cases in which security paper played a role. Some will be of the amusing variety and some of the nearly undetectable variety. In addition, as in the Paper Examination session above, participants will be invited to share case summaries.

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**Paper, Watermark, Security**

## **W6 Practical Ethics in Forensic Science – A Multidisciplinary Call to Arms**

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After attending this presentation, attendees will better understand the nature of practical applied ethics, including the history, practice, enforcement, and expectations from a multidisciplinary view. Attendees will understand potential dangers associated with unethical conduct.

This presentation will impact the forensic science community by providing a better understand of the history and nature of ethics as it relates to the practice of forensic science.

“Tainted Science and Testimony Leads to Re-opening of 120 Cases over Last 15 years,” “Man Freed After Serving 21 years in Prison Due to Lab ‘Oversight?’”, “State Crime Lab Employee Accused of Biased Analyses and Testimony”

All of these headlines can confront and scare legitimate practicing forensic scientists. At the heart of this fear is wondering how such events could ever occur, since inherent in the practice of forensic science is the requirement for each practitioner to be truthful and beyond reproach. A failure of forensic scientists to act ethically results in serious adverse outcomes. The word “ethical” can be defined merely as proper conduct – doing the right thing, the right way, for the right reason. While seemingly simple to define, the application of being “ethical” is somewhat more obscure. The 2009 National Academy of Sciences Report on the status of the forensic sciences argued for the need for enhanced ethics training within all forensic disciplines. Tacit within this commentary is that there is a significant problem related to practitioner behavior. As most involved in the day-to-day practice of forensic science would argue that they are complying with all extant codes of conduct, there seems to be a disconnect between theory and practice which needs to be addressed. Thus, the question becomes when is ethical, “ethical,” and when is it not? As a non-partisan in the adversarial justice system, the scientist should have no stake in case outcomes.

Clearly, as part of the legal system, there must be room for differences of opinion in the forensic sciences. What is not clear, however, is when such differences are so divergent that individuals’ ethics are drawn into question. In this workshop, the role of ethics in the forensic sciences from different perspectives will be addressed with the intent of approaching an understanding of when the proverbial ethical line is crossed.

The workshop will include a discussion of practical ethics in forensic disciplines from multiple points-of-view, including historians, theologians, educators, practitioners, law enforcement, witnesses, attorneys, and judges.

Presentations will include comparisons to canons of ethics in the existing forensic organizations and in other fields, with possible mechanisms for enforcement of standards presented. The unrealistic expectations of the public may be at least partially responsible for the present intense interest in the field. Recognition of ethical concerns by those in the field and vehicles for whistle-blowing to bring unacceptable behaviors to an end are addressed. Potential outcomes of unethical behavior, especially in the forensic sciences, are presented in a series of case studies.

In the final analysis, all forensic practitioners should remain keenly aware of the inherent ethical dilemmas in the nature of the work and should maintain the highest standards of personal and professional conduct. Transgressions sully the entire field and should not be tolerated. The first step involves knowing the basics and where dangers might lie. This workshop intends to present such a primer.

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**Ethics, Whistle-Blower, Dilemmas**

## **W7 Preparation and Strategic Planning for Accreditation of Forensic Laboratories Based on the ISO/IEC 17025 International Standard**

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The goal of this workshop is to provide attendees with a general overview of the recourses to meet ISO/IEC 17025 requirements and explain how they may be applied in the context of forensic laboratories to include medical examiner laboratories. After attending the workshop the participants will understand the accreditation process as well as the general requirements to prepare for ISO/IEC 17025 accreditation of forensic laboratories.

This presentation will impact the forensic science community by providing an overview of the accreditation of a multi-discipline forensic laboratory based on ISO/IEC 17025. The workshop is geared towards managers and analysts of international forensic laboratories with little or no exposure to the accreditation process. This workshop will cover definitions of common quality assurance terms, the accreditation process, various accrediting bodies, and the general ISO/IEC 17025 and ILAC G-19 guidelines for forensic laboratories. The workshop will provide realistic expectations of what is required to achieve accreditation and provide an achievable roadmap. The workshop will allow international forensic laboratories to create a strategic plan and timetable to achieve accreditation based on the ISO/IEC 17025 Standard.

Laboratory accreditation is a tool used to evaluate the general competence of a laboratory, and as such, is increasingly expected by clients of forensic and medical examiner laboratories and by the courts. The number of states in the United States and countries internationally mandating laboratory accreditation is growing. The value of accreditation increases in the investigation of international crimes when the accreditation program is based on an internationally recognized standard. Often evidence analyzed by 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. Multi-country investigations and prosecutions require that the criminal justice systems in one country can use and trust forensic laboratory reports issued by laboratories in other countries. The concept of and mechanism for international uniformity, consistency, and competence of laboratories

through Regional Multi-Lateral Recognition Arrangements and the International Laboratory Accreditation Cooperation (ILAC) Mutual Recognition Arrangements will be explained including many of the acronyms and buzz words in the accreditation world (ISO, ILAC, IAAC, APLAC, AB, CAB, regional cooperations, MRA, and MLA to name a few).

The International Organization for Standardization has thousands of standards that are used worldwide. The standard ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories is the ISO standard currently in wide use for forensic laboratories. How this standard is applied to the accreditation of forensic laboratories will be covered. The workshop will include a general overview of the Management and Technical Requirements sections of ISO/IEC 17025:2005 covering the main issues of each section.

ISO/IEC 17025:2005 covers general concepts that have been proven to provide a sound foundation for effective organizations performing testing and calibrations; however, ISO/IEC 17025:2005 is not specific to forensic science. ILAC's Guidelines for Forensic Science Laboratories (ILAC G19), is an example of an amplification document for forensic laboratories, and will be reviewed to demonstrate the relationship between an ISO standard and an amplification document.

Following the general overview, detailed presentations will be provided on Document Control, Traceability, Measurement Uncertainty, and Internal Audits.

**Document Control:** The requirements for document control are an excellent area to begin with if a laboratory is just beginning to prepare for accreditation. Specific requirements and strategies for compliance will be provided.

**Traceability and Measurement Uncertainty:** These topics are new to many in forensic science and specifically to the area of toxicology. Establishing traceability has a significant impact on the quality of laboratory results and measurement uncertainty provides a mechanism to compare test or calibration results between laboratories. Specific applications will be provided in the area of toxicology.

Internal audits are a requirement in ISO/IEC 17025:2005; however, internal audits are also a useful tool in preparing your laboratory for accreditation. How to plan and conduct global, vertical, and horizontal audits will be covered.

Months have been spent preparing for ISO/IEC based assessment and now it is time to submit the application to an accrediting body. Understanding the assessment process minimizes the fear associated with an external assessment. The workshop will step through the process from application through accreditation being granted. The development of the assessment plan will be covered. The methods used by assessors to document conformance with requirements will be presented. These will include document review, case record review, observation of testing or calibration activities, and interviews of laboratory personnel. The general process for remediation of findings of non-conformance will be provided. In addition, tips and techniques for making this process as easy as it can be will be provided.

The workshop will conclude with discussion of the most common deficiencies found during accreditation assessments of various forensic testing laboratories.

This workshop will provide valuable practical guidance to national and international forensic laboratories working towards and maintaining accreditation based on the ISO/IEC 17025:2005 standard.

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#### **ISO/IEC 17025, Accreditation, Requirements**

## **W8 Examination and Analysis of Explosives and Device Construction/Components**

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After attending this presentation, attendees will be familiar with forensic explosive analysis, microscopical analysis, instrumental analysis, and sample preparation techniques. The attendees will also become familiar with the investigative leads generated by the characteristics of and components used in a device, its container housing, and its firing train.

This presentation will impact the forensic science community by providing an understanding of the principles and practices of explosive material identification, explosive device construction, component characterization, and report writing. This training will provide exposure to a variety of experts and testing approaches that can be utilized in local, state, and federal laboratories.

The increase of domestic and foreign terrorist activities has brought awareness to explosive analysis in local, state, and federal forensic science service providers. From offering general awareness of materials to investigators and first responders to laboratory analysis of explosives, explosive residues, and device components, analysts in this specialized field continue to work together to share information and intelligence. This networking is valuable for sourcing materials and identifying domestic and foreign terrorists, potentially leading to their arrest or capture. Although national and international agencies are involved in this type of work, this workshop will focus on the forensic science practitioner and training in the forensic science testing of explosive materials and device construction/components.

The workshop will provide the foundational knowledge of explosive terms, types of explosions, specific types of explosive compounds and their characteristics. The workshop will present the attendees with an understanding of common approaches to the examination and analysis of explosive residues and materials. The ensuing presentations and activities over the two days will cover physical examinations and chemical analyses, tips on sample isolation and preparation, improvised explosive devices (IEDs), low explosives, high explosives, and homemade explosives (HME's). Microscopical examination and preparation techniques will be enhanced with hands-on exercises. Workshop attendees will have opportunities to reconstruct devices from found evidence and debris. Faculty presenters will discuss specific cases and offer their conclusions. The panel and participants will conclude this session with discussions on appropriate wording and report writing guidelines that will reflect an understanding in the criminal justice, forensic science, and judicial communities.

In addition to the foundational knowledge, the workshop will provide a short review of explosive blast physics (thermal effects, pressure wave, etc.), the types of explosions followed by the types of explosives (primary, high, and low). Individual compounds of each type will be reviewed including their brief history, development, and modern usage. Explosive materials such as black powder, black powder substitutes, flash powders, and smokeless powders will be examined and characterized as a part of a hands-on exercise. The high explosives will focus on commonly seen compounds such as TNT, C-4, and PETN. Plastic bonded explosives, emulsions and modern blasting agents will also be covered. Analytical

approaches will be discussed and the instrumental techniques utilized such as microscopy, FT-IR, GC/MS, and X-ray techniques.

Microscopical analyses have been intimately tied to forensic science since its inception. However, some modern trends have resulted in moving away from the microscope in explosives analysis. This session will focus on the extreme power the microscope can offer the examiner. From searching debris for small pieces of fragmentation and particles to microcrystalline techniques that can identify specific explosive compounds, the microscope is an invaluable tool in the examiner's toolbox.

In the last few years, new trends in explosives have resulted in an increase in the number of homemade explosives (HMEs). This session will familiarize attendees with the current analytical practices related to HMEs. How these devices are developed and used, the types of explosive materials in them, and the chemistry behind their devastating effects will be presented. Lastly, the workshop will offer a brief insight into potential future testing methods to include instrumentation and innovations in explosives detection and analysis that could impact this field.

At the conclusion of this workshop, the attendees will have the knowledge, understand techniques required to pursue the analysis of explosives, and understand of the importance of the materials used in the construction of explosive devices. Participants will be able to utilize the principles taught in the lectures and offered in the hands-on exercises to hone their skills and bring these resources back to their organizations thereby improving on their existing explosive analysis programs.

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**Explosives, HME, IED**

## **W9 What Did You Just Step In?! Use of Forensic Soil Examinations to Find Out**

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After attending this presentation, attendees will be equipped with sufficient knowledge of soil analysis to either initiate this type of service in their laboratory or suggest improvements to their existing methods for soil analysis. Situational awareness of the need to utilize the analytical services of an outside laboratory for select soil comparisons will also be gained.

This presentation will impact the forensic science community by providing education to those desiring training in forensic soil examinations, as well serving as a reminder about a service that more laboratories may wish to add to their suite of analytical services. This workshop will help to make soil analysis more accessible to the forensic community by demonstrating the limited instrumentation necessary to make this service available. Additionally, the workshop will provide a forum for examiners to discuss the differences in existing techniques, the need for standardization, and the impact of accreditation.

A quick search of the internet for "soil analysis laboratories in the United States" returns a long list of laboratories providing soil analysis for agricultural concerns. However, laboratories providing comparative soil

examinations for forensic applications are rare. Though soil analysis is a well-established field of forensic examination, the air of mystery and difficulty has made the "forensic soil examiner" a rare breed.

Proficiency in the field is only maintained through constant practice and continuing education; however, training courses in the discipline are infrequently offered and often cancelled due to a lack of attendees. Laboratories often cite the lack of instrumentation, time, or personnel as the reasons for not providing this service. Much of this can be overcome with proper and efficient training; for the underpinning work in such examinations can be performed without high-end instrumentation. In fact, much of the comparative work can be performed with instrumentation that most forensic laboratories already have available (i.e., polarized light microscopes, automated scanning electron microscope/energy dispersive spectrometer, Fourier transform infrared spectrometers, and x-ray fluorescence spectrometers).

During this two-day workshop, attendees will gain insight into the geological and historical foundations for soil examinations and their practical applications in forensic science. They will gain experience in the application of a variety of laboratory techniques commonly used for forensic soil examinations. Lectures and "hands-on" sessions will include, but are not limited to, the following: the historical basis for forensic soil examinations; geology as related to the formation of soil; soil collection methods; comparative color and texture analyses; the preparation and separation of soil samples for the isolation of the botanical, anthropogenic (man-made), clay, and mineral components of soil; the application of x-ray diffraction and Fourier transform infrared spectroscopy for the identification and comparison of clays; fractionation of the heavy and light mineral components for identification purposes; mounting methods for identification of soil minerals by polarized light microscopy; methods for percent composition comparisons of the heavy and light mineral content; the utilization of automated scanning electron microscopy-energy dispersive x-ray analysis for automated mineral analysis; determination of provenance based upon the sum total of the components identified in soil; report writing verbiage; and significance evaluations.

Attendees will gain, at a minimum, a theoretical and practical foundation for incorporating soil analyses into their laboratory's suite of analytical services. They will also obtain the skills necessary to detect, collect, and preserve soil evidence and to perform minimal comparisons, thereby enabling preliminary evaluation of such evidence for use in their own cases.

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**Soil, Mineral Analysis, Microscopy**

## **W10 Drug Enforcement Administration U.S. Customs and Border Protection Forensic Mobile Device Workshop**

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The goal of the presentation is to assist attendees in the examination of mobile devices in a forensically sound manner through discussion of relevant topics: (1) handling and preservation of mobile devices: attendees will understand proper procedures for collecting and handling mobile devices so as to preserve data for forensic examination; (2) validation of mobile device examination methods: this discussion will present a proposed framework for validating mobile device examination methods and software tools in light of agency accreditation requirements; and, (3) by learning to leverage available resources against their limitations, attendees will be able to relate the *Daubert* standards to the challenges specific to codifying methodologies in an ever-evolving discipline.

## W11 Digital Photography for Forensic Document Examiners

This presentation will impact the forensic science community by providing sound strategies and methodologies that can be directly applied to the examination of mobile devices and further the development of the digital forensic discipline.

The following topics will be covered: handling and preservation of mobile devices from seizure to analysis and methods validation with a proposed framework for validating mobile forensic methods and tools.

Attendees will: (1) understand the importance and applicability of the *Daubert* Standards in mobile forensic methods; (2) develop and apply an organizational methodology for software tools validation within the framework of accreditation; and, (3) be able to leverage available resources while understanding their limitations and understand the challenges of codifying methodologies in an ever-evolving discipline.

A review of all cell phone forensic tools and the Cell Phone and GPS Forensic Tool Classification System will be covered in depth. Our world has become saturated with inexpensive digital devices. The ubiquity of these devices has changed the way we do everything, from staying in touch with friends on our smartphones to finding the nearest gas station with GPS technology. Criminals also use these devices to aid in the commission of crimes. GPS devices are used for human and narcotics smuggling, while cell phones are used to deliver text messages coordinating the next terrorist attack. Most electronic devices themselves contain a wealth of information and intelligence for investigators, though the field of digital device forensics is still in its infancy. There is a need for a common framework to classify the plethora of tools released into the commercial marketplace in the last five years. Given the exaggerated claims of software manufacturers, the field of mobile forensics has long since clamored for an understanding of not only how these tools work, but when they should be used. Attendees will be able to categorize any mobile device acquisition tool within a classification system. In addition, an overview of many commercial tools for cell phone data extraction currently available will be discussed. This presentation will provide a common framework for the digital device data extraction tool classification system for the entire digital forensics community.

The Apple iPhone®, utilizes SQLite Databases to store user data and applications that have been installed on the device. A digital forensic examiner trying to access this information through traditional means may miss large amounts of relevant data still present within the database. Because of the way inactive data is marked for deletion, it may be impossible to extract this data through typical database queries. Fragmented sections of these databases may also be found in unused space on the device. While the complete file is not readable by traditional methods, forensic recovery may still be possible. The key to gaining access to this information is to understand how the data is organized within the database structure and the manner in which it has been stored and encoded. Attendees will gain an understanding of how to obtain digital evidence from SQLite databases. Attendees will also gain an understanding of how this information is stored, how active and deleted content within these databases can be extracted, and how to decode the extracted information. An explanation of SQLite data storage structure, encoded data, the use of regular expressions to search for and identify records, and the ability to decode the stored values within the recovered records will also be presented. The storage of call history and text messages utilized by Apple's iOS® on the iPhone® will be specifically addressed and used as an example for how to identify and decode deleted records.

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**Mobile Forensics, Apple iOS®, Validation**

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After attending this presentation, attendees will be able to: (1) successfully demonstrate the effective documentation of forensic digital capture techniques as well as explain how digital technologies are used in evidentiary photography processes; (2) successfully complete a hands-on exercise demonstrating the comparison of image resolution and screen (display) resolution, demonstrating the artifacts caused by improper resolution settings and parameters; and (3) successfully complete a hands-on exercise demonstrating the artifacts caused by improper image processing techniques (such as rotating an image, compressing an image, printing an image, etc.). Participant achievement of the training objectives will be evaluated by observing participant behavior in the classroom and assessment of demonstrated practical skills by the instructor.

This presentation will impact the forensic science community by addressing the need for knowledge about the legal ramifications of digital evidentiary photography and best practices for digital evidentiary photography to include acceptable file formats and standards for capturing, storing, printing, transferring, and preserving digital/digitized evidence. It will also provide participants with the acceptable methods and techniques for comparing imaging technologies. The activities will include instructor-led demonstrations and hands-on exercises using image-editing software on the laptop computers participants bring for use in the course.

The Digital Photography for Forensic Document Examiners Training Program is intended for individuals working in a law enforcement field who have limited knowledge and experience using digital technologies in a forensic environment. Upon completion of this program, attendees will have a basic understanding of forensic digital imaging concepts and how various image capture techniques can aid in their investigative process.

It is recommended that attendees taking part in this course have a basic understanding of the Microsoft Windows XP operating system. In addition, it is recommended that all class participants be actively involved in a law enforcement discipline that uses digital imaging technologies.

This is a one-day training program that includes both lecture and hands-on training including note taking. Course instruction also includes a review of best practices and procedures as well as a comprehensive discussion and application of digital imaging techniques.

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**Digital Photography, Questioned Documents, Digital Imaging**

## **W12 Humanitarian Forensic Science: The Forensic Investigation of Human Remains From Armed Conflicts and Catastrophes**

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After attending this presentation, attendees will become familiar with the main practical considerations for large-scale forensic investigations for the search of missing persons from armed conflicts and catastrophes.

This presentation will impact the forensic science community by exploring the application of forensic science in humanitarian contexts and outlining some of the unique challenges posed to the wider forensic community by investigations into persons gone missing. It will also present some of the solutions identified for assisting forensic practitioners, institutions, and service providers involved in these investigations. A multidisciplinary panel of international experts will share their experiences, lessons learned and recommendations regarding practical considerations for large-scale forensic investigations in the search for missing persons from armed conflicts and catastrophes.

The humanitarian scope of forensic investigations applied in the search of missing persons world-wide requires awareness and consideration of factors rarely encountered in the domestic setting. International public law provisions must be well understood and socio-political factors impacting investigations must be taken into consideration. Forensic practitioners must be aware of the health and safety issues associated with working in foreign environments, as well as unique health and safety precautions necessary in potentially dangerous contexts. The psycho-social needs of the families related to the investigation of victims of conflict should be addressed, including the expectations of the bereaved. The selection and integration of identification methods should be planned with care and should be suitable to the specific requirements of the investigation, including the biological profile-related characteristics of the victims and the needs of the investigation. Resources for large-scale investigations, which are usually limited, should be appropriately managed and suitable for logistically-challenged settings while maintaining quality assurance and ethical conduct. Data gathered during investigative processes must be properly managed, taking into consideration chain-of-custody and confidentiality of the data. It must be decided in advance to whom the data will be shared with and under what circumstances. Utilization of local expertise and resources should be balanced against the needs for confidentiality, neutrality, and impartiality in the investigation at the same time recognizing that long-term investigation and identification processes require local involvement to ensure sustainability. Finally,

specific ethical dilemmas related to forensic work and management of the dead in diverse contexts must be addressed, taking into consideration all other planning challenges and constraints. For example, political or prosecutorial pressures may place emphasis on investigative processes for judicial purposes leaving reduced resources for identification efforts. Likewise, reduced resources may force investigators to selectively target specific graves for investigation while leaving other known gravesites untouched, or necessitate the recovery of only a sample of remains within a grave.

As forensic practitioners increasingly become involved in international humanitarian operations, they must adapt their working methods to the specific needs of the investigation, which may differ from the domestic setting. In addition, they must integrate their technical skills into multi-disciplinary teams in order to address the various needs of the investigation and the victims to which the investigations relate. Therefore, it is imperative that practitioners working in large-scale forensic investigations for the search of missing persons from armed conflicts and catastrophes become familiar with the unique challenges that these operations may pose.

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### **Humanitarian, Conflicts, Catastrophes**

## **W13 Estimating Uncertainty in Weights: Hands-On Workshop Using SWGDRUG Document SD3**

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After attending this presentation, attendees will be able to: (1) reference the SWGDRUG website and all associated resources; (2) understand the concept of uncertainty estimation in the seized drug context; (3) evaluate their weighing procedures and protocols using several different uncertainty estimation tools; (4) design a simple uncertainty budget using Excel; (5) design an uncertainty budget utilizing laboratory control charts; and (6) defend a reasonable uncertainty estimation before their peers and before triers of fact.

This presentation will impact the forensic science community by showing how estimation of uncertainty is a key element of any quantitative data and should be estimated when quantitative data is obtained. The 2009 National Academy of Sciences Report emphasized the need for estimation of uncertainty and accredited laboratories will soon face issues of uncertainty estimation and reporting. This workshop will assist forensic scientists in developing reasonable and defensible uncertainty estimations for an important category of quantitative data.

This workshop will include a hands-on tutorial designed to assist forensic analysts in estimating the uncertainty of weights obtained in a seized drug analysis. Although the target audience is drug analysts, anyone interested in uncertainty estimation will find this workshop useful and valuable. The workshop will be taught at an introductory level. No chemical knowledge is required or assumed. The workshop is built around the Scientific Working Group for Seized Drug Analysis (SWGDRUG, [www.swgdrug.org](http://www.swgdrug.org)) Supplemental Document SD-3, which presents several examples for making reasonable and defensible uncertainty estimates for weight measurements.

Uncertainty is a fundamental part of any quantitative measurement. Uncertainty is not the same thing as error, and recording data with an estimation of uncertainty does not imply that data is somehow suspect. The opposite is true; a quantitative value such as a weight of a drug exhibit, along with an uncertainty estimate, is more valuable in many cases, both to

the laboratory and to the customers it serves. The exercise of estimating the uncertainty associated with an instrument or procedure assists laboratories in improving practices and facilitating more effective and efficient method validation policies. The workshop will present examples of how these tasks can work together to improve laboratory operation.

The first part of the workshop program will include a general introduction to SWGDRUG Recommendations - Part IV C (Quality Assurance/Uncertainty) and Supplemental Document SD-3, followed by a discussion on the basic principles of uncertainty estimation. Statistical and metrological resources will be presented and key statistical concepts will be reviewed. Attendees will also receive useful information related to the selection, requirements, operation, calibration, and maintenance of laboratory balances.

The second part of the workshop will be dedicated to discussions of SWGDRUG Supplemental Document SD-3 (Measurement Uncertainty for Weight Determinations in Seized Drug Analysis). A copy of this document will be provided to all attendees and its purpose and background, within the scope of SWGDRUG's mission, will be discussed. This document contains specific examples of how to calculate the uncertainty associated with weight measurements performed in a laboratory. The basis, assumptions, and background behind each one of these examples will be presented in a format that provides for active discussion with the workshop audience. The workshop presenters will also provide assistance and suggestions on how to present and defend uncertainty estimates to peers as well as legal professionals.

The third part of the workshop will be dedicated to open discussions. Attendees are strongly encouraged to bring examples of specific laboratory models for presentation during this section of the workshop. Examples will be discussed and assistance provided on developing uncertainty budgets. Attendees are also welcomed to bring a laptop computer and data from their laboratory for use during the hands-on portion of the workshop.

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#### **Uncertainty Estimation, Seized Drugs, SWGDRUG**

## **W14 Using Pharmacokinetics to Analyze Forensic Toxicology Cases**

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After attending this presentation, attendees will: (1) summarize the basic physiology, pharmacology, and pharmacokinetics required to interpret drug blood and urine levels which have been obtained both before and after death; (2) present confounding issues that limit the interpretation of many quantitative blood test results such as postmortem redistribution, and pharmacokinetics in children; and (3) review approaches to analyzing the complicated Forensic Toxicology case.

This presentation will impact the forensic science community by showing how 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 she/he impaired?", and/or "Did it injure him/her?" Such questions are very difficult to answer on the strength of one test result. The workshop begins with a review of the physiology, pharmacology, and pharmacokinetics of drugs, followed by presentations involving postmortem redistribution, postmortem pharmacokinetics, tissue distribution in the ante- and postmortem settings, and the significance of using various sampling sites for blood.

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of drugs. In many instances, determination of the cause and manner of death may be elucidated by analyzing the correlation

of the drug's pharmacokinetics and the drug's effect (pharmacodynamics). 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 injure or kill him/her?", or "Was she/he impaired?" These questions are very difficult to answer on the strength of one test result. In many instances, the investigating forensic scientist would like to develop an adequate history regarding the time the last dose was taken, the amount, the route of administration, and whether the final dose was a single acute OD, a large OD, or an OD that occurred due to drug accumulation over time, but access to those data are unavailable. In addition, genetic factors regulating drug metabolism, drug interactions, and differences in a drug's pharmacokinetics during toxic dosing (toxicokinetics) impact the analysis of the drug's blood levels and toxic effects.

Though many cases involve the analysis of blood samples obtained from living subjects, in some cases, the blood samples are obtained after death. In the postmortem state, multiple samples obtained from different body sites (e.g., femoral, iliac, subclavian, and cardiac) often show different quantitative results. This may be due to partial absorption from the gastrointestinal tract, incomplete distribution of the drug after ingestion but prior to death, or postmortem redistribution. Cardiac blood is the least reliable. There are four chambers of the heart, and on analysis, all may give different results. Results of blood samples taken from the left side of the heart are less reliable than results obtained from blood samples taken from the right side of the heart because the left side fills from the pulmonary vein (and the lungs are known to accumulate drugs), thus causing the blood to contain higher levels of drug than found in the right heart, which fills from the systemic circulation (vena cavae).

When blood samples have been obtained after death or at autopsy, changes in postmortem redistribution, putrefaction, bacterial contamination, and postmortem production of ethanol may confound the interpretation of the results, unless blood sampling from various body sites and tissues was carried out. In the postmortem state, determining the cause and manner of death may be quite difficult. Moreover, in the postmortem setting, the pharmacokinetics of the drug is altered, as is the volume of distribution (Vd). Using PK and Vd data from antemortem studies and applying them to postmortem data, or using Vd data from adults and applying them to children may lead to major errors in the interpretation of the data and incorrect conclusions about the cause of impairment or death. Attendees will learn how to better apply the principles of PK in their cases, and raise the level of the application of the principles of PK in their practice of forensic toxicology.

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#### **Pharmacokinetics, Postmortem Redistribution, Forensic Toxicology**

## **W15 Hell on Earth — Just Another Day at Work: An Overview of the Tri-State Crematory Catastrophe**

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After attending this presentation, attendees will be able to understand how to approach a mass disaster created when large numbers of bodies are not cremated in accordance with standard funeral practice. Participants will also gain an understanding of how multiple agencies interact in dealing with a complex mass disaster. Approaches to public and media involvement will also be discussed.

This presentation will impact the forensic science community by showing lessons learned from a unique incident which created social havoc

throughout Georgia, Tennessee, and Alabama. The impact was felt among thousands of individuals who had sent the bodies of their loved ones to be cremated. This incident also captured national and international attention in the forensic and non-forensic communities.

On the otherwise typical afternoon of February 15, 2002, during the course of the AAFS meeting in Atlanta, GA, a call came in that changed an otherwise beautiful and mild, deep-South winter's day into one of the singularly most macabre events ever imagined. A decade later, the memories are vivid.

Acting on an anonymous call, investigators responded to a scene in the Noble Community of rural Walker County, GA, just 100 miles from Atlanta, the heart of the "new south." A passerby had discovered what were believed to be human remains in a heavily wooded area, adjacent to several residences and the family-owned Tri-State Crematory, serving northeast Alabama, south central Tennessee, and northwest Georgia.

Investigators arrived to find a scene almost defying description – dozens of bodies, in various stages of decomposition, were laid out in a garage. As investigators recovered from the initial shock and continued exploring the property, dozens of bodies, then hundreds were discovered. Eventually, bodies were found strewn in the woods, comingled in pits, comingled in casket vaults, and within several buildings and vehicles on the property. The crematory owner had maintained essentially no records regarding his supposed cremation practices. A full-scale mass death scene investigation ensued, requiring the combined resources of local, state, and national agencies. The National Disaster Mortuary Response Team (D-MORT) was deployed to assist with the processing the unidentified bodies and partial sets of remains. It was eventually determined to represent remains of some 334 individuals. The recovery process alone lasted for three weeks. Eventually, this case became the largest criminal investigation in the history of the state of Georgia, involving over 500 individuals from more than 55 local, state, and federal agencies in the recovery and identification of these remains. Successful and continuous multi-jurisdictional agency interaction was essential to the resolution of the matter, particularly in handling a problem that literally grew with each passing day.

The scale of the recovery and identification process was hindered by multiple factors, including: location, multiple clusters of mini-scenes with varied conditions, lack of defined scene boundaries, unknown and varied postmortem interval of individual cases, and unknown numbers of remains. All of these factors, combined with the intense international interest in the case led to one of the most unusual death investigation cases in the annals of death investigation. This overview touches on the various aspects of the case including initial response, scene assessment and triage, body recovery, identification, family interaction, media interaction, and criminal investigation.

**Families and media:** The macabre nature of the event resulted in immediate and eventually world-wide interest in the case. Given the intense interest in the case, media interaction became an important factor. A balance had to be struck between the families' rights to be kept informed and public information. Daily interactions included meetings with grieving family members and the national and international press. Dignitaries concerned enough to visit the site included the state's Governor and both U.S. Senators. Superimposed was a criminal investigation, involving some 700 charges and three states. The balance was struck to inform families of progress first and to have one public face associated with the media. The importance of a "family-first" philosophy facilitated a rapid and successful resolution.

**Initial response/scene:** The challenging nature of the sheer volume (literally hundreds of bodies, including many co-mingled and partial remains) was compounded by the ill-defined crime scene area and the lack of any kind of tentative manifest to facilitate with recovering and processing of the remains. The key to a successful outcome in such a massive undertaking is effective assessment and planning.

**Body recovery:** The archaeology of body recovery from mass graves and pits, combined with the need for preservation of evidence for potential criminal prosecution, is a fortunately rare occurrence. The early recognition of the nature and scope of the case allowed strategic placement of sufficient resources in a timely manner. The latter, combined with a strong team

concept, kept morale high and expedited the initial processing of remains.

**Identification:** The unique nature of such an aggregate of an unknown number of unidentified partial and complete remains raving from minimally decomposed to skeletonized was compounded by the lack of knowledge regarding numbers of bodies present. Identification concerns were paramount to the families, and as such, rapid protocols for body identification were required. A balance had to be struck between speed and science, with accuracy unquestioned. The identification techniques employed and success/concerns is summarized. The identification process included collection of thousands of DNA samples from family members and lasted almost two years, although an internet inventory of remains with possible identifiers still results in occasional requests for possible identification.

**Investigation/Prosecution:** The ultimate cost of this man-made disaster to the State of Georgia was approximately 10 million dollars and generated over 100,000 pages of documentation. The perpetrator was charged with 787 counts, including theft by deception, abuse of a corpse, fraud, and giving false statements. He allegedly faced the potential of over 8,000 years in prison if convicted. He eventually pled guilty and was sentenced to twelve years, with credit for time served. Approximately 1,700 family members whose loved ones' bodies had been abandoned joined a class action suit. Settlements reached totaled well over \$100 million but were later significantly reduced to approximately \$54 million

In the final analysis, this case was about hundreds of families whose loved ones' bodies were left to decompose in the foothills of North Georgia. The overall investigation and close teamwork between the law enforcement, medical examiner, anthropologist, and district attorney resulted in a guilty plea in one of the most bizarre criminal cases of all time and a rapid resolution to the ubiquitous civil litigations.

**Motive:** "To those of you who may have come here today looking for answers, I cannot give you." said by Ray Brent Marsh upon entering his guilty plea. His attorneys would later allege that the admitted perpetrator of the crime resulting in largest investigation in the history of the state was a victim of mercury poisoning, allegedly from exposure caused by the dental amalgams from cremated bodies.

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**Crematory, Media, Identification**

## W16 Applications of 2D and 3D Geometric Morphometrics in Forensic Comparisons

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The goal of this workshop is to familiarize attendees with the possibilities for morphometric comparison in both 2D and 3D formats as well as limitations and possibilities for use in forensic science are discussed.

This presentation will impact the forensic science community by showing methods that are used for pattern recognition in forensic comparison with error rates.

Last spring, the Forensic Sciences Foundation's (FSF) Theoretical Forensic Science Committee sponsored a contest. Mary Bush, DDS from the State University of New York at Buffalo was the winner of this "New Science or Technology to Forensic Science" competition.

Based on the contest, this workshop has been developed to discuss and present different aspects of morphometric comparison in forensic science, with application to odontology, footwear impressions, bullets, fingerprints, facial comparison, wound analysis, and 3D reconstruction.

This workshop will: (1) provide an overview of geometric morphometric (GM) analysis; and, (2) demonstrate the methods for evaluation, measurement, and statistical comparison of shape change/distortion of an object in question. The generation of matching criteria and error rates based on a statistical model are discussed in relation to the work of Dr. Bush. Application of GM methods to bitemarks, fingerprints, shoeprints to 3D visualization of 3D bullet comparison, medical forensics, and facial comparison will be presented. Advantages and disadvantages of the different approaches on comparison of shapes based on pattern recognition in digital images will be discussed.

A well-developed method to describe shape variation between biological specimens is geometric morphometric analysis. GM analysis involves placement of landmark points, curves, or outlines on either two- or three-dimensional images. The landmark data can be extracted and analyzed statistically as a unit. GM methods allow for a quantitative examination of shape by capturing the geometry of morphological structures of interest and preserving this information through statistical analysis. Shape information can be visualized by plotting landmark positions in Procrustes superimposition, a method of optimally matching one shape to another. This can be performed with or without scale. Procrustes distances can be used to summarize variations in populations, to express the degree of similarity of individual specimens, means of populations, or to search for matches between specimens.

Amongst the tools available for statistical analysis is principal component analysis (PCA) with which the principal variations of shape can be plotted and visualized. This allows for determination of which shape aspect is responsible for the most variation. Canonical variates analysis (CVA) can also be used to determine if shape information can distinguish between different categories of data. A range of other standard multivariate statistical methods can also be applied to shape data, allowing applications to a wide range of research questions and practical problems.

There has been much recent discussion in forensic forums concerning what constitutes a match, or what defines two objects as being indistinguishable. It might be stated that two objects are identical when differences in measured attributes fall below measurement error levels, when the differences seen are no longer distinguishable from random effects. Error rates can be established by repeated measures studies, in which the same object is measured multiple times. The Procrustes distance derived from GM analysis can be used as a quantitative descriptor of error, and can be used as a threshold value below which objects might be said to match.

Clearly measurement resolution also depends on the scale of the object being measured, and the resolution (smallest object measurable) of the means used to image the object. For example, in crime scene or accident reconstruction, the resolution required might be fractions of a meter; in a shoeprint, individualizing detail might be separated by cm, in a bitemark the achievable measurement resolution might be on a mm scale, fingerprints on a micron scale, and tool marks potentially submicron.

With these examples in mind, careful attention to the issues of magnification (scale) and resolution is of the first importance. However, GM methods can be applied regardless of scale, as long as the nature of the data is understood. At each level of scale, error rates can be established and quantified.

Using GM methods, large datasets can be statistically compared to explore the issues stated above. Ideally, quantification of the range and types of distortion produced will provide forensic practitioners with

quantifiable validations of the quality of example items in the pattern evidence disciplines.

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## Morphometric, Pattern Recognition, Comparison

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### W17 Advances in Asphyxia by Strangulation for Pathologists and Anthropologists

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After attending this presentation, attendees will have a better understanding and knowledge of the new standardized INFOR classification of asphyxia, best practices of autopsy dissections in strangulation cases, radiology aspect of these cases, anthropology best approach, and updates on the pathophysiology.

This presentation will impact the forensic science community by informing the forensic pathologists and anthropologists of the updates on strangulation and the best practices in these cases.

The classification of asphyxia and the definitions of subtypes are far from being uniform, varying widely from one textbook to another and from one paper to the next. Unfortunately, similar research designs can lead to totally different results depending on the definitions used. Closely comparable cases are called differently by equally competent forensic pathologists/medico-legal doctors. In response to this situation, a unified system of classification was recently proposed. This new standardized classification of asphyxia, called the INFOR classification, will be presented. In the INFOR classification, asphyxia in the forensic context is divided in suffocation (subdivided in choking, smothering, and confined spaces/entrapment/vitiated atmosphere), strangulation (subdivided in hanging, ligature strangulation, and manual strangulation), positional and traumatic asphyxia, and drowning. The controversies around the term mechanical asphyxia will be discussed. Asphyxial games will be presented in relation to this new classification.

The external and internal examination findings in strangulation will be discussed. A proper neck dissection is a key element in the investigation of strangulation deaths. Despite the usefulness of x-rays and computed tomography as ancillary techniques, manual dissection of the neck structures remains the most widely used technique to assess the integrity of neck structures. There are two ways to dissect the neck: *in situ* or *ex-situ*. The *ex-situ* method follows the removal of the neck structures by cutting the muscles insertions of the base of the mouth and gently removing this group of structures from the vertebral column. This *ex-situ* method is superior to the *in-situ* method and the reasons for this will be explained. The method will be described step-by-step, with images of external and internal injuries. The common pitfalls in the interpretation of autopsy findings will be discussed: anatomical variations (e.g., triticeal cartilage), normal mobility or bending of a cartilage or joint (with considerations on the rate of ossification of the hyoid and thyroid horns and the sinostosis of the horn/body joint of the hyoid), and postmortem changes.

The incidence of bone fractures in suicidal versus homicidal hangings and manual strangulation will be presented, along with the explanations for these injuries. The accuracy of anthropologist/pathologist interpretations of evidence obtained during autopsy and analysis of bones will be discussed.

The modern understanding of the pathophysiology of hanging and strangulation was significantly changed by the creation of the Working Group on Human Asphyxia in 2006. The results from this ongoing study will be presented. The study of filmed hangings and strangulation has clearly established the agonal responses in these deaths: rapid loss of consciousness (10 s ± 3 s), mild generalized convulsions (14 s ± 3 s),

decrease rigidity (19 s ± 5 s), beginning of deep rhythmic abdominal respiratory movements (19 s ± 5 s), decorticate rigidity (38 s ± 15 s), loss of muscle tone (1 min 17 s ± 25 s), end of deep abdominal respiratory movements (1 min 51 s ± 30 s), and last muscle movement (4 min 12 s ± 2 min 29 s). The time to irreversible damages and death in hanging and strangulation will be discussed, based on the study of non-lethal filmed events. The implication of the advances from the Working Group on Human Asphyxia on the understanding of asphyxial games will be discussed.

#### **Asphyxia, Strangulation, Forensic**

### **W18 Deadly by Design: Forensic Toxicology, Adverse Effects of Synthetic Cannabinoids, and Novel Designer Drugs (“Bath Salts”)**

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The goals of this presentation are to: (1) make the forensic science community aware of the prevalence and toxicity of the products; (2) educate forensic professionals about the challenges involved in their analysis including sample preparation and analysis; (3) familiarize forensic pathologists and toxicologists with the adverse effects of the chemicals; (4) provide reference case details of circumstances surrounding impairment and criminal acts committed under the influence of the drugs; and, (5) present evidence that the drugs can cause death.

This presentation will impact the forensic science community by increasing awareness of the latest categories of abused drugs which will require changes in current forensic practice and by providing evidence to support the scheduling and control of these dangerous new chemicals, many of which are currently available without restriction.

This program will present information regarding the various classes of synthetic drugs which have recently become popular within the recreational drug using community. Exploration of the highs associated with these drugs has become known as the “research chemicals movement.” Compounds used by these groups include synthetic cannabinoid agonists, cathinone derivatives, pyrovalerone derivatives, and many others. Use of these chemicals has significant adverse effects and forensic consequences.

The program builds on basic analytical information presented at the AAFS Annual Meeting in 2011, and will cover the latest developments in the availability, analysis, and forensic toxicology of these new illicit drugs. The scope will include information about the chemicals in circulation, how they are detected and measured, adverse event reports presented to Poison Control Centers, and the involvement of the drugs in impaired driving, suicide, assault, and homicide cases.

Specific presentations will cover the current scope of drugs present in these materials, their legal status, and how the analog and homolog aspects of the federal law should be interpreted. The remainder of the workshop will include a review of current analytical approaches to the identification of the chemicals in seized materials, blood, urine, and tissue, and will include pharmacokinetic data in blood, urine, and oral fluid from controlled administration of a range of the synthetic cannabinoid drugs. The focus of the remainder of the session will be on adverse effects associated with the various compound classes from various medical and forensic perspectives.

This will include data from Poison Control Centers derived from calls from the public and medical professionals that have documented hypertension, agitation, paranoia, and seizures following use of synthetic cannabinoid and other legal high drugs. The program also includes case reports of suicides, assaults, and homicides following use of the unscheduled drugs mephedrone and MDPV sold as “Bath Salts” but intended for recreational use. Finally, a series of deaths in which either the synthetic cannabinoid or stimulant type drugs appear to have played a role will be discussed along with the difficulties in testing for use of the compounds and assessing their role in the causation of death.

At the conclusion of the workshop, attendees will be able to describe the various categories of drugs in the synthetic cannabinoid and designer drug categories; select or order appropriate analytical tests to ensure their detection; apply aids to the interpretation of designer drug findings in forensic casework; and recognize adverse effects of the drugs through evaluation of signs and symptoms of use.

#### **Designer Drugs, Synthetic Cannabinoids, Forensic Toxicology**

### **W19 The Anatomy of Error: Dissecting Adverse Events to Strengthen the Forensic Sciences**

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After attending this presentation, attendees will: (1) learn the fundamentals of human error analysis; (2) define error, active error, and latent error; (3) discuss the origins of error in human and system perspectives; (4) understand human factors impacting performance; (5) describe and apply the different models of failure; and, (6) identify and initiate steps to resolve potential “error traps” in the work environment.

This presentation will impact the forensic science community by providing an overview of the field of human error, illuminate how human factors such as fatigue and bias can negatively impact performance, and educate the attendees on the application of human factor codes to items analyzed in a high profile murder case.

Reviewing the actions and accomplishments of other professions that have successfully dealt with human errors will assist attendees in understanding the fundamentals of error analysis. The medical and aviation professions have conducted extensive research on the subconscious to identify how human performance is helped or hindered by the design of equipment, work environment, and peer pressure. Making corrections or changes according to these revelations have improved performance and mitigated errors within these professions.

Fatigue has long been associated with decreases in human performance. While this is a problem that challenges most industries, those that are human-centric are at an even greater threat. The forensic sciences remain a field that heavily relies on the performance of its analysts and requires 100% accuracy. Unfortunately, crime scene technicians and analysts are just as susceptible to fatigue as other professionals. Operator fatigue has been shown to effect performance after just 18 hours of sustained wakefulness. While reductions in alertness and performance are a problem for workers in general, those who perform tasks, which demand high levels of cognition, are even more susceptible to the effects of fatigue. Fortunately, there are a number of management techniques, countermeasures, and interventions that will be discussed and defined to help mitigate these negative effects.

One aspect of the management technique is the education of staff on the difference between active errors, those events when individuals are in direct contact with physical evidence and latent errors, when the underlying support system comes into question. These topics have been part of the two year effort assigned to the Expert Working Group on Human Factors in Latent Print Analysis, an effort that has recently completed its task. Evaluating approaches to reducing errors in terms of their efficacy, appropriateness, cost, scientific basis, feasibility, associated risks, and quality of evidence have been documented and will be offered.

The class participation portion of the workshop provides an opportunity for the attendees to take the provided information and apply it to an actual criminal case. Applying lessons learned (when back at the crime scene, in the laboratory, or managing staff), work flow is increased when provided an opportunity to experience direct application. Part of the direct application will be segmenting levels of forensic work, using error coding techniques and assigning mitigation activities. A 1992 murder case, the conviction of Ray Krone and the associated bitemark evidence, will be used for this activity.

Therefore, this workshop has direct application to individuals who collect, handle, analyze, manage, and store forensic evidence. It introduces the concept of applying error mitigation techniques to the forensic science profession and provides established guidance learned in other professional fields. An actual criminal case will be presented allowing each attendee to gain experience in the actual error coding process and understand the underlying basis and resulting influence of errors.

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**Error, Cognitive Bias, Quality Assurance Quality Control**

## **W20 Flawed Forensics: Recognizing and Challenging Misleading Forensic Evidence and Disingenuous Expert Testimony**

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After attending this presentation, the attendees will be better acquainted with the current problems and challenges facing expert witnesses in various disciplines of forensic science. Attendees will learn how expert testimony or evidence is being challenged as unreliable, or misleading and disingenuous, what methods lawyers use to impeach such testimony or evidence, and the legal basis for excluding such evidence.

This presentation will impact the forensic science community by providing forensic scientists with examples of the problems and challenges they can face when testifying in court. All stakeholders in the administration of criminal or civil justice – experts, lawyers, and judges – will benefit from learning about scientific and evidentiary challenges to expert testimony, testimonial practices that experts should avoid, and the means by which lawyers challenge and judges exclude forensic expert testimony. This workshop will assist forensic scientists and lawyers in recognizing when the testimony of a particular expert witness is misleading and disingenuous and how to challenge and effectively impeach or preclude such testimony.

A multidisciplinary faculty will review actual testimony of forensic specialists in different disciplines. Discussion of cases and reported court decisions will illustrate the problems and ongoing challenges facing forensic

scientists testifying in criminal and civil cases. Critical knowledge about when and under what circumstances, testimony or evidence introduced by forensic scientists will be challenged as being misleading or disingenuous, and how such challenges take place in the adversarial process of our judicial system will be discussed.

The uses and limits of cross-examination to reveal weaknesses in the testimony of forensic scientists will be explored, and examples involving misleading and disingenuous testimony by toxicologists, pathologists, and other forensic scientists will be examined and discussed from a scientific and legal standpoint, and from the perspective of the forensic scientist, the trial lawyer, and the judge. Actual cases in which deficiencies in report writing and incomplete or misleading testimony by forensic practitioners contributed to wrongful convictions will be reviewed and analyzed, and recommended solutions designed to correct this problem will be discussed.

Workshop attendees will learn about the true meaning of the different types of casework “peer review” occurring within the forensic community, the methods lawyers can use to impeach disingenuous testimony concerning such peer reviews, and the legal basis for excluding peer review testimony by the actual reviewer.

High profile criminal defense attorneys will discuss cases involving flawed and fabricated scientific evidence. The lead defense attorney in the recent, highly-publicized capital murder prosecution of Casey Anthony, which resulted in not guilty verdicts on all charges involving the death of the defendant’s 2-year-old daughter, will discuss the challenges he faced before and during the lengthy jury trial, as well as the methods used to impeach the forensic evidence presented by the prosecution.

Attendees will become acquainted with the principles and pitfalls involving courtroom testimony in the wake of the National Academy of Sciences (NAS) Report and recent court decisions defining the parameters of forensic testimony. Specific examples of courtroom testimony will be utilized to illustrate problems in fingerprint comparison, tool mark identification, and bitemark analysis. Various aspects of testimonial evidence will be specifically targeted, such as the use of terminology in rendering a conclusion. The validity of subjective analysis and the intermingling of scientific principles will be addressed in the context of probability assertions. How the forensic practitioner handles the dual problems of explaining key forensic discipline definitions and producing appropriate back-up materials in buttressing validity will also be examined.

Finally, attendees will learn about the misleading use journal articles, treatises, and studies or experiments to substantiate or attack the basis for an expert opinion in the pre-trial and trial phases of a court proceeding, and the manner in which judges scrutinize and evaluate such evidence. Attendees will also learn about the ethical implications for a trial judge faced with disingenuous expert testimony and the nature of the ensuing judicial response.

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**Flawed Forensics, Misleading Evidence, Disingenuous Testimony**

## **W21 Innovation in Forensic Image and Video Analysis**

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After attending this presentation, attendees will understand how to validate forensic methods in image restoration and limitations and possibilities of forensic multimedia investigation.

This presentation will impact the forensic science community by providing an overview of the new developments and needs in forensic research.

During this workshop information will be provided on new developments of forensic investigation of (digital) images and video streams and the use of 3D computer modeling in forensic investigations. The workshop will be interactive and many examples of case material will be shown.

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), quality assurance, and validation aspects. During the last decades, the use of CCTV-camera systems and digital cameras on phones has become widespread. Typical questions concern the quality and the selection of images from a specific camera in a multi-camera-recording, and combining the information. 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. In addition, questions about image interpretation like facial recognition, body height, car speed (often in low resolution), time lapse, or compressed images have increased. A special discussion will be given on image analysis.

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? Gait analysis, 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 measurement in images are presented and discussed. Special attention will be given to measurement uncertainties of the results and the impact on the conclusions from these investigations.

Common sources of video streams and images are video recordings from handy cams, digital photo cameras, the 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 state-of-the-art image enhancement techniques such as contrast, stretching, and deblurring, as well as super resolution, stabilizing, and automatic tracking methods. It will also cover the issue of erased video files, and how to recover these when they are partly erased.

The state-of-the-art methods for camera comparison will also be presented, examples of comparing Photo Response Non Uniformity with software. With this method a camera can be linked to a camera. Also the cautions of identification and limitations of the technique are discussed, and solved with likelihood ratios.

The methods are discussed also in relation to forensic medical image analysis and possibilities within pathology, with many practical examples. Computing speed for some of the image analysis methods (especially for high volume of data), and the possibilities of fast parallel computing with GPU's and CUDA for forensic video applications are discussed.

Finally, an overview on the use of 3D computer modeling in forensic investigations will be provided because 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, the methods for visualizing uncertainties, and possibly erroneous suggestions coming from these visualizations. 3D video fusion for use in casework is demonstrated.

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**Multimedia, Video, Medical**





ATLANTA 2012

# SPECIAL SESSIONS



ATLANTA 2012

## S1 Interdisciplinary Symposium - International and Interdisciplinary Symposium ... Now Boarding

Laura L. Liptai, PhD\*, BioMedical Forensics HQ CA/FL, 1660 School Street, Suite 103, Moraga, CA 94556; Carla M. Noziglia, MS\*, United States Department of Justice, ICITAP, 305 Ascot Drive, Aiken, SC 29803-7833; Kathryn Bomberger, MA\*, International Commission on Missing Persons, 45A Alipasina, Sarajevo, BOSNIA-HERZEGOVINA; Daniel D. Garner, PhD\*, United States Department of Justice, ICITAP/Criminal Division, 1331 F Street Northwest, Suite 500, Washington, DC 20530; Morris V. Tidball-Binz, MD\*, 4 Chemin des Fleurs, Ferney Voltaire, L'Ain 01210, FRANCE; Duarte N. Vieira, PhD, MD\*, Rua Antonio Jose de Almeida, No 117, Coimbra, 3000-044, PORTUGAL; Eddy De Valek, DDS\*, Parklaan 10, Beigem, B-1852, BELGIUM; Zeno J. Geradts, PhD\*, Netherlands Forensic Institute, Ministry of Justice, Laan van Ypenburg 6, Den Haag, SH 2497 GB, NETHERLANDS; A.W. Jones, PhD, DSc\*, National Lab Forensic Chemistry, , 12 Artillerigatan, Linkoping, 58758, SWEDEN; Deog-Im Kim, PhD\*, Catholic University of Korea, Department of Anatomy, 505, Banpo-dong, Seocho-gu, Seoul, 137701, KOREA; Klaus C. Neudecker, MD\*, Schirmgasse 268, Landshut, Bavaria D-84028, GERMANY; Rosalind J. Spencer, BSc\*, Canada Border Services Agency, 14 Colonnade Road, Suite 280, Ottawa, ON K2E 7M6, CANADA; and Douglas H. Ubelaker, PhD\*, Smithsonian Institution, Department of Anthropology, NMNH - MRC 112, Washington, DC 20560

**Educational Objective:** After attending this presentation, attendees will be introduced to a diversity of views, a variety of topics and a wealth of knowledge in the field of forensic science that can be used to improve our methodologies as well as promote further research, collaboration, and understanding.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the practice, elevating the standards, and advancing forensic sciences worldwide.

Worldwide forensic research in action will come to you! Preeminent forensic research scientists from ten countries will amaze you. It's a once in a lifetime opportunity to hear from:

**International Committee of the Red Cross, Switzerland**

**International Commission on Missing Persons,**

Bosnia/Herzegovina

**International Criminal Investigative Training Assistance Program,**

Worldwide

**National Institute for Legal Medicine, Portugal**

**Panel Host: President, Douglas Ubelaker, Smithsonian Institution, Washington, DC**

The American Academy of Forensic Sciences (AAFS) serves a distinguished and diverse membership of over 6,200 members, divided into eleven forensic sections representing all 50 United States, Canada, and 62 other countries worldwide. What is our "Forensic Science Edge?" Our advantage or "Edge" is access to preeminent forensic experts worldwide. This symposium encourages international interdisciplinary collegial collaboration that professionally advances us by reinforcing, challenging and/or transforming our thinking.

This symposium is the stuff of which legends are made. See forensic research around the world in action. Toxicology in Sweden! Questioned Documents in Canada! Psychiatry & Behavioral Science in Germany! Physical Anthropology in Korea! Odontology in Belgium! Digital & Multimedia Sciences in the Netherlands! Pathology/Biology in Portugal!

General in Switzerland! Jurisprudence in Bosnia/Herzegovina! Criminalistics from ICITAP! And, Engineering from the United States!  
**International Forensic Science, Victim Identification, Accident Reconstruction**

## S2 Contemporary Topics in the Forensic Science Community

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**Educational Objective:** After attending this presentation, attendees will have a better understanding of the scope of the different fields of forensic science. Participants will learn how the different fields of forensic science work together to each play a significant role in casework. Both casework and research will be presented by the speakers. These presentations will show how casework and research are intertwined and how they both contribute to advancing forensic science. In addition, attendees will learn about each section represented by the AAFS and about the benefits of membership in the Academy. Participants will learn about various cases and research being done by their peers at the posters and slides sessions, and they will learn valuable skills needed to secure a job within the forensic science field at the breakfast session.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing encouragement, tools, resources, and support needed to give new and future professionals the ability to positively contribute to the forensic science field.

For more than a decade, the Young Forensic Scientists Forum (YFSF) has provided a program for new and young forensic scientists ranging from students in both undergraduate and graduate programs to professionals new to their career in forensic science with five years experience or less. YFSF is designed to attract both members and non-members of the American Academy of Forensic Sciences (AAFS). The continuing goal is to provide this audience with topics relevant to their education, training, and skill levels. The program is also designed to provide a comfortable environment for students and new professionals to present to their peers as well as an opportunity to effectively and efficiently network with experienced members and Fellows of the AAFS. The opportunities to present range from presenting at the YFSF Bring Your Own Slides (BYOS) session or the YFSF Bring Your Own Posters (BYOP) session and to showcase emerging forensic scientists. The Emerging Forensic Scientist Award winner is always invited to present his/her award winning paper.

For the AAFS 64th Annual Scientific Meeting in Atlanta, Georgia, the YFSF Special Session will present the theme: "Contemporary Topics in the Forensic Science Community." The special session to be held on Tuesday, February 21, will include speakers who will discuss trends currently observed among various forensic disciplines. With the rise of different types and variations of evidence being submitted to various laboratories, as well as new technologies in evidence examination, the focus of the 2012 forum will be to highlight and educate young forensic scientists on these trends. Lunch is provided to both attendees and speakers who are registered for the special session.

The annual YFSF BYOS, scheduled for Wednesday evening, includes presentations from students and new forensic scientists. The program will continue Thursday morning with the annual YFSF breakfast session with the theme: "Creating the Resume That Will Get Your Foot in The Door." The breakfast session is included in the registration for the special session held on Tuesday. YFSF does not require presenters of BYOS or BYOP to be members of AAFS and does not require they attend the special session but we do encourage them to do so.

One of the goals of the YFSF is to foster relationships between the participants of the session with their 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.

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**Forensics, Evidence Submission, Current Trends**

## ES1 The Casey Anthony Trial — From the Defense, Medical, and Scientific Viewpoint

*Linda K. Baden, JD\*, Law Office of Linda Kenney Baden, 15 West 53 Street, Apartment 18 B/C, New York, NY 10019; Jose A. Baez, JD\*, 522 Simpson Road, Kissimmee, FL 34744; Haskell M. Pitluck, JD\*, 573 Lake Avenue, Crystal Lake, IL 60014; Jane H. Bock, PhD\*, Forensic Botany, Limited Liability Company, 5224 Lighthouse Point Court, Loveland, CO 80537-7916; Anthony B. Falsetti, PhD\*, ICMP, Alipasina 45a, Sarajevo, 71000, BOSNIA-HERZEGOVINA; Kenneth G. Furton, PhD\*, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199; Timothy E. Huntington, PhD\*, 175 East Seward Street, Seward, NE 68434; Lawrence Kobilinsky, PhD\*, John Jay College of Criminal Justice/CUNY, Department of Sciences, 445 West 59th Street, Room 3263A, New York, NY 10019; Kathleen J. Reichs, PhD\*, University of North Carolina Charlotte, Department of Anthropology, Charlotte, NC 28223; Barry K. Logan, PhD\*, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; and Werner U. Spitz, MD\*, Forensic Pathology/Toxicology, 23001 Greater Mack Avenue, Saint Clair Shores, MI 48080*

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**Educational Objective:** After attending this presentation attendees will understand that experts at trial must limit their opinions and conclusions to those which are scientifically supportable despite pressures to tailor testimony to popular community and media sentiment.

**Impact on the Forensic Science Community:** This presentation will seek to educate all as to the limits of the areas of forensic science testified to by the defense experts in the Casey Anthony trial.

The biggest media case, arguably larger than O.J. Simpson, was the 2011 trial of Casey Marie Anthony for first-degree death penalty murder. This was the first trial that not only engulfed the media but also occurred in the climate of burgeoning social networking, such as Facebook® and twitter®. As a result, everyone worldwide became an armchair juror or lawyer, influenced by rating-conscious talking heads with little understanding of the scientific evidence. Despite the recent pronouncement by the National Academy of Sciences (NAS) Report, the court allowed forensic testimony that had not been validated. The pressure on experts to put forth supportable medical and scientific testimony, despite the unpopularity of the defendant in this case, was great; however, the opinions of scientific experts must be independent of the desires of those who call upon them - unlike the attorneys, they are not advocates for either side.

In 2011 in Orlando, Florida, Casey Anthony was tried for the murder of her two-year-old child Caylee Marie Anthony. Jurors who had not been subject to pretrial publicity were picked by both sides from a different county and sequestered in Orlando, hearing about six weeks of evidence. The result is widely known – that Casey Anthony was acquitted of death penalty murder and all lesser included charges related to the death of her daughter. In order to bolster the emotional argument that the prosecution presented against Ms. Anthony, various forensic disciplines were embraced for the trial. These included not only medical and pathology testimony but also anthropological, entomological, chemistry, toxicology, botanical, and DNA opinions.

The defense claimed that much of the medical and scientific testimony was novel and had exceeded the boundaries of validated forensic science and should not have been allowed in by the gatekeeper. The judge, who did not accept the NAS Report as authoritative, allowed the jury to hear from experts whose opinions and conclusions had never been utilized in court, had not been subject to rigorous error rates, failed to have peer review protocols or quality control, and appeared to fall short of United States Supreme Court standards.

The defense prepared their own scientific conclusions beginning with a second autopsy and anthropological examination. It is important for all forensic experts to understand that at trial the role of the expert and

the jury's acceptance of expert testimony have substantially changed in the past decade.

The controversial nature of some of the testimony allowed to be presented to the jury in this matter, along with the actual systematic assistance provided by the experts for the defense, will be explored so that future forensic experts testifying in any trial, especially a high profile trial, will better understand why jurors accept or reject their conclusions.

This presentation will be a constructive examination of rapidly developing scientific technologies and the National Academy of Sciences concerns as to courtroom admissibility.

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**Casey Anthony Trial, NAS Report, Unvalidated Forensic Science**

## **ES2 National Missing and Unidentified Persons System (NamUs) Best Practices for System Use**

*Bruce E. Anderson, PhD\*, Pima County Office of the Medical Examiner, 2825 East District Street, Tucson, AZ 85714; Randy L. Hanzlick, MD\*, Fulton County, Medical Examiner Center, 430 Pryor Street, Southwest, Atlanta, GA 30312; J.C. Upshaw Downs, MD\*, Georgia Bureau of Investigation, Medical Examiner, 925 A Mohawk Drive, Savannah, GA 31419; Daniel J. Warren, MS\*, Florida Department of Law Enforcement, 4700 Terminal Drive, Suite 1, Fort Myers, FL 33907; Kevin Lothridge, MSM, National Forensic Science Technology Center, 7881 114th Avenue, Largo, FL 33773; and C.W. Billy Young II, BS\*, and Carrie B. Sutherland, BS\*, National Forensic Science Technology Center, , 7881 114th Avenue, North, Largo, FL 33773*

**Educational Objectives:** After attending this presentation, attendees will learn best practices for using the NamUs system including case entry, case enhancement, and utilization of NamUs forensic services. At the conclusion of the session, attendees will be able to efficiently navigate, operate, and utilize the NamUs system to assist their agency in applying NamUs to resolve missing and unidentified person's cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how increased awareness and use of NamUs offers the promise of reducing backlog of unidentified remains cases in the United States and streamlining the investigation of missing persons by rapidly matching cases across state lines.

The National Missing and Unidentified Persons System, NamUs, combines two databases to assist law enforcement, medical examiners, coroners, and family members in the search for missing persons and the identification of unidentified human remains. The system, to date, has helped resolve over 150 cases of missing persons and unidentified remains. The information presented in the session offers agencies proven best practices for using NamUs to successfully resolve these cases.

This presentation, developed in conjunction with the National Association of Medical Examiners, Georgia Bureau of Investigation, and the Florida Department of Law Enforcement, will outline the benefits and best practices for using NamUs. Death investigators and law enforcement will see firsthand how the NamUs system benefits practitioners in their field. This presentation will be given by registered users from the medical examiner, coroner, and law enforcement fields and will prove vital to the attendees' agencies success with the NamUs program.

First, attendees will be walked through the registration process for becoming a secure user such as law enforcement or a medical examiner or coroner. Once through the registration process, basic and advanced case entry and searching will be demonstrated. Attendees will learn how to enter the best possible case information and use advanced searching techniques based on forensic identifiers such as dental records and tattoos.

Next, the secure user dashboard will be presented, illustrating the benefits of NamUs and its ease of use for users and agencies. The exclusions process and exclusions menu, a significant benefit of the system, will be explained and demonstrated. Under this menu, law enforcement, medical examiners, and coroners can track potential case matches that have been excluded based on forensic examination. The cases are archived so that users can actively monitor these cases for any changes or future comparison.

NamUs rates cases on a star system, from one to five stars, based on the amount of identifiers present in the case record. Five star cases have a higher likelihood of reaching resolution through the system. Identifiers found in five star cases include DNA, fingerprints, and dental records. This presentation will demonstrate how to enter a five star case, or enhance an existing case to five star status. Attendees will also learn how to import all agency cases that reside in the FBI's NCIC database into NamUs electronically, saving them time and money by avoiding manual case entry.

Through NamUs, the National Institute of Justice also offers a wide variety of free forensic service assistance including dental and anthropological services, fingerprint examination, and DNA sampling. This presentation will address how these services are provided, protocols for obtaining the services, and methods for case enhancement using forensic services.

The final topic of the presentation will present system successes. Cases resolved with the assistance of NamUs will be highlighted, demonstrating how the system helps family members, medical examiners/coroners, and law enforcement work together to resolve agency cold cases, bring closure to families and give people their identities back.

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**NamUs, Identification, Missing Persons**



ATLANTA 2012

# BREAKFAST SEMINARS



ATLANTA 2012

## BS1 Postmortem Examination and Personal Identification of Victims of the Great East Japan Earthquake

*Yasuhiro Aoki, MD, PhD\*, Department Forensic Medicine, Nagoya City University School of Medicine, 1 Kawasumi Mizuho-cho Mizuho-k, Nagoya, 467-8601, JAPAN*

**Educational Objective:** The goal of this presentation is to familiarize attendees with an outline of the forensic investigation of victims of the Great East Japan Earthquake Disaster which was carried out with the assistance of members of the Japanese Society of Legal Medicine (JSLM).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing foundational knowledge on the features of the Japanese medico-legal system and mass disaster management, and highlighting the role of forensic personnel in the response to overwhelming natural disasters.

A massive earthquake of magnitude 9.0 struck eastern Japan at 2:46 p.m. on March 11, 2011. The epicenter was approximately 130 km off the Pacific Coast of northeast Japan. Strong tremors were observed across a wide area. However, both human casualties and property damage were concentrated on three prefectures (government jurisdictions in Japan), Iwate, Miyagi, and Fukushima, located along the Pacific Coast of the northeastern part of the main island of Japan (Honshu). This was primarily due to the huge tsunami triggered by the earthquake, over 15 m in amplitude and 40 m in run-up height, which engulfed the coastal areas of those prefectures.

Faced with the devastation, the JSLM established the *ad hoc* Disaster Response Headquarters on March 12, 2011, and dispatched member pathologists, physicians, and dentists to the three prefectures in cooperation with the National Police Agency. This was the first time such a headquarters had been established since the society issued a guideline in 1997 for an integrated support system of mass disaster management based on the experience of the 1995 great Hanshin-Awaji (Kobe) earthquake. The first response team consisting of three pathologists and three dentists departed Tokyo at 10:00 p.m. on the same day, traveling in police vehicles due to paralysis of the public transportation network. Examination of victims in Rikuzentakata, Iwate began the following afternoon. Most of the remains were immersed in water and covered with mud, and some victims had suffered extensive burns. Hypothermia was also the cause of death in some cases. The Headquarters successively organized and dispatched JSLM members through July 6, 2011. Some 122 pathologists and physicians contributed a total of 1,090 person-days of work, and 31 dentists performed a total of 298 person-days of work at the disaster sites. Aside from local physicians and dentists associations, the Japan Dental Association and Japan Self Defense Force also sent support teams to the affected areas.

As of July 29, 2011, the remains of 15,645 victims, including 27 non-Japanese, had been recovered, and another 4,984 people were still listed as missing. Approximately 90% of the victims were positively identified, in most cases from personal belongings and body features including dentition. In some cases, identity was established by DNA profiling, and more extensive identification attempts using a computer-assisted dental comparison system and kinship analysis of DNA profiles, which will be important for identification of as yet unidentified victims and yet to be discovered victims, are now in process. The latter is being conducted by the National Research Institute of Police Science and scientific criminal investigation laboratories of regional police headquarters. Fingernails and blood are the first choice source of DNA.

No fewer than 25 countries, regions, and international organizations have sent rescue teams and other specialists to the disaster areas; however, partly because of the language barrier, direct overseas assistance in the examination and identification of bodies has not been possible thus far.

Recently, the Japanese police departments have been increasing the number of prefectural police officers, especially those in charge of the investigation of death scenes and victims. This was effective in handling the large number of remains through wide-range mobilization from outside of the devastated areas. On the other hand, the medico-legal investigation system, such as the medical examiner system, is immature and remains an issue to be addressed from the standpoint of mass disaster management.

**Natural Disaster, Personal Identification, Japan**

## BS2 The Cleveland Cyanide Murder Case: A Multidisciplinary Approach to Crime Investigation Including Chemical Identification, Cause of Death, Capture, and Court Proceedings

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**Educational Objective:** The goal of the presentation, is to offer attendees insight into the investigation of a high profile homicide and the cooperation between various governmental agencies and forensic disciplines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the complexities of a murder case and a successful team approach to investigation.

On February 24, 2005, a married, 38-year-old mother of two, driving to meet her sister at a movie theater, called a friend to inform her she was feeling nauseous. The female said that she had taken a calcium pill that her husband, a Cuyahoga County physician, gave her just before she left their house. Shortly after the female's cell phone conversation with her friend, she was involved in a low speed automobile accident and was rushed to a local hospital.

When the female arrived at the hospital, she exhibited shallow breathing, an erratic heartbeat, and was unresponsive; however, she had no major signs of trauma. After 30 to 40 minutes of attempting to revive her, the attending physician saw no signs of improvement and pronounced her dead. At the time of autopsy, there was no indication of external injury, and an internal examination showed no evidence of trauma. As a result, the coroner was unable to determine cause of death and awaited toxicology results. Routine toxicology testing, which included volatile, OTC, prescription, and illicit drug screens, did not reveal any unusual substances in the decedent's system.

On March 17, 2005, the husband voluntarily met with the investigating detective and provided a statement concerning his wife's death. In the course of the interview, the husband stated that his wife had been taking prenatal vitamins and calcium supplements. Later that evening, the detective retrieved these items from the home. After this meeting, the husband fled the United States, leaving his children in the care of the decedent's family.

On March 22, 2005, the calcium capsules that were retrieved by the detective were examined by a local county crime laboratory. The chemical examination revealed that nine of the 56 capsules submitted contained cyanide. On April 21, 2005, additional toxicology testing performed on the decedent's specimens revealed 9.1 mg/L of cyanide in her blood. On April 22, 2005, the coroner concluded that the cause and manner of death was homicide by acute cyanide intoxication.

On October 6, 2006, authorities in Cyprus arrested the husband while he was traveling under a false Lebanese passport. He fought extradition in Cyprus until December 12, 2008, when the Cypriot Minister of Justice issued an official surrender warrant. The husband was returned to the United States on January 9, 2009, and arraigned on January 14, 2009.

A jury trial commenced on January 19, 2010, and lasted until March 8, 2010, when the jury returned a verdict of guilty of aggravated murder, as charged in the indictment. The husband appealed his conviction. On May 26, 2011, after a review of the record and pertinent law, the Court of Appeals of Ohio affirmed the appellant's conviction for the aggravated murder of his wife.

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### Cyanide, Homicide, Multidisciplinary Approach

## BS3 Forensic Science and Cultural Heritage – Examination of the Gettysburg Address

*Henry Swofford, BS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and Fenella France, PhD\*, United States Library of Congress, 101 Independence Avenue SE, Washington DC, DC 20540*

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**Educational Objective:** The goal of this presentation, is to provide attendees with an understanding of a new application of hyperspectral imaging as a non-destructive technique to detect latent fingerprints, specifically on an historical document.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through a discussion of the detection of latent fingerprints on the Nicolay Copy of the Gettysburg Address – one of our nation's most precious documents – using hyperspectral imaging. Through a collaborative effort between the Preservation Research and Testing Division (PRTD) of the United States Library of Congress, and the Latent Print Branch of the United States Army Criminal Investigation Laboratory, progress is being made in the recovery of additional artifacts and information of historical significance through forensic examinations using hyperspectral imaging techniques.

Hyperspectral imaging involves a spectroscopic analysis of materials to distinguish certain materials, one from another, as a function of their differential reflectance and absorption properties across a multitude of wavelengths in the electromagnetic spectrum. The particular wavelengths at which materials reflect and absorb will differ based on the chemical composition of the material giving it a characteristic reflectance spectrum. It is this characteristic reflectance spectrum which distinguishes certain materials. The normal human eye can distinguish the difference in materials based on their color. The color of a material is a descriptive property corresponding to the particular wavelength of electromagnetic energy that is reflected back towards the eye. The brain then detects and defines this reflected energy as a particular color depending on the wavelength of the reflected energy. The human eye is sensitive to distinguishing electromagnetic energy between 390nm and 750nm – commonly referred to as the “visible spectrum.” The visible spectrum represents only a very small portion of the entire electromagnetic spectrum which is infinite and continuous. Hyperspectral imaging relies on hundreds of narrow and contiguous wavelengths across the electromagnetic spectrum resulting in characteristic reflectance spectra for each pixel in a digital image allowing the viewer to see the image as a function of the differential reflectance spectra. The hyperspectral imaging technique is undergoing rapid improvements with continuous developments of more

powerful imagery sensors and image processing algorithms expanding the technique to be used for a multitude of additional applications.

In 2008, the United States Library of Congress PRTD began utilizing spectral imaging to examine a number of documents having historical interest to our nation including original drafts of the Declaration of Independence, the Gettysburg Address, and the Waldseemüller 1507 World Map, the first map to refer to the continent as America. The application and development of hyperspectral imaging for the preservation of cultural heritage materials and analysis of historic documents provides a powerful non-invasive technique for assessing documents. This technique utilizes an imaging system that captures the spectral response of materials from the ultraviolet, visible and near infrared regions of the spectrum (UV-VIS-NIR) and also reveals obscured or hidden information. The Library imaging system comprises a MegaVision 39 Megapixel monochrome camera (7216 x 5412) E6 back, and APO-Digitar 5, 6/120 lens, integrated through customized software with light emitting diode (LED) illumination panels that span the spectral range of 365nm to 1000nm for reflected, transmitted and raking (side-lighting) imaging modes. Recent advances include the development of a lens that had increased sensitivity in the UV region.

Non-destructive spectral imaging can be used to characterize historic documents by capturing the unique chemical spectral response of composite materials including substrates (paper, parchment, photographic materials) and media (inks, pigments, colorants). Capturing UV, VIS, and NIR spectral data in various illumination orientations minimizes handling of fragile items and allows greater capacity for materials analysis and post-acquisition processing to uncover hidden and obscured text and information. All images are accurately registered, enabling almost unlimited combinations of spectral wavebands for further processing. The integrated system uses low heat and reduced light exposure on the document, ensuring preservation of original materials.

During the examination of these historic documents, multiple latent fingerprints were developed on the Nicolay and Hay copies of the Gettysburg Address. The United States Library of Congress and the United States Army Criminal Investigation Laboratory began a collaborative effort to obtain additional items of historical significance pertaining to President Lincoln for non-destructive forensic examinations with the intent of developing additional latent fingerprints to compare with those on the Gettysburg Address. This presentation will give the attendees a chance to become familiar with the application of hyperspectral imaging for latent print development, view the latent fingerprints which were developed on the Gettysburg Address, and discuss the imaging and processing techniques used to capture and non-invasively develop these prints.

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### Hyperspectral Imaging, Forensic Documents, Latent Prints

## BS4 Theater of the Absurd – Ethics and the Truth Versus the Fiction of the Courtroom

*J.C. Upshaw Downs, MD\*, ForensX, LLC, 11511 Abercorn Street Ext #182, Savannah, GA 31419; and Anjali R. Swienton, JD\*, SciLawForensics, Limited, 12447 Great Park Circle, #210, Germantown, MD 20876*

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**Educational Objective:** The objective of this presentation is to introduce the contrasting fictional portrayal of the court in media versus the reality of actual trial practices with the goal of allowing forensic witnesses to benefit from lessons learned—both good and bad—by the presenters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing the attendee to better understand unrealistic portrayals and expectations placed on the forensic witness by attorneys, juries, and others based on a comparison with media presentations.

The forensic practitioner is challenged with expectation placed on him/her by many interests. These often come to light most directly in the courtroom where counsel for both parties have a vested interest in

substantiating their version of the facts. This adversarial system draws in the neutral practitioner who is faced with expectations of performance from the judge, law enforcement, jury, and others. In the modern era, the media has periodically gone through apparent feeding frenzies of interest in high-profile “case of the century” vignettes with ever-increasing frequency. The particulars of day-to-day forensic work have been glamorized on television in the form of fictional crime dramas and reality television, leaving many viewers expecting investigative miracles from the forensic practitioner. The “CSI effect” is an all too real phenomenon.

Using a compare and contrast case study basis with use of video clips from dramatic courtroom events and actual sworn testimony, the dilemmas for the forensic witness will be presented. The ethical dilemmas for the witness in how to properly convey complex scientific testimony to a lay jury while attempting to stay neutral and objective is a continuing challenge for the witness – even if well-experienced. Trying to ensure that the jury has a complete picture of the science underlying the testimony, while not reading in real or perceived shortcomings, is a challenge. At times, counsel might not ask the appropriate questions or the jury might believe that certain evidence “should be present” when it is not. These are all too familiar scenarios to those who have been in the witness box.

The 2009 National Academy of Sciences Report stressed the need for reinforcing ethics in forensic practice. Trial testimony is often where one’s moral code can be put to the test by not only what one says but what is not said and how these ends are achieved. The impartial scientist should hold fast to the purity of the science and, while understanding the possible motives of parties involved, not become an advocate. The witness’s use of logic should remain that and not become mere rationalization, intended to raise doubt. To this end, the practitioner must be able to convey the difference between “hard” and “soft” science, between reality and illusion.

Areas of specific inclusion would relate to the strength of opinion (that is overstating or understating testimony), misrepresenting facts or underlying principles, and the ethics of being a private versus governmental employee. While any ethical lapses may end with the net result that the ideal of “the truth, the whole truth, and nothing but the truth” is not fully conveyed to the jury, the difference between outright lies, deception, and misleading testimony is an important consideration. Media versions may allow some blurring of an ethical line that must remain clear if the foundation of the science is to remain sound.

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#### **Testimony, Courtroom, Ethics**

### **BS5 The Battle of Gettysburg: How Today’s Technology Connected Jennie Wade to Abraham Lincoln After 145 Years**

*Rod Englert, BS\*, Englert Forensic Consultants, PO Box 605, West Linn, OR 97068; and John Sotos, MD\*, 1788 Oak Creek Drive, Palo Alto, CA 94304*

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**Educational Objective:** During this presentation, attendees will learn of historical events being affected by today’s technology involving blood patterns, luminol, DNA, bullet trajectory, and clues to President Lincoln’s medical condition.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by relating historical events to modern-day scientific technology.

Can state-of-the-art forensic technology shed new light on mysteries that have remained unsolved for more than a century? This presentation reopens two intriguing historic events—the deaths of President Abraham Lincoln and Jennie Wade, the Battle of Gettysburg’s only civilian casualty—and puts them under the lens of modern science, subjecting 19th-century evidence to 21st-century technology including DNA analysis and luminescent chemical testing as well as advanced medical diagnostics and bullet trajectory analysis.

The first case discussed will be the shooting of Mary Virginia “Jennie” Wade during the Civil War in 1863. A team of crime scene reconstruction and DNA experts conducted a detailed examination of the Jennie Wade House in Gettysburg and discovered convincing evidence that historic accounts had the facts wrong. Attendees will be walked through significant points in this historic sniper shooting scene, revealing how trajectory analysis can be applied to bullet holes in the original wooden doors to determine that the bullet responsible for killing 20-year-old Wade came from a different angle and locale than originally believed. This presentation will include a reenactment of the crime as it unfolded. The forensic tests performed on the floorboards and the bread trough Wade was said to be leaning over when she was shot to death will also be discussed. Use of Luminol revealed chemical reactions suggesting the presence of bloodstains. Core samples were submitted for advanced DNA testing, which may ultimately determine after 148 years not only whether the blood is human but whether it belonged to Wade.

Equally fascinating from a forensic standpoint are other bloodstained artifacts from the Civil War era, including a lock of hair found in a private safe at the Wade House, which was allegedly preserved from President Abraham Lincoln’s autopsy. Medical historian and obscure-diagnosis expert John Sotos of the University of North Carolina School of Medicine has conducted exhaustive research on Lincoln and concluded that, based on his features and symptoms, the president suffered from a rare genetic cancer syndrome that would likely have killed him within months had he not been assassinated. By examining bloodstained Lincoln artifacts, this presentation may be able to expand science’s understanding of the sixteenth president’s medical condition.

In his presentation, Dr. Sotos will explain how a master diagnostician can analyze clues and pinpoint telltale details in photographic and other historical evidence to determine that Lincoln and three of his four sons had multiple endocrine neoplasia type 2B (MEN2B). The hallmarks of the disease and how they are recognizable in such distinctive characteristics as Lincoln’s unusual height and long limbs, sagging face and bumpy lips, fatigue and headaches, perpetually cold hands and feet, as well as the death of three of Lincoln’s sons before age 20 and his mother’s death at 34, will also be discussed.

The discussion will delve into the applications—and potential breakthroughs—that scientific technology offers to solve crimes, broaden our understanding of history, expand medical knowledge, and provide answers to questions that have eluded experts for generations.

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#### **Luminol, Trajectory, Lincoln**

### **BS6 Tables Turned: An Unsuccessful Case of a Newborn Kidnapping by Caesarian Section**

*Robert J. Morton, MS\*, Federal Bureau of Investigation, National Center for Analysis of Violent Crimes, Critical Incident Response Group, 2501 Investigation Parkway, Quantico, VA 22135*

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**Educational Objective:** After attending this presentation, attendees will understand the unique dynamics involved in cases of offenders who attack pregnant women to steal their fetuses. This case will highlight the need for forensic sciences to assist in unraveling complex homicide crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the complexities involved in homicide scenes and the need for forensic sciences to assist law enforcement in ascertaining the sequence of events involved in equivocal death investigations.

This presentation is designed to discuss a very rare and bizarre type of homicide where an offender attacks a pregnant woman and forcibly performs a crude caesarian section, removing the victim’s fetus, usually resulting in the death of the mother. A case will be discussed where a victim was stalked and attacked by a woman who was intending to steal her fetus

by caesarian section. In this instance, the victim was able to successfully fight off the offender, killing her. Additionally, this presentation will explore the history of newborn kidnapping by caesarian section, as well as the research that has been conducted on this unusual type of crime.

The FBI's National Center for the Analysis of Violent Crime (NCAVC) is routinely consulted by federal, state, local, international law enforcement, and criminal justice authorities in a variety of cases of unusual, bizarre, and repetitive violent crimes, especially homicides. NCAVC assistance was requested by local authorities in regards to a case of a pregnant woman who was attacked by an offender attempting to steal her fetus.

The victim did not previously know the offender. The offender concocted a ruse where a store had mistakenly delivered a package to her residence that was addressed to the victim. The offender was able to engage the victim in conversation regarding the victim's pregnancy learning that the victim was due to deliver in the next couple days.

When the victim went to pick up a second package, she became suspicious and attempted to leave the offender's residence. The offender attacked the victim with a knife, but the victim managed to disarm her, stabbing the offender three times. The victim fled the apartment with the knife and the offender's cordless telephone. The victim was able to contact the police and the offender was transported to the hospital where she died. The victim suffered minor defensive injuries and subsequently delivered a healthy baby.

The police conducted an equivocal death investigation into the case and discovered the offender had previously claimed to have been pregnant four other times. According to the offender, she lost the pregnancies due to miscarriage or stillborn deaths. No one in the offender's family ever visited her in the hospital or attended any funerals for the babies. Further, there were no medical records that substantiated the pregnancies. The offender had gone to great lengths to convince her family she was pregnant. The offender purchased furniture, baby supplies, diapers, and had set up a nursery in her residence. She even wore maternity clothing that was padded to resemble a pregnancy. The offender had a "delivery" kit containing surgical gloves, hemostats, surgical scissors, absorbent gauze, and a plastic clip to secure an umbilical cord.

The investigation revealed the offender was sexually and emotionally abused while growing up. Both of the offender's parents were alcoholics and the offender became the main caregiver to her other siblings. The offender had claimed her father had sexually abused her from ages 7 to 12 years of age, and he was subsequently convicted of molestation and sentenced to 20 years.

This case highlights the unusual dynamics involved in newborn kidnapping cases by caesarian section. It reinforces the role of forensic science, as well as the extensive investigative effort required in equivocal death investigations.

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**Forensic Science, Fetus Theft, Equivocal Death**

## **BS7 The Tale of the Black Cow, Granny, and How Animal DNA and Branding Experts Exonerated a Man From Wikieup, AZ**

*Jason D. Ricke, JD, LLM\*, Mohave County Public Defender's Office, PO Box 7000, Kingman, AZ 86402*

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**Educational Objective:** After attending this presentation, attendees will understand the impact of animal DNA in a criminal case and how jurors may ignore what we, as insiders, perceive as the strongest piece of scientific evidence in a case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing unique topics of cattle DNA and branding experts. It is imperative to recognize that as our understanding of DNA and comparison science evolves, it reaches into topics previously foreign to these types of scientific analysis.

This presentation will highlight a case arising out of events that took place in Wikieup, AZ, a small ranch town of 150 residents in Northwest Arizona. Everybody sees cattle when driving down country roads, and nearly everyone buys beef in the local grocery store, but how does that meat get from point A to point B? How do we decide who owns livestock that graze out on the open range? What happens when someone is accused of cattle rustling and then lies about it under oath?

When a lawyer takes on a unique area of law, they themselves must become experts in that field. This story highlights one attorney's journey into unfamiliar territory and how experts eventually led to a not guilty verdict at trial.

The defendant was caught with two cattle on his ranch that the State says were not his, one of which had an altered brand. The State asserted that the defendant altered or obliterated the brand on the animal, and then at a cattle ownership hearing in the Kingman/Cerbat Justice Court, lied under oath about when he branded the animal. At the outset of the case, the State had bovine DNA testing done to prove paternity of the two cattle involved, and was going to call as witnesses an animal DNA expert, a branding expert, as well as a veterinary cattle expert. The case looked bad for the defense.

Everything changed when a package from California arrived at the doorstep of the Public Defender's Office containing tissue and teeth of what the defendant claimed to be the mother of the cow with the altered brand, thereby proving these were his cows. In the end, the defense used the same animal DNA expert originally called by the State as well as their own retired branding expert to deal with the evidence against him which resulted in a not guilty verdict.

The greatest surprise of all was not the verdict at trial, but how the jurors perceived the scientific evidence that was presented. The State focused their closing argument on how the defense story was a bunch of bull. The defense waived their hand the other direction and said look at the DNA. Once the verdict was returned, some of the jurors explained that their entire decision was based on something that neither side saw coming.

Attendees may walk away reevaluating their own cases based on the story of the black cow, granny, and a small time ranch hand from Wikieup, AZ.

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**Animal DNA, Cattle Branding, Comparison Science**

## **BS8 Thomas Krauss Memorial Bitemark Breakfast – Fantasy of Forensics: How Junk Science Failed to Persuade the Jury in the Casey Anthony Case**

*Jose A. Baez, JD\*, 522 Simpson Road, Kissimmee, FL 34744*

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**Educational Objective:** During this presentation, attendees will learn firsthand how several attempts to introduce junk science in the courtroom in this mega high-profile case were rejected by the jury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for sound forensic science and the use of the judicial system as a gatekeeper.

What was dubbed "The Trial of the New Century," the *State of Florida vs. Casey Marie Anthony* posed several problems to law enforcement and state forensic experts. The daily barrage of news coverage in all forms of media resulted in unprecedented coverage. This was the first high profile case that involved social media such as blogs, Facebook®, and twitter®. Instant access was a must. This coverage placed undue pressure on state experts to render several opinions, which were at times questionable, to opinions that fell outside of their area of expertise. Expert witnesses felt this pressure and delivered for the prosecution in the media but delivered more so for the defense during trial.

In July 2008, the worldwide media began to focus on the case of a missing child under what many believed were mysterious circumstances. Caylee Marie Anthony (born August 9, 2005) was reported missing by her

grandmother, Cindy Anthony, on July 15, 2008. Casey Anthony was indicted on charges of first-degree murder and pled not guilty on October 14, 2008. Caylee's skeletal remains were discovered in a wooded area near the family home on December 11, 2008. The prosecution sought the death penalty and the trial lasted for six weeks. On July 5, 2011, the jury found Casey Anthony not guilty of murder, aggravated child abuse, and aggravated manslaughter of a child, but guilty of four misdemeanor counts of providing false information to a law enforcement officer.

In the center of it all was the defendant's vehicle that law enforcement said had the "smell of death." The remains of Caylee Marie Anthony were found less than a quarter mile from the Anthony home. What resulted in trial was a classic battle of the experts with a cutting edge twist. Flying in the face of the recent report issued by the National Academy of Sciences, novel and unproven science was allowed in the courtroom, which resulted in a battle involving every single discipline of forensic science. The prosecution asked the jury to engage in a "fantasy of forensics," which they ultimately rejected. They asked the jury to consider a phantom heart shaped sticker, a phantom stain of decomposition fluid, unverified dog alerts, false computer reports, novel science of air samples purporting to contain the odor of human decomposition, and even a video superimposition of the death of the child where no cause of death was determined. Despite this free-for-all of forensics, and despite the overwhelming inflammatory coverage of this case, the jury saw right through it and rejected the State of Florida's fantasy of forensics. Jose Baez, the defense attorney for Casey Anthony, explores this fascinating case examining not only the use of forensic science within the case but also the role of the media – with the case now being cited as an example of the unfairness of prejudicial pretrial publicity – and the impact that this could have on the rights of defendants.

**Forensic, Judicial, Defense**



ATLANTA 2012

# LUNCHEON SEMINARS



ATLANTA 2012

## L1 An Analysis of a Mine Incident That Led to Deaths Due to an Unknown Confined Space Hazard

*Gerald A. MacIntyre, DVM\*, 2700 1st Avenue South, Cranbrook, BC VIC 6Y3, CANADA*

**Educational Objectives:** After attending this presentation, attendees will understand how we are all at risk when dealing with inadequate and erroneous communications. This incident provides attendees with the knowledge that what you are told is not necessarily the facts in scene attendance. We are reliant on exact information that we are given. The information given may result in your own death from complacency.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting cases in which what you are told may kill you. This is a multidisciplinary death investigation of environmental concerns within a mine reclamation.

On December 21, 2001, the Sullivan lead, silver, and zinc mine production was discontinued after 100 years of production. The mine, which is located in Kimberley, British Columbia, Canada, had the dubious reputation of being one of the longest producing mines in the world.

Following production, a reclamation process began and the staff gradually diminished over the following years to a small skeleton staff for maintenance.

The reclamation of the mine site included covering tailings dumps with glacial till. Water drains were placed at the toe of this dump for water quality testing within the dump. A contractor was hired to monitor water quality.

On May 15, 2006, the contractor responsible for monitoring water quality failed to return to his home that evening. On May 17, 2010, the management at the mine site was alerted to the contractor's disappearance. Two employees were dispatched to search for Mr. Ericksen in separate vehicles. Mr. Bob Newcome found Mr. Erickson's truck at the water sampling shed at the toe of number one waste dump. He observed Mr. Ericksen collapsed in the water sampling shed and lying in the shallow water. He called 911 and reported the finding of an unconscious male lying in water. He entered the shed attempting to assess the physical state of Mr. Ericksen. Mr. Newcome also contacted his fellow employee to meet the ambulance at the main entrance gate. When the ambulance arrived, the attendants entered the shed separately. The senior attendant collapsed as she climbed down to assess Mr. Newcome and Mr. Ericksen. The second mine employee stated to the second ambulance attendant that his partner had collapsed. The attendant, in an effort to rescue his partner, fell into unconsciousness.

A Coroner's Inquest was held in July 2009 to investigate the deaths of Robert Newcome, Kimberley Weitzel, Shawn Currier, and Doug Ericksen. Several recommendations were submitted by the inquest jury to prevent a similar occurrence. The intention of this presentation is to discuss the cause of this incident and enlighten the membership of the various communication difficulties that occurred during this tragedy.

The investigation revealed that all four individuals died from an extremely anoxic environment. The cause of the anoxia was determined to include variable environmental and geochemical factors. The glacial till became compacted over the years and did not allow the dump to breathe. The chemical reactions created by acidophilic bacteria required oxygen to complete the oxidation of iron within the dump and consequently, oxygen within the sampling shed was utilized and drawn into the dump through the water sampling pipe. The oxygen void within the sampling shed appeared to be replaced with nitrogen from within the dump. There was no indication

of anoxia to any of the workers because no odor is associated with this phenomenon. The physiological sequence of events associated with anoxia resulted in hypercapnia and death.

The initial call to the ambulance did, in retrospect, contain all the relevant information regarding a confined space scenario but unfortunately a drowning scenario was used to dispatch the ambulance attendants. They were not prepared mentally to assess an anoxic hazard when they attended the scene and unfortunately died in an attempt to rescue two men that were down upon their arrival.

**Environmental Asphyxia, Anoxia, Environmental Anoxia**

## L2 Odor Mortis: What Is It Anyway?

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**Educational Objectives:** After attending this presentation, attendees will learn the significance of the use of *Odor Mortis* to identify sites where human decomposition has occurred, how this evidence is gathered, analyzed in the laboratory, and how this forms as a basis for expert opinion. The attendees will learn applicable rules of circumstantial evidence which govern the use of *Odor Mortis* in court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the emergence of *Odor Mortis* capture and analysis will establish a scientific basis for qualified witness at a crime scene or in a laboratory to specifically identify the "smell of death" as they recognize it from their experience and have that identification confirmed by laboratory analysis.

The presentation of a case based upon circumstantial evidence is all about putting the pieces of the puzzle together. Where that case is based largely on scientific evidence, one is faced with an additional challenge. Before the pieces can be arranged in such a way as to show the picture of the defendant's guilt the jury must first understand the evidence. The attorney presenting the evidence must know enough about the science to understand it themselves without allowing their presentation to become so immersed in the science that they lose the jury. This is the true challenge in the presentation of a complex scientific circumstantial evidence case. It is meeting this challenge in general and in particular the use of the concept of *Odor Mortis* in the case of *State of Florida v. Casey Marie Anthony* that will be discussed. Additional discussion will concentrate on the admissibility of the underlying scientific basis of *Odor Mortis* analysis, the expert testimony based upon that analysis, and the use of an expert's sense of smell to identify *Odor Mortis*.

In addition, specific discussion of scientific evidence in this presentation will focus on the development of the decompositional odor analysis database and will include collection methodologies, experimental design, controls, and ramifications of air collection protocols at crime scenes. *Odor Mortis* will be discussed and how the chemical signature of death changes over time. This discussion will end with a summary of how these analytical procedures were used as circumstantial evidence in the Casey Anthony case to confirm the presence of human remains in the trunk of the defendant's car.

For those individuals who smell decomposing bodies on an almost daily basis, they know it is a pungent, unique, and for many, an objectionable odor. How unique is the odor? Most experienced people can say it smells nothing like rotting food, small animals, or fish; however, is that partly because of the quantity of the rotting material we smell? Medical examiners are routinely confronted by these issues. For example, two scenarios involving a medical examiner will be discussed where large quantities of rotting material were associated with a case. The first is where a closed thermal cooler was found by the side of a road with decomposed, liquidized tissue of approximately a foot deep in which bones had sunk to the bottom. Is the smell of the inside of this cooler enough to enable a medical examiner to tell whether this was a rotting deer and not a human? The second scenario involves a driver of a dump truck filled with mildly rotting cow entrails from a slaughterhouse that was in a collision with a freight train on a hot summer day in Texas. Both the driver and the train engine became covered with the entrails that continued to rot in the summer heat while investigators worked the train crash. The smell was pungent and objectionable, but was it unique based on human smell alone? It might be difficult to make an *Odor Mortis* determination in either situation without some sort of scientific determination.

Lastly, toxicological analysis of decomposed remains will be discussed. Such toxicological analysis is routinely performed by forensic toxicology laboratories as an essential component of the medicolegal death investigation process. The presence of drugs and drug metabolites can be used to support a toxicological cause of death. A wide range of specimens are typically available, but the type and condition of the specimen is dependent on the decedent's state of decomposition. The usual specimens include decomposition fluid, solid organ tissue, cranial wash, bone, bone marrow, hair and nails. Toxicological analysis of specimens can also be used to identify products of decomposition. While these findings are not typically reported to the medical examiner, the results may prove useful in investigation and be used to support an *Odor Mortis* finding.

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**Odor Mortis, Human Decomposition, Circumstantial Evidence**



ATLANTA 2012

# WORKSHOPS



ATLANTA 2012

## W1 High-Profile Cases: The Los Angeles Experience

*Christopher B. Rogers, MD\*, Lakshmanan Sathyavagiswaran, MD\*, and Edward Winter, BA\*, Los Angeles County Department of Coroner, 1104 North Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will be familiar with techniques used in handling high-profile cases including media relations, office security, and preparation of the autopsy report.

This presentation will impact the forensic science community by providing practical techniques for handling high-profile cases.

Most medical examiners periodically handle high-profile cases. These cases can be especially challenging because of increased media attention and the need to maintain the security of the medical examiner's office. A thoughtfully prepared autopsy report may help to avoid unanswered questions in the future.

Los Angeles County has seen a number of prominent people become coroner's cases, as well as other cases that have attracted public attention such as officer-involved shootings, deaths in custody, and serial murders. Taking examples from these cases, this presentation will demonstrate methods of handling cases that generate high public interest.

Members of the media need accurate and current information about medical examiner's cases. In order to prepare for high-profile cases, the medical examiner should develop a media relations plan. In larger offices, there should be a few people responsible for media calls and public statements. Smaller offices may specify in advance the resources necessary to handle a large number of media calls. At times the number of calls is so great that it is a full-time job to deal with them. There should be an advance agreement about which section of government will be responsible for media releases in high-profile cases. This will avoid the confusion of having several officials announce different information.

Although there may be pressure to announce results, press releases and other documents should never be prepared in haste. Part of the media plan should give sample press releases for various situations. There is an increasing need to monitor social media, as internet sites can spread misinformation rapidly. The medical examiner's office may need to disseminate accurate information, starting as soon as possible, through social media outlets.

A media relations plan should include provisions for office security. In some cases a large number of news vehicles, grieving fans, or demonstrators block access for employees and others. The decedent must be protected from photographers and curious members of the staff and public. The medical examiner's office must, however, allow access to employees and those whose business is unrelated to the high-profile case. In the past, the police have assisted with security by limiting access to the parking lots.

During the autopsy, as in many forensic cases, details that seem small at first can assume great significance later. The autopsy surgeon should re-read and carefully follow office procedures. Any extraneous photographs (by surveillance cameras, cell phone cameras, etc.) should be prohibited. Photographs, x-rays, documents, and computer files should be secured. Toxicologists and other consultants need to be aware of questions that may come up concerning the case. The typed autopsy report should be proofread several times and checked against primary sources of data. It is possible to find flaws in even the most carefully prepared autopsy report, but they should be minimized to the fullest extent possible.

**High-Profile Case, Media Relations, Security**

## W2 Postmortem Monocular Indirect Ophthalmoscopy Workshop

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After attending this presentation, attendees will: (1) differentiate between direct and indirect ophthalmoscopy noting advantages and limitations of each technique for the postmortem detection of fundal hemorrhages; (2) discuss the fundal location of retinal hemorrhages relative to their projected aerial image during monocular indirect ophthalmoscopy; and, (3) on a fundal diagram, accurately draw retinal abnormalities observed during monocular indirect ophthalmoscopy with a simple ocular model.

This presentation will impact the forensic community by providing an overview of postmortem monocular indirect ophthalmoscopy, facilitating skill acquisition, and evaluating practical training.

Postmortem examination of the retina has relied on ocular evisceration. In most medical examiner/coroner jurisdictions, ocular enucleation is not a standard autopsy procedure unless child abuse is suspected, thus creating observational bias when citing the prevalence of postmortem fundal findings such as retinal hemorrhages (preretinal, flame-shaped or splinter, and dot/blot), perimacular retinal folds, retinoschisis, and postmortem artifactual retinal folds. Postmortem Monocular Indirect Ophthalmoscopy (PMIO) permits examination of the decedent's posterior fundus and portions of the peripheral retina. The required equipment necessary for PMIO is relatively inexpensive and when compared to direct ophthalmoscopy. This technique is less affected by corneal clouding, lens opacity, or vitreous hemorrhage. PMIO uses a focal light source and an aspheric, convex condensing lens. An excellent source of coaxial illumination is a halogen, xenon surgical, or procedural headlamp. This light source creates a collimated beam of light and permits the examiner to stabilize the condensing lens with both hands. Current aspheric lenses range from +14 to +40 diopters and come in different diameters permitting a field of view of 35°-55°. Postmortem corneal opacity may cause the fundus to appear hazy; however, by gently removing the epithelial layer of the cornea, the emergent image is usually of adequate quality to readily detect lesions such as fundal hemorrhages and retinal folds.

Learning how to perform and become proficient at PMIO can be perplexing and intimidating. Most pathology residents and forensic pathology fellows have limited exposure to indirect ophthalmoscopy. Because the projected aerial image is inverted and laterally reversed, precise descriptions or recording of fundal abnormalities can be challenging. Unlike binocular indirect ophthalmoscopy with a teaching mirror attachment, an instructor and the fellow or resident cannot view the projected aerial image simultaneously during PMIO. To address these learning obstacles, it is necessary to develop tools and models to facilitate skill acquisition. An hour or two with an inexpensive ocular model can shift the learning curve of the resident, fellow, or forensic pathologist substantially to the right demonstrating how to correctly position the light source and hold the indirect lens.

This workshop consists of three sessions. An initial discussion reviews the technique of PMIO, highlighting the optics, the equipment, and examples of abnormal fundal findings found at autopsy by PMIO. Next, attendees will have a realistic learning experience by practical hands-on training with a procedural headlamp, an aspheric indirect lens, and a simple ocular model containing a variety of retinal abnormalities observed at autopsy. The ocular models have variably sized “pupillary” openings and some will have clear acetate over the openings to simulate corneal glare. Facilitators will assist attendees in positioning the procedural headlamp, holding the indirect lens, viewing the projected aerial image, and accurately recording the retinal abnormalities. Following practice visualizing and diagramming numerous fundal images, attendees will be evaluated with a series of unknowns. Self-assessment of technical skill training and review of the unknown retinal findings concludes the workshop.

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**Forensic Science, Postmortem Monocular Indirect Ophthalmoscopy, Retinal Hemorrhages**

### **W3 Advanced DNA Mixture Interpretation and Statistical Approaches**

*Debra E. Glidewell, MS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297-5205; Debra A. Figarelli, BS\*, 7881 North 114th Avenue, North, Largo, FL 33773; Timothy S. Kalafut, PhD\*, and Joel D. Sutton, MSFS\*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297-5205; and Robert I. O'Brien, BS\*, 7881 114th Avenue, North, Largo, FL 33773*

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After attending this presentation, attendees will be able to: (1) describe the importance and use of statistics for DNA mixtures; (2) describe all mixture statistical formulas and mixture interpretation approaches outlined in the SWGDAM DNA Mixture Interpretation Guidelines (approved 1/14/10); (3) use multiple approaches to DNA mixture deconvolution and statistical applications; and, (4) be familiar with one software tool for DNA mixture deconvolution.

This presentation will impact the forensic science community by providing attendees with a better understanding of complex mixture deconvolution techniques, the United States Army Criminal Investigation Laboratory (USACIL) software tool, and the application of statistical formulae outlined in the SWGDAM DNA Mixture Interpretation Guidelines.

DNA mixture deconvolution and the application of statistical methods is one of the most challenging aspects of forensic DNA analysis. This mixture workshop will be a hands-on workshop using both qualitative and quantitative approaches to mixture deconvolution. This workshop will review the SWGDAM Guidelines to include the use of analytical and stochastic thresholds, heterozygous peak balance, percent contribution to assess mixture proportions, and stutter considerations.

The NFSTC DNA Services team has trained over 100 forensic DNA analysts in DNA mixture interpretation and statistical applications using the 2010 DNA Mixture Interpretation Guidelines. NFSTC will provide devices for computerized interactive participation by attendees which will allow attendee opinions to be recorded anonymously and seen on a screen. This on screen anonymous feedback allows attendees to see how other laboratories are approaching DNA mixture deconvolution. This information can then be taken back to their laboratories to share with the analytical staff.

Due to the nature of the cases routinely received, the USACIL has years of experience in complex mixture interpretation. USACIL presenters will discuss their methods and demonstrate a software program used by USACIL staff. ArmedXpert™ is a commercial DNA data analysis program developed by the U.S. Army Criminal Investigation Laboratory and licensed to NicheVision Forensics, LLC. The program uses data tables generated from data analysis tools. The user is able to quickly compare samples from multiple tables to one another in a case, between cases, to a

laboratory staff database, QC databases, and create tables or cmf files for CODIS entry. In addition, the software uses a proportional allele method to deconvolute two and three contributor mixtures.

USACIL will provide real case examples of complex mixture data that will be worked through as interactive group activities. The panel of instructors will discuss and show how to apply all interpretation and statistical approaches outlined in the SWGDAM Guidelines for each case example. These case examples will then be deconvoluted using the software (ArmedXpert™) employed by USACIL in order to compare the manual versus automated deconvolution results.

When drafting the 2010 SWGDAM DNA Mixture Interpretation Guidelines, one of the goals of SWGDAM was to provide information that would enable greater consistency and accuracy among analysts within a laboratory. This workshop is designed to provide attendees with hands on applications of scientifically acceptable methods for mixture deconvolution. The interactive component is intended to encourage analysts and technical leaders to review their standard operating procedures and validation data in light of the training on these guidelines and to update their procedures as needed.

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**DNA, Mixture, Workshop**

### **W4 Sex-Related Homicide and Death Investigation: Practical and Clinical Perspectives—Significance of Pornography, Sexual Deviance, Autoerotic Fatalities, Signature and MO, Serial Murder Investigation, as Well as the Increase in African American Serial Killers Involved in These Events**

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After attending this presentation, attendees will: (1) understand the role of fantasy in sex-related death; (2) collect and preserve physical and psychological evidence in sex-related incidents; (3) determine the MO and signature characteristics in crime scenes; (4) understand investigative and behavioral analysis in criminal profiling; and (5) understand serial murder trends.

This presentation will impact the forensic science community by informing the attendees of the dynamics and proper procedures in the investigation of sex-related homicides and death investigations.

Sex-related homicides and deaths occurring during sexual events have drastically increased over the years and claim victims from all walks-of-life (men or women, lovers or strangers, elders, or children). These fatalities may occur from recreational misadventures or sex-related crimes, which are perpetrated by sex offenders and represent the most horrific crimes imaginable. The internet has certainly provided society with technological advances but has also resulted in the proliferation of pornography and easy access to sex-related materials to anyone with a computer or computer access. It is significant that the sex industry, which consists of commercial enterprises providing adult entertainment, earns over \$13 billion a year in the United States and how that may influence the increase in sex-related events.

Attendees of this presentation will better understand the significance of sexual deviancy, fantasy, and pornography in sex-related events as well as the investigative and behavioral analysis applied to these types of incidents. Attendees will understand the importance of the collection and preservation of evidence in sex-related events and the attendees will appreciate the impact of Signature characteristics and *modus operandi* as applied to sex-related homicides.

## W5 Paper Fundamentals for Forensic Document Examiners

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In the first segment, the presenters will provide examples of sexual deviance as well as the paraphiliac considerations in these type death investigations. There are over 35 Paraphilias described in the literature and a number of Paraphilias are cross-associated with sexual homicides. These will be explored at length in the presentation. The presenters will also explore the connection between Pedophilia and the sexual homicide of children with appropriate case studies provided for illustration. The attendees will then be apprised of the current knowledge regarding autoerotic fatalities.

Attendees will be apprised of the current knowledge regarding autoerotic fatalities including definitions, incidence, crime scene characteristics, typical and atypical methods, and victims. It will be demonstrated that the widely-cited incidence of 500 to 1,000 autoerotic deaths-per-year in the United States is no longer accurate. It will be explained that an incidence of 0.2 to 0.5 cases per million inhabitants per year is a better estimate of the incidence of autoerotic deaths. New epidemiological data have demonstrated that this incidence is higher in big cities compared to rural areas. There is no clear evidence of a preferential time-of-day for these deaths, but there appears to be slightly more incidences of autoerotic deaths during summer. Recently, the typical and atypical methods of autoerotic deaths have also been revisited based on the new standardized classification of asphyxia. New data on the crime scene characteristics will also be presented, along with a discussion of four types of atypical victims: non-white female, children, adolescent, and the elderly. This presentation will provide a comprehensive and practical illustration of specific examples and cases with a discussion of the investigative and clinical considerations, which will include pathology, pathophysiology, as well as the investigative and behavioral and forensic aspects of these events.

The second segment of the workshop will focus on the dynamics of sex-related homicides, which includes rape, lust murder, and serial killing. Using a case history format, the importance of “signature” and “*modus operandi*” will be demonstrated to illustrate the application of Investigative and Behavioral Analysis to the crime scene examination.

Current research regarding the frequency of sexual posing in homicide crime scenes and the phenomena of African-American sexual serial killers in the United States will be included. The myth that there are no African-American serial killers has been perpetuated based on media coverage on television and/or Hollywood movies such as: *The Red Dragon*, *Silence of the Lambs*, and *Hannibal* which exclusively focus on the white serial killers. However, there has been a drastic increase in the number of African-American serial killers since the 1980's. Only recently, with the arrest and conviction of Anthony Sowell in Cleveland, Ohio and the identification through the use of familial DNA of Lonnie Franklin, Jr. “The Grim Sleeper” that the media has acknowledged the fact that there are African-American serial killers reported on these cases. The current research indicates over 150 African-American sexual serial killers as of July, 2011.

The overall goal will be to provide comprehensive and practical information which will serve an investigative guide in sex-related homicide and death inquiries.

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### Sex-Related Homicide, Autoerotic Deaths, Serial Murder Trends

After attending this presentation, attendees will have a better understanding of ASTM training requirements. Individuals with little previous exposure to the subject area will have a comprehensive introduction, those with moderate exposure will have an updated refresher, and those with significant exposure will have had an opportunity to share their knowledge and case experiences with the other participants.

This presentation will impact the forensic science community by establishing and implementing standards for training and practice, and providing a more predictable skillset. The “questioned document” profession has established minimum training requirements which are documented as an ASTM standard; this includes training regarding paper. As both a supplemental initial training and continuing education opportunity, this workshop provides access to technical expertise that geographically dispersed individuals and groups could not realize.

Forensic document examiners are qualified through an apprenticeship process. The types of training required were determined by the profession and are described in the ASTM Standard *E2388-05 Standard Guide for Minimum Training Requirements for Forensic Document Examiners*.

In order to ensure that examiners are up-to-date regarding paper, the all-day workshop will be divided into four sessions: the first will include the history of paper and the paper manufacturing process. The second will cover techniques and casework that involve paper. The third will introduce security papers. The final session will include techniques for the examination of security papers, and casework examples in which security paper played a role.

**History:** Using extensive artwork and historical images, the “Paper History Timeline” will be shared with workshop participants. In Asia, the Middle East, and Europe, cultures were striving to have something on which to write! Fraud was soon to follow.

**Manufacturing Process:** Using extensive video, images, and marginally technical explanations, speakers will ensure the workshop participants comprehend the technical complexities that have radically changed the simple process that is papermaking.

**Paper Properties and Basic Examinations:** Various characteristics are inherent in a single piece of paper given the materials and process used. Knowing what they are, identifying differences, identifying reasonable variations, and deciding what they mean are the fundamental basis of paper examinations. In this session, how the characteristics are affected when paper is acted upon, and methods for assessing the condition of those characteristics will be described. Indented handwriting, fracture match, and paper fiber impressions will be discussed. Participants will be invited to share case summaries.

**Security Papers:** As the value of a paper document increases, the need to protect that document also increases. Over time, many elements have been changed or added to make documents more difficult to alter or counterfeit. These elements may be printed material, chemical additives, or other security materials (such as fibers or planchettes.) In this session, the entire range of security papers will be described.

**Security Papers – Examination Techniques and Case Examples:** Counterfeiters have only one job: to fool the person they need to fool. Typically, the person receiving a counterfeit item is not a forensic examiner and the counterfeiter knows this. Many efforts to duplicate security papers are amusing to the skilled document examiner; however, they are sufficient to be accepted as genuine. In this session, technical information regarding

examination techniques and equipment will be provided, as well as examples of cases in which security paper played a role. Some will be of the amusing variety and some of the nearly undetectable variety. In addition, as in the Paper Examination session above, participants will be invited to share case summaries.

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**Paper, Watermark, Security**

## **W6 Practical Ethics in Forensic Science – A Multidisciplinary Call to Arms**

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After attending this presentation, attendees will better understand the nature of practical applied ethics, including the history, practice, enforcement, and expectations from a multidisciplinary view. Attendees will understand potential dangers associated with unethical conduct.

This presentation will impact the forensic science community by providing a better understand of the history and nature of ethics as it relates to the practice of forensic science.

“Tainted Science and Testimony Leads to Re-opening of 120 Cases over Last 15 years,” “Man Freed After Serving 21 years in Prison Due to Lab ‘Oversight?’”, “State Crime Lab Employee Accused of Biased Analyses and Testimony”

All of these headlines can confront and scare legitimate practicing forensic scientists. At the heart of this fear is wondering how such events could ever occur, since inherent in the practice of forensic science is the requirement for each practitioner to be truthful and beyond reproach. A failure of forensic scientists to act ethically results in serious adverse outcomes. The word “ethical” can be defined merely as proper conduct – doing the right thing, the right way, for the right reason. While seemingly simple to define, the application of being “ethical” is somewhat more obscure. The 2009 National Academy of Sciences Report on the status of the forensic sciences argued for the need for enhanced ethics training within all forensic disciplines. Tacit within this commentary is that there is a significant problem related to practitioner behavior. As most involved in the day-to-day practice of forensic science would argue that they are complying with all extant codes of conduct, there seems to be a disconnect between theory and practice which needs to be addressed. Thus, the question becomes when is ethical, “ethical,” and when is it not? As a non-partisan in the adversarial justice system, the scientist should have no stake in case outcomes.

Clearly, as part of the legal system, there must be room for differences of opinion in the forensic sciences. What is not clear, however, is when such differences are so divergent that individuals’ ethics are drawn into question. In this workshop, the role of ethics in the forensic sciences from different perspectives will be addressed with the intent of approaching an understanding of when the proverbial ethical line is crossed.

The workshop will include a discussion of practical ethics in forensic disciplines from multiple points-of-view, including historians, theologians, educators, practitioners, law enforcement, witnesses, attorneys, and judges.

Presentations will include comparisons to canons of ethics in the existing forensic organizations and in other fields, with possible mechanisms for enforcement of standards presented. The unrealistic expectations of the public may be at least partially responsible for the present intense interest in the field. Recognition of ethical concerns by those in the field and vehicles for whistle-blowing to bring unacceptable behaviors to an end are addressed. Potential outcomes of unethical behavior, especially in the forensic sciences, are presented in a series of case studies.

In the final analysis, all forensic practitioners should remain keenly aware of the inherent ethical dilemmas in the nature of the work and should maintain the highest standards of personal and professional conduct. Transgressions sully the entire field and should not be tolerated. The first step involves knowing the basics and where dangers might lie. This workshop intends to present such a primer.

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**Ethics, Whistle-Blower, Dilemmas**

## **W7 Preparation and Strategic Planning for Accreditation of Forensic Laboratories Based on the ISO/IEC 17025 International Standard**

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The goal of this workshop is to provide attendees with a general overview of the recourses to meet ISO/IEC 17025 requirements and explain how they may be applied in the context of forensic laboratories to include medical examiner laboratories. After attending the workshop the participants will understand the accreditation process as well as the general requirements to prepare for ISO/IEC 17025 accreditation of forensic laboratories.

This presentation will impact the forensic science community by providing an overview of the accreditation of a multi-discipline forensic laboratory based on ISO/IEC 17025. The workshop is geared towards managers and analysts of international forensic laboratories with little or no exposure to the accreditation process. This workshop will cover definitions of common quality assurance terms, the accreditation process, various accrediting bodies, and the general ISO/IEC 17025 and ILAC G-19 guidelines for forensic laboratories. The workshop will provide realistic expectations of what is required to achieve accreditation and provide an achievable roadmap. The workshop will allow international forensic laboratories to create a strategic plan and timetable to achieve accreditation based on the ISO/IEC 17025 Standard.

Laboratory accreditation is a tool used to evaluate the general competence of a laboratory, and as such, is increasingly expected by clients of forensic and medical examiner laboratories and by the courts. The number of states in the United States and countries internationally mandating laboratory accreditation is growing. The value of accreditation increases in the investigation of international crimes when the accreditation program is based on an internationally recognized standard. Often evidence analyzed by 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. Multi-country investigations and prosecutions require that the criminal justice systems in one country can use and trust forensic laboratory reports issued by laboratories in other countries. The concept of and mechanism for international uniformity, consistency, and competence of laboratories

through Regional Multi-Lateral Recognition Arrangements and the International Laboratory Accreditation Cooperation (ILAC) Mutual Recognition Arrangements will be explained including many of the acronyms and buzz words in the accreditation world (ISO, ILAC, IAAC, APLAC, AB, CAB, regional cooperations, MRA, and MLA to name a few).

The International Organization for Standardization has thousands of standards that are used worldwide. The standard ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories is the ISO standard currently in wide use for forensic laboratories. How this standard is applied to the accreditation of forensic laboratories will be covered. The workshop will include a general overview of the Management and Technical Requirements sections of ISO/IEC 17025:2005 covering the main issues of each section.

ISO/IEC 17025:2005 covers general concepts that have been proven to provide a sound foundation for effective organizations performing testing and calibrations; however, ISO/IEC 17025:2005 is not specific to forensic science. ILAC's Guidelines for Forensic Science Laboratories (ILAC G19), is an example of an amplification document for forensic laboratories, and will be reviewed to demonstrate the relationship between an ISO standard and an amplification document.

Following the general overview, detailed presentations will be provided on Document Control, Traceability, Measurement Uncertainty, and Internal Audits.

**Document Control:** The requirements for document control are an excellent area to begin with if a laboratory is just beginning to prepare for accreditation. Specific requirements and strategies for compliance will be provided.

**Traceability and Measurement Uncertainty:** These topics are new to many in forensic science and specifically to the area of toxicology. Establishing traceability has a significant impact on the quality of laboratory results and measurement uncertainty provides a mechanism to compare test or calibration results between laboratories. Specific applications will be provided in the area of toxicology.

Internal audits are a requirement in ISO/IEC 17025:2005; however, internal audits are also a useful tool in preparing your laboratory for accreditation. How to plan and conduct global, vertical, and horizontal audits will be covered.

Months have been spent preparing for ISO/IEC based assessment and now it is time to submit the application to an accrediting body. Understanding the assessment process minimizes the fear associated with an external assessment. The workshop will step through the process from application through accreditation being granted. The development of the assessment plan will be covered. The methods used by assessors to document conformance with requirements will be presented. These will include document review, case record review, observation of testing or calibration activities, and interviews of laboratory personnel. The general process for remediation of findings of non-conformance will be provided. In addition, tips and techniques for making this process as easy as it can be will be provided.

The workshop will conclude with discussion of the most common deficiencies found during accreditation assessments of various forensic testing laboratories.

This workshop will provide valuable practical guidance to national and international forensic laboratories working towards and maintaining accreditation based on the ISO/IEC 17025:2005 standard.

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#### **ISO/IEC 17025, Accreditation, Requirements**

## **W8 Examination and Analysis of Explosives and Device Construction/Components**

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After attending this presentation, attendees will be familiar with forensic explosive analysis, microscopical analysis, instrumental analysis, and sample preparation techniques. The attendees will also become familiar with the investigative leads generated by the characteristics of and components used in a device, its container housing, and its firing train.

This presentation will impact the forensic science community by providing an understanding of the principles and practices of explosive material identification, explosive device construction, component characterization, and report writing. This training will provide exposure to a variety of experts and testing approaches that can be utilized in local, state, and federal laboratories.

The increase of domestic and foreign terrorist activities has brought awareness to explosive analysis in local, state, and federal forensic science service providers. From offering general awareness of materials to investigators and first responders to laboratory analysis of explosives, explosive residues, and device components, analysts in this specialized field continue to work together to share information and intelligence. This networking is valuable for sourcing materials and identifying domestic and foreign terrorists, potentially leading to their arrest or capture. Although national and international agencies are involved in this type of work, this workshop will focus on the forensic science practitioner and training in the forensic science testing of explosive materials and device construction/components.

The workshop will provide the foundational knowledge of explosive terms, types of explosions, specific types of explosive compounds and their characteristics. The workshop will present the attendees with an understanding of common approaches to the examination and analysis of explosive residues and materials. The ensuing presentations and activities over the two days will cover physical examinations and chemical analyses, tips on sample isolation and preparation, improvised explosive devices (IEDs), low explosives, high explosives, and homemade explosives (HME's). Microscopical examination and preparation techniques will be enhanced with hands-on exercises. Workshop attendees will have opportunities to reconstruct devices from found evidence and debris. Faculty presenters will discuss specific cases and offer their conclusions. The panel and participants will conclude this session with discussions on appropriate wording and report writing guidelines that will reflect an understanding in the criminal justice, forensic science, and judicial communities.

In addition to the foundational knowledge, the workshop will provide a short review of explosive blast physics (thermal effects, pressure wave, etc.), the types of explosions followed by the types of explosives (primary, high, and low). Individual compounds of each type will be reviewed including their brief history, development, and modern usage. Explosive materials such as black powder, black powder substitutes, flash powders, and smokeless powders will be examined and characterized as a part of a hands-on exercise. The high explosives will focus on commonly seen compounds such as TNT, C-4, and PETN. Plastic bonded explosives, emulsions and modern blasting agents will also be covered. Analytical

approaches will be discussed and the instrumental techniques utilized such as microscopy, FT-IR, GC/MS, and X-ray techniques.

Microscopical analyses have been intimately tied to forensic science since its inception. However, some modern trends have resulted in moving away from the microscope in explosives analysis. This session will focus on the extreme power the microscope can offer the examiner. From searching debris for small pieces of fragmentation and particles to microcrystalline techniques that can identify specific explosive compounds, the microscope is an invaluable tool in the examiner's toolbox.

In the last few years, new trends in explosives have resulted in an increase in the number of homemade explosives (HMEs). This session will familiarize attendees with the current analytical practices related to HMEs. How these devices are developed and used, the types of explosive materials in them, and the chemistry behind their devastating effects will be presented. Lastly, the workshop will offer a brief insight into potential future testing methods to include instrumentation and innovations in explosives detection and analysis that could impact this field.

At the conclusion of this workshop, the attendees will have the knowledge, understand techniques required to pursue the analysis of explosives, and understand of the importance of the materials used in the construction of explosive devices. Participants will be able to utilize the principles taught in the lectures and offered in the hands-on exercises to hone their skills and bring these resources back to their organizations thereby improving on their existing explosive analysis programs.

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**Explosives, HME, IED**

## **W9 What Did You Just Step In?! Use of Forensic Soil Examinations to Find Out**

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After attending this presentation, attendees will be equipped with sufficient knowledge of soil analysis to either initiate this type of service in their laboratory or suggest improvements to their existing methods for soil analysis. Situational awareness of the need to utilize the analytical services of an outside laboratory for select soil comparisons will also be gained.

This presentation will impact the forensic science community by providing education to those desiring training in forensic soil examinations, as well serving as a reminder about a service that more laboratories may wish to add to their suite of analytical services. This workshop will help to make soil analysis more accessible to the forensic community by demonstrating the limited instrumentation necessary to make this service available. Additionally, the workshop will provide a forum for examiners to discuss the differences in existing techniques, the need for standardization, and the impact of accreditation.

A quick search of the internet for "soil analysis laboratories in the United States" returns a long list of laboratories providing soil analysis for agricultural concerns. However, laboratories providing comparative soil

examinations for forensic applications are rare. Though soil analysis is a well-established field of forensic examination, the air of mystery and difficulty has made the "forensic soil examiner" a rare breed.

Proficiency in the field is only maintained through constant practice and continuing education; however, training courses in the discipline are infrequently offered and often cancelled due to a lack of attendees. Laboratories often cite the lack of instrumentation, time, or personnel as the reasons for not providing this service. Much of this can be overcome with proper and efficient training; for the underpinning work in such examinations can be performed without high-end instrumentation. In fact, much of the comparative work can be performed with instrumentation that most forensic laboratories already have available (i.e., polarized light microscopes, automated scanning electron microscope/energy dispersive spectrometer, Fourier transform infrared spectrometers, and x-ray fluorescence spectrometers).

During this two-day workshop, attendees will gain insight into the geological and historical foundations for soil examinations and their practical applications in forensic science. They will gain experience in the application of a variety of laboratory techniques commonly used for forensic soil examinations. Lectures and "hands-on" sessions will include, but are not limited to, the following: the historical basis for forensic soil examinations; geology as related to the formation of soil; soil collection methods; comparative color and texture analyses; the preparation and separation of soil samples for the isolation of the botanical, anthropogenic (man-made), clay, and mineral components of soil; the application of x-ray diffraction and Fourier transform infrared spectroscopy for the identification and comparison of clays; fractionation of the heavy and light mineral components for identification purposes; mounting methods for identification of soil minerals by polarized light microscopy; methods for percent composition comparisons of the heavy and light mineral content; the utilization of automated scanning electron microscopy-energy dispersive x-ray analysis for automated mineral analysis; determination of provenance based upon the sum total of the components identified in soil; report writing verbiage; and significance evaluations.

Attendees will gain, at a minimum, a theoretical and practical foundation for incorporating soil analyses into their laboratory's suite of analytical services. They will also obtain the skills necessary to detect, collect, and preserve soil evidence and to perform minimal comparisons, thereby enabling preliminary evaluation of such evidence for use in their own cases.

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**Soil, Mineral Analysis, Microscopy**

## **W10 Drug Enforcement Administration U.S. Customs and Border Protection Forensic Mobile Device Workshop**

*Rhesa G. Gilliland, MS\*, Drug Enforcement Administration, Digital Evidence Laboratory, 10555 Furnace Road, Lorton, VA 22079; Samuel I. Brothers, BBA\*, United States Customs & Border Protection, 7501 Boston Boulevard, Room 113, Springfield, VA 20598; and Lam D. Nguyen, MS\*, and Scott D. Roffman, MS\*, Drug Enforcement Administration, Digital Evidence Laboratory, 10555 Furnace Road, Lorton, VA 22079*

The goal of the presentation is to assist attendees in the examination of mobile devices in a forensically sound manner through discussion of relevant topics: (1) handling and preservation of mobile devices: attendees will understand proper procedures for collecting and handling mobile devices so as to preserve data for forensic examination; (2) validation of mobile device examination methods: this discussion will present a proposed framework for validating mobile device examination methods and software tools in light of agency accreditation requirements; and, (3) by learning to leverage available resources against their limitations, attendees will be able to relate the *Daubert* standards to the challenges specific to codifying methodologies in an ever-evolving discipline.

## W11 Digital Photography for Forensic Document Examiners

This presentation will impact the forensic science community by providing sound strategies and methodologies that can be directly applied to the examination of mobile devices and further the development of the digital forensic discipline.

The following topics will be covered: handling and preservation of mobile devices from seizure to analysis and methods validation with a proposed framework for validating mobile forensic methods and tools.

Attendees will: (1) understand the importance and applicability of the *Daubert* Standards in mobile forensic methods; (2) develop and apply an organizational methodology for software tools validation within the framework of accreditation; and, (3) be able to leverage available resources while understanding their limitations and understand the challenges of codifying methodologies in an ever-evolving discipline.

A review of all cell phone forensic tools and the Cell Phone and GPS Forensic Tool Classification System will be covered in depth. Our world has become saturated with inexpensive digital devices. The ubiquity of these devices has changed the way we do everything, from staying in touch with friends on our smartphones to finding the nearest gas station with GPS technology. Criminals also use these devices to aid in the commission of crimes. GPS devices are used for human and narcotics smuggling, while cell phones are used to deliver text messages coordinating the next terrorist attack. Most electronic devices themselves contain a wealth of information and intelligence for investigators, though the field of digital device forensics is still in its infancy. There is a need for a common framework to classify the plethora of tools released into the commercial marketplace in the last five years. Given the exaggerated claims of software manufacturers, the field of mobile forensics has long since clamored for an understanding of not only how these tools work, but when they should be used. Attendees will be able to categorize any mobile device acquisition tool within a classification system. In addition, an overview of many commercial tools for cell phone data extraction currently available will be discussed. This presentation will provide a common framework for the digital device data extraction tool classification system for the entire digital forensics community.

The Apple iPhone®, utilizes SQLite Databases to store user data and applications that have been installed on the device. A digital forensic examiner trying to access this information through traditional means may miss large amounts of relevant data still present within the database. Because of the way inactive data is marked for deletion, it may be impossible to extract this data through typical database queries. Fragmented sections of these databases may also be found in unused space on the device. While the complete file is not readable by traditional methods, forensic recovery may still be possible. The key to gaining access to this information is to understand how the data is organized within the database structure and the manner in which it has been stored and encoded. Attendees will gain an understanding of how to obtain digital evidence from SQLite databases. Attendees will also gain an understanding of how this information is stored, how active and deleted content within these databases can be extracted, and how to decode the extracted information. An explanation of SQLite data storage structure, encoded data, the use of regular expressions to search for and identify records, and the ability to decode the stored values within the recovered records will also be presented. The storage of call history and text messages utilized by Apple's iOS® on the iPhone® will be specifically addressed and used as an example for how to identify and decode deleted records.

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**Mobile Forensics, Apple iOS®, Validation**

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After attending this presentation, attendees will be able to: (1) successfully demonstrate the effective documentation of forensic digital capture techniques as well as explain how digital technologies are used in evidentiary photography processes; (2) successfully complete a hands-on exercise demonstrating the comparison of image resolution and screen (display) resolution, demonstrating the artifacts caused by improper resolution settings and parameters; and (3) successfully complete a hands-on exercise demonstrating the artifacts caused by improper image processing techniques (such as rotating an image, compressing an image, printing an image, etc.). Participant achievement of the training objectives will be evaluated by observing participant behavior in the classroom and assessment of demonstrated practical skills by the instructor.

This presentation will impact the forensic science community by addressing the need for knowledge about the legal ramifications of digital evidentiary photography and best practices for digital evidentiary photography to include acceptable file formats and standards for capturing, storing, printing, transferring, and preserving digital/digitized evidence. It will also provide participants with the acceptable methods and techniques for comparing imaging technologies. The activities will include instructor-led demonstrations and hands-on exercises using image-editing software on the laptop computers participants bring for use in the course.

The Digital Photography for Forensic Document Examiners Training Program is intended for individuals working in a law enforcement field who have limited knowledge and experience using digital technologies in a forensic environment. Upon completion of this program, attendees will have a basic understanding of forensic digital imaging concepts and how various image capture techniques can aid in their investigative process.

It is recommended that attendees taking part in this course have a basic understanding of the Microsoft Windows XP operating system. In addition, it is recommended that all class participants be actively involved in a law enforcement discipline that uses digital imaging technologies.

This is a one-day training program that includes both lecture and hands-on training including note taking. Course instruction also includes a review of best practices and procedures as well as a comprehensive discussion and application of digital imaging techniques.

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**Digital Photography, Questioned Documents, Digital Imaging**

## **W12 Humanitarian Forensic Science: The Forensic Investigation of Human Remains From Armed Conflicts and Catastrophes**

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After attending this presentation, attendees will become familiar with the main practical considerations for large-scale forensic investigations for the search of missing persons from armed conflicts and catastrophes.

This presentation will impact the forensic science community by exploring the application of forensic science in humanitarian contexts and outlining some of the unique challenges posed to the wider forensic community by investigations into persons gone missing. It will also present some of the solutions identified for assisting forensic practitioners, institutions, and service providers involved in these investigations. A multidisciplinary panel of international experts will share their experiences, lessons learned and recommendations regarding practical considerations for large-scale forensic investigations in the search for missing persons from armed conflicts and catastrophes.

The humanitarian scope of forensic investigations applied in the search of missing persons world-wide requires awareness and consideration of factors rarely encountered in the domestic setting. International public law provisions must be well understood and socio-political factors impacting investigations must be taken into consideration. Forensic practitioners must be aware of the health and safety issues associated with working in foreign environments, as well as unique health and safety precautions necessary in potentially dangerous contexts. The psycho-social needs of the families related to the investigation of victims of conflict should be addressed, including the expectations of the bereaved. The selection and integration of identification methods should be planned with care and should be suitable to the specific requirements of the investigation, including the biological profile-related characteristics of the victims and the needs of the investigation. Resources for large-scale investigations, which are usually limited, should be appropriately managed and suitable for logistically-challenged settings while maintaining quality assurance and ethical conduct. Data gathered during investigative processes must be properly managed, taking into consideration chain-of-custody and confidentiality of the data. It must be decided in advance to whom the data will be shared with and under what circumstances. Utilization of local expertise and resources should be balanced against the needs for confidentiality, neutrality, and impartiality in the investigation at the same time recognizing that long-term investigation and identification processes require local involvement to ensure sustainability. Finally,

specific ethical dilemmas related to forensic work and management of the dead in diverse contexts must be addressed, taking into consideration all other planning challenges and constraints. For example, political or prosecutorial pressures may place emphasis on investigative processes for judicial purposes leaving reduced resources for identification efforts. Likewise, reduced resources may force investigators to selectively target specific graves for investigation while leaving other known gravesites untouched, or necessitate the recovery of only a sample of remains within a grave.

As forensic practitioners increasingly become involved in international humanitarian operations, they must adapt their working methods to the specific needs of the investigation, which may differ from the domestic setting. In addition, they must integrate their technical skills into multi-disciplinary teams in order to address the various needs of the investigation and the victims to which the investigations relate. Therefore, it is imperative that practitioners working in large-scale forensic investigations for the search of missing persons from armed conflicts and catastrophes become familiar with the unique challenges that these operations may pose.

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### **Humanitarian, Conflicts, Catastrophes**

## **W13 Estimating Uncertainty in Weights: Hands-On Workshop Using SWGDRUG Document SD3**

*Suzanne Bell, PhD\**, West Virginia University, 1600 University Avenue, Oglebay Hall, Room 208, Morgantown, WV 26506-6121; *Sandra E. Rodriguez-Cruz, PhD\**, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081; *Linda C. Jackson, MS\**, Department of Forensic Science, 700 North 5th Street, Richmond, VA 23219; and *Scott R. Oulton, BS\**, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081

After attending this presentation, attendees will be able to: (1) reference the SWGDRUG website and all associated resources; (2) understand the concept of uncertainty estimation in the seized drug context; (3) evaluate their weighing procedures and protocols using several different uncertainty estimation tools; (4) design a simple uncertainty budget using Excel; (5) design an uncertainty budget utilizing laboratory control charts; and (6) defend a reasonable uncertainty estimation before their peers and before triers of fact.

This presentation will impact the forensic science community by showing how estimation of uncertainty is a key element of any quantitative data and should be estimated when quantitative data is obtained. The 2009 National Academy of Sciences Report emphasized the need for estimation of uncertainty and accredited laboratories will soon face issues of uncertainty estimation and reporting. This workshop will assist forensic scientists in developing reasonable and defensible uncertainty estimations for an important category of quantitative data.

This workshop will include a hands-on tutorial designed to assist forensic analysts in estimating the uncertainty of weights obtained in a seized drug analysis. Although the target audience is drug analysts, anyone interested in uncertainty estimation will find this workshop useful and valuable. The workshop will be taught at an introductory level. No chemical knowledge is required or assumed. The workshop is built around the Scientific Working Group for Seized Drug Analysis (SWGDRUG, [www.swgdrug.org](http://www.swgdrug.org)) Supplemental Document SD-3, which presents several examples for making reasonable and defensible uncertainty estimates for weight measurements.

Uncertainty is a fundamental part of any quantitative measurement. Uncertainty is not the same thing as error, and recording data with an estimation of uncertainty does not imply that data is somehow suspect. The opposite is true; a quantitative value such as a weight of a drug exhibit, along with an uncertainty estimate, is more valuable in many cases, both to

the laboratory and to the customers it serves. The exercise of estimating the uncertainty associated with an instrument or procedure assists laboratories in improving practices and facilitating more effective and efficient method validation policies. The workshop will present examples of how these tasks can work together to improve laboratory operation.

The first part of the workshop program will include a general introduction to SWGDRUG Recommendations - Part IV C (Quality Assurance/Uncertainty) and Supplemental Document SD-3, followed by a discussion on the basic principles of uncertainty estimation. Statistical and metrological resources will be presented and key statistical concepts will be reviewed. Attendees will also receive useful information related to the selection, requirements, operation, calibration, and maintenance of laboratory balances.

The second part of the workshop will be dedicated to discussions of SWGDRUG Supplemental Document SD-3 (Measurement Uncertainty for Weight Determinations in Seized Drug Analysis). A copy of this document will be provided to all attendees and its purpose and background, within the scope of SWGDRUG's mission, will be discussed. This document contains specific examples of how to calculate the uncertainty associated with weight measurements performed in a laboratory. The basis, assumptions, and background behind each one of these examples will be presented in a format that provides for active discussion with the workshop audience. The workshop presenters will also provide assistance and suggestions on how to present and defend uncertainty estimates to peers as well as legal professionals.

The third part of the workshop will be dedicated to open discussions. Attendees are strongly encouraged to bring examples of specific laboratory models for presentation during this section of the workshop. Examples will be discussed and assistance provided on developing uncertainty budgets. Attendees are also welcomed to bring a laptop computer and data from their laboratory for use during the hands-on portion of the workshop.

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#### **Uncertainty Estimation, Seized Drugs, SWGDRUG**

## **W14 Using Pharmacokinetics to Analyze Forensic Toxicology Cases**

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After attending this presentation, attendees will: (1) summarize the basic physiology, pharmacology, and pharmacokinetics required to interpret drug blood and urine levels which have been obtained both before and after death; (2) present confounding issues that limit the interpretation of many quantitative blood test results such as postmortem redistribution, and pharmacokinetics in children; and (3) review approaches to analyzing the complicated Forensic Toxicology case.

This presentation will impact the forensic science community by showing how 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 she/he impaired?", and/or "Did it injure him/her?" Such questions are very difficult to answer on the strength of one test result. The workshop begins with a review of the physiology, pharmacology, and pharmacokinetics of drugs, followed by presentations involving postmortem redistribution, postmortem pharmacokinetics, tissue distribution in the ante- and postmortem settings, and the significance of using various sampling sites for blood.

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of drugs. In many instances, determination of the cause and manner of death may be elucidated by analyzing the correlation

of the drug's pharmacokinetics and the drug's effect (pharmacodynamics). 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 injure or kill him/her?", or "Was she/he impaired?" These questions are very difficult to answer on the strength of one test result. In many instances, the investigating forensic scientist would like to develop an adequate history regarding the time the last dose was taken, the amount, the route of administration, and whether the final dose was a single acute OD, a large OD, or an OD that occurred due to drug accumulation over time, but access to those data are unavailable. In addition, genetic factors regulating drug metabolism, drug interactions, and differences in a drug's pharmacokinetics during toxic dosing (toxicokinetics) impact the analysis of the drug's blood levels and toxic effects.

Though many cases involve the analysis of blood samples obtained from living subjects, in some cases, the blood samples are obtained after death. In the postmortem state, multiple samples obtained from different body sites (e.g., femoral, iliac, subclavian, and cardiac) often show different quantitative results. This may be due to partial absorption from the gastrointestinal tract, incomplete distribution of the drug after ingestion but prior to death, or postmortem redistribution. Cardiac blood is the least reliable. There are four chambers of the heart, and on analysis, all may give different results. Results of blood samples taken from the left side of the heart are less reliable than results obtained from blood samples taken from the right side of the heart because the left side fills from the pulmonary vein (and the lungs are known to accumulate drugs), thus causing the blood to contain higher levels of drug than found in the right heart, which fills from the systemic circulation (vena cavae).

When blood samples have been obtained after death or at autopsy, changes in postmortem redistribution, putrefaction, bacterial contamination, and postmortem production of ethanol may confound the interpretation of the results, unless blood sampling from various body sites and tissues was carried out. In the postmortem state, determining the cause and manner of death may be quite difficult. Moreover, in the postmortem setting, the pharmacokinetics of the drug is altered, as is the volume of distribution (Vd). Using PK and Vd data from antemortem studies and applying them to postmortem data, or using Vd data from adults and applying them to children may lead to major errors in the interpretation of the data and incorrect conclusions about the cause of impairment or death. Attendees will learn how to better apply the principles of PK in their cases, and raise the level of the application of the principles of PK in their practice of forensic toxicology.

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#### **Pharmacokinetics, Postmortem Redistribution, Forensic Toxicology**

## **W15 Hell on Earth — Just Another Day at Work: An Overview of the Tri-State Crematory Catastrophe**

*Kris L. Sperry, MD\**, Georgia Bureau of Investigation, 3121 Panthersville Road, PO Box 370808, Decatur, GA 30037-0808; *J.C. Upshaw Downs, MD\**, Georgia Bureau of Investigation Medical Examiner's Office, 925 A Mohawk Drive, Savannah, GA 31419; *Mark A. Koponen, MD*, University of North Dakota, Department of Pathology, 501 North Columbia Road, Mailstop 9037, Grand Forks, ND 58282-9037; and *Greg Ramey, MA\**, Georgia Bureau of Investigation, 3121 Panthersville Road, Decatur, GA 30034

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After attending this presentation, attendees will be able to understand how to approach a mass disaster created when large numbers of bodies are not cremated in accordance with standard funeral practice. Participants will also gain an understanding of how multiple agencies interact in dealing with a complex mass disaster. Approaches to public and media involvement will also be discussed.

This presentation will impact the forensic science community by showing lessons learned from a unique incident which created social havoc

throughout Georgia, Tennessee, and Alabama. The impact was felt among thousands of individuals who had sent the bodies of their loved ones to be cremated. This incident also captured national and international attention in the forensic and non-forensic communities.

On the otherwise typical afternoon of February 15, 2002, during the course of the AAFS meeting in Atlanta, GA, a call came in that changed an otherwise beautiful and mild, deep-South winter's day into one of the singularly most macabre events ever imagined. A decade later, the memories are vivid.

Acting on an anonymous call, investigators responded to a scene in the Noble Community of rural Walker County, GA, just 100 miles from Atlanta, the heart of the "new south." A passerby had discovered what were believed to be human remains in a heavily wooded area, adjacent to several residences and the family-owned Tri-State Crematory, serving northeast Alabama, south central Tennessee, and northwest Georgia.

Investigators arrived to find a scene almost defying description – dozens of bodies, in various stages of decomposition, were laid out in a garage. As investigators recovered from the initial shock and continued exploring the property, dozens of bodies, then hundreds were discovered. Eventually, bodies were found strewn in the woods, comingled in pits, comingled in casket vaults, and within several buildings and vehicles on the property. The crematory owner had maintained essentially no records regarding his supposed cremation practices. A full-scale mass death scene investigation ensued, requiring the combined resources of local, state, and national agencies. The National Disaster Mortuary Response Team (D-MORT) was deployed to assist with the processing the unidentified bodies and partial sets of remains. It was eventually determined to represent remains of some 334 individuals. The recovery process alone lasted for three weeks. Eventually, this case became the largest criminal investigation in the history of the state of Georgia, involving over 500 individuals from more than 55 local, state, and federal agencies in the recovery and identification of these remains. Successful and continuous multi-jurisdictional agency interaction was essential to the resolution of the matter, particularly in handling a problem that literally grew with each passing day.

The scale of the recovery and identification process was hindered by multiple factors, including: location, multiple clusters of mini-scenes with varied conditions, lack of defined scene boundaries, unknown and varied postmortem interval of individual cases, and unknown numbers of remains. All of these factors, combined with the intense international interest in the case led to one of the most unusual death investigation cases in the annals of death investigation. This overview touches on the various aspects of the case including initial response, scene assessment and triage, body recovery, identification, family interaction, media interaction, and criminal investigation.

**Families and media:** The macabre nature of the event resulted in immediate and eventually world-wide interest in the case. Given the intense interest in the case, media interaction became an important factor. A balance had to be struck between the families' rights to be kept informed and public information. Daily interactions included meetings with grieving family members and the national and international press. Dignitaries concerned enough to visit the site included the state's Governor and both U.S. Senators. Superimposed was a criminal investigation, involving some 700 charges and three states. The balance was struck to inform families of progress first and to have one public face associated with the media. The importance of a "family-first" philosophy facilitated a rapid and successful resolution.

**Initial response/scene:** The challenging nature of the sheer volume (literally hundreds of bodies, including many co-mingled and partial remains) was compounded by the ill-defined crime scene area and the lack of any kind of tentative manifest to facilitate with recovering and processing of the remains. The key to a successful outcome in such a massive undertaking is effective assessment and planning.

**Body recovery:** The archaeology of body recovery from mass graves and pits, combined with the need for preservation of evidence for potential criminal prosecution, is a fortunately rare occurrence. The early recognition of the nature and scope of the case allowed strategic placement of sufficient resources in a timely manner. The latter, combined with a strong team

concept, kept morale high and expedited the initial processing of remains.

**Identification:** The unique nature of such an aggregate of an unknown number of unidentified partial and complete remains raging from minimally decomposed to skeletonized was compounded by the lack of knowledge regarding numbers of bodies present. Identification concerns were paramount to the families, and as such, rapid protocols for body identification were required. A balance had to be struck between speed and science, with accuracy unquestioned. The identification techniques employed and success/concerns is summarized. The identification process included collection of thousands of DNA samples from family members and lasted almost two years, although an internet inventory of remains with possible identifiers still results in occasional requests for possible identification.

**Investigation/Prosecution:** The ultimate cost of this man-made disaster to the State of Georgia was approximately 10 million dollars and generated over 100,000 pages of documentation. The perpetrator was charged with 787 counts, including theft by deception, abuse of a corpse, fraud, and giving false statements. He allegedly faced the potential of over 8,000 years in prison if convicted. He eventually pled guilty and was sentenced to twelve years, with credit for time served. Approximately 1,700 family members whose loved ones' bodies had been abandoned joined a class action suit. Settlements reached totaled well over \$100 million but were later significantly reduced to approximately \$54 million

In the final analysis, this case was about hundreds of families whose loved ones' bodies were left to decompose in the foothills of North Georgia. The overall investigation and close teamwork between the law enforcement, medical examiner, anthropologist, and district attorney resulted in a guilty plea in one of the most bizarre criminal cases of all time and a rapid resolution to the ubiquitous civil litigations.

**Motive:** "To those of you who may have come here today looking for answers, I cannot give you." said by Ray Brent Marsh upon entering his guilty plea. His attorneys would later allege that the admitted perpetrator of the crime resulting in largest investigation in the history of the state was a victim of mercury poisoning, allegedly from exposure caused by the dental amalgams from cremated bodies.

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**Crematory, Media, Identification**

## W16 Applications of 2D and 3D Geometric Morphometrics in Forensic Comparisons

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The goal of this workshop is to familiarize attendees with the possibilities for morphometric comparison in both 2D and 3D formats as well as limitations and possibilities for use in forensic science are discussed.

This presentation will impact the forensic science community by showing methods that are used for pattern recognition in forensic comparison with error rates.

Last spring, the Forensic Sciences Foundation's (FSF) Theoretical Forensic Science Committee sponsored a contest. Mary Bush, DDS from the State University of New York at Buffalo was the winner of this "New Science or Technology to Forensic Science" competition.

Based on the contest, this workshop has been developed to discuss and present different aspects of morphometric comparison in forensic science, with application to odontology, footwear impressions, bullets, fingerprints, facial comparison, wound analysis, and 3D reconstruction.

This workshop will: (1) provide an overview of geometric morphometric (GM) analysis; and, (2) demonstrate the methods for evaluation, measurement, and statistical comparison of shape change/distortion of an object in question. The generation of matching criteria and error rates based on a statistical model are discussed in relation to the work of Dr. Bush. Application of GM methods to bitemarks, fingerprints, shoeprints to 3D visualization of 3D bullet comparison, medical forensics, and facial comparison will be presented. Advantages and disadvantages of the different approaches on comparison of shapes based on pattern recognition in digital images will be discussed.

A well-developed method to describe shape variation between biological specimens is geometric morphometric analysis. GM analysis involves placement of landmark points, curves, or outlines on either two- or three-dimensional images. The landmark data can be extracted and analyzed statistically as a unit. GM methods allow for a quantitative examination of shape by capturing the geometry of morphological structures of interest and preserving this information through statistical analysis. Shape information can be visualized by plotting landmark positions in Procrustes superimposition, a method of optimally matching one shape to another. This can be performed with or without scale. Procrustes distances can be used to summarize variations in populations, to express the degree of similarity of individual specimens, means of populations, or to search for matches between specimens.

Amongst the tools available for statistical analysis is principal component analysis (PCA) with which the principal variations of shape can be plotted and visualized. This allows for determination of which shape aspect is responsible for the most variation. Canonical variates analysis (CVA) can also be used to determine if shape information can distinguish between different categories of data. A range of other standard multivariate statistical methods can also be applied to shape data, allowing applications to a wide range of research questions and practical problems.

There has been much recent discussion in forensic forums concerning what constitutes a match, or what defines two objects as being indistinguishable. It might be stated that two objects are identical when differences in measured attributes fall below measurement error levels, when the differences seen are no longer distinguishable from random effects. Error rates can be established by repeated measures studies, in which the same object is measured multiple times. The Procrustes distance derived from GM analysis can be used as a quantitative descriptor of error, and can be used as a threshold value below which objects might be said to match.

Clearly measurement resolution also depends on the scale of the object being measured, and the resolution (smallest object measurable) of the means used to image the object. For example, in crime scene or accident reconstruction, the resolution required might be fractions of a meter; in a shoeprint, individualizing detail might be separated by cm, in a bitemark the achievable measurement resolution might be on a mm scale, fingerprints on a micron scale, and tool marks potentially submicron.

With these examples in mind, careful attention to the issues of magnification (scale) and resolution is of the first importance. However, GM methods can be applied regardless of scale, as long as the nature of the data is understood. At each level of scale, error rates can be established and quantified.

Using GM methods, large datasets can be statistically compared to explore the issues stated above. Ideally, quantification of the range and types of distortion produced will provide forensic practitioners with

quantifiable validations of the quality of example items in the pattern evidence disciplines.

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## Morphometric, Pattern Recognition, Comparison

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### W17 Advances in Asphyxia by Strangulation for Pathologists and Anthropologists

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After attending this presentation, attendees will have a better understanding and knowledge of the new standardized INFOR classification of asphyxia, best practices of autopsy dissections in strangulation cases, radiology aspect of these cases, anthropology best approach, and updates on the pathophysiology.

This presentation will impact the forensic science community by informing the forensic pathologists and anthropologists of the updates on strangulation and the best practices in these cases.

The classification of asphyxia and the definitions of subtypes are far from being uniform, varying widely from one textbook to another and from one paper to the next. Unfortunately, similar research designs can lead to totally different results depending on the definitions used. Closely comparable cases are called differently by equally competent forensic pathologists/medico-legal doctors. In response to this situation, a unified system of classification was recently proposed. This new standardized classification of asphyxia, called the INFOR classification, will be presented. In the INFOR classification, asphyxia in the forensic context is divided in suffocation (subdivided in choking, smothering, and confined spaces/entrapment/vitiated atmosphere), strangulation (subdivided in hanging, ligature strangulation, and manual strangulation), positional and traumatic asphyxia, and drowning. The controversies around the term mechanical asphyxia will be discussed. Asphyxial games will be presented in relation to this new classification.

The external and internal examination findings in strangulation will be discussed. A proper neck dissection is a key element in the investigation of strangulation deaths. Despite the usefulness of x-rays and computed tomography as ancillary techniques, manual dissection of the neck structures remains the most widely used technique to assess the integrity of neck structures. There are two ways to dissect the neck: *in situ* or *ex-situ*. The *ex-situ* method follows the removal of the neck structures by cutting the muscles insertions of the base of the mouth and gently removing this group of structures from the vertebral column. This *ex-situ* method is superior to the *in-situ* method and the reasons for this will be explained. The method will be described step-by-step, with images of external and internal injuries. The common pitfalls in the interpretation of autopsy findings will be discussed: anatomical variations (e.g., triticeal cartilage), normal mobility or bending of a cartilage or joint (with considerations on the rate of ossification of the hyoid and thyroid horns and the sinostosis of the horn/body joint of the hyoid), and postmortem changes.

The incidence of bone fractures in suicidal versus homicidal hangings and manual strangulation will be presented, along with the explanations for these injuries. The accuracy of anthropologist/pathologist interpretations of evidence obtained during autopsy and analysis of bones will be discussed.

The modern understanding of the pathophysiology of hanging and strangulation was significantly changed by the creation of the Working Group on Human Asphyxia in 2006. The results from this ongoing study will be presented. The study of filmed hangings and strangulation has clearly established the agonal responses in these deaths: rapid loss of consciousness (10 s ± 3 s), mild generalized convulsions (14 s ± 3 s),

decrebrate rigidity (19 s ± 5 s), beginning of deep rhythmic abdominal respiratory movements (19 s ± 5 s), decorticate rigidity (38 s ± 15 s), loss of muscle tone (1 min 17 s ± 25 s), end of deep abdominal respiratory movements (1 min 51 s ± 30 s), and last muscle movement (4 min 12 s ± 2 min 29 s). The time to irreversible damages and death in hanging and strangulation will be discussed, based on the study of non-lethal filmed events. The implication of the advances from the Working Group on Human Asphyxia on the understanding of asphyxial games will be discussed.

#### **Asphyxia, Strangulation, Forensic**

### **W18 Deadly by Design: Forensic Toxicology, Adverse Effects of Synthetic Cannabinoids, and Novel Designer Drugs (“Bath Salts”)**

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The goals of this presentation are to: (1) make the forensic science community aware of the prevalence and toxicity of the products; (2) educate forensic professionals about the challenges involved in their analysis including sample preparation and analysis; (3) familiarize forensic pathologists and toxicologists with the adverse effects of the chemicals; (4) provide reference case details of circumstances surrounding impairment and criminal acts committed under the influence of the drugs; and, (5) present evidence that the drugs can cause death.

This presentation will impact the forensic science community by increasing awareness of the latest categories of abused drugs which will require changes in current forensic practice and by providing evidence to support the scheduling and control of these dangerous new chemicals, many of which are currently available without restriction.

This program will present information regarding the various classes of synthetic drugs which have recently become popular within the recreational drug using community. Exploration of the highs associated with these drugs has become known as the “research chemicals movement.” Compounds used by these groups include synthetic cannabinoid agonists, cathinone derivatives, pyrovalerone derivatives, and many others. Use of these chemicals has significant adverse effects and forensic consequences.

The program builds on basic analytical information presented at the AAFS Annual Meeting in 2011, and will cover the latest developments in the availability, analysis, and forensic toxicology of these new illicit drugs. The scope will include information about the chemicals in circulation, how they are detected and measured, adverse event reports presented to Poison Control Centers, and the involvement of the drugs in impaired driving, suicide, assault, and homicide cases.

Specific presentations will cover the current scope of drugs present in these materials, their legal status, and how the analog and homolog aspects of the federal law should be interpreted. The remainder of the workshop will include a review of current analytical approaches to the identification of the chemicals in seized materials, blood, urine, and tissue, and will include pharmacokinetic data in blood, urine, and oral fluid from controlled administration of a range of the synthetic cannabinoid drugs. The focus of the remainder of the session will be on adverse effects associated with the various compound classes from various medical and forensic perspectives.

This will include data from Poison Control Centers derived from calls from the public and medical professionals that have documented hypertension, agitation, paranoia, and seizures following use of synthetic cannabinoid and other legal high drugs. The program also includes case reports of suicides, assaults, and homicides following use of the unscheduled drugs mephedrone and MDPV sold as “Bath Salts” but intended for recreational use. Finally, a series of deaths in which either the synthetic cannabinoid or stimulant type drugs appear to have played a role will be discussed along with the difficulties in testing for use of the compounds and assessing their role in the causation of death.

At the conclusion of the workshop, attendees will be able to describe the various categories of drugs in the synthetic cannabinoid and designer drug categories; select or order appropriate analytical tests to ensure their detection; apply aids to the interpretation of designer drug findings in forensic casework; and recognize adverse effects of the drugs through evaluation of signs and symptoms of use.

#### **Designer Drugs, Synthetic Cannabinoids, Forensic Toxicology**

### **W19 The Anatomy of Error: Dissecting Adverse Events to Strengthen the Forensic Sciences**

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After attending this presentation, attendees will: (1) learn the fundamentals of human error analysis; (2) define error, active error, and latent error; (3) discuss the origins of error in human and system perspectives; (4) understand human factors impacting performance; (5) describe and apply the different models of failure; and, (6) identify and initiate steps to resolve potential “error traps” in the work environment.

This presentation will impact the forensic science community by providing an overview of the field of human error, illuminate how human factors such as fatigue and bias can negatively impact performance, and educate the attendees on the application of human factor codes to items analyzed in a high profile murder case.

Reviewing the actions and accomplishments of other professions that have successfully dealt with human errors will assist attendees in understanding the fundamentals of error analysis. The medical and aviation professions have conducted extensive research on the subconscious to identify how human performance is helped or hindered by the design of equipment, work environment, and peer pressure. Making corrections or changes according to these revelations have improved performance and mitigated errors within these professions.

Fatigue has long been associated with decreases in human performance. While this is a problem that challenges most industries, those that are human-centric are at an even greater threat. The forensic sciences remain a field that heavily relies on the performance of its analysts and requires 100% accuracy. Unfortunately, crime scene technicians and analysts are just as susceptible to fatigue as other professionals. Operator fatigue has been shown to effect performance after just 18 hours of sustained wakefulness. While reductions in alertness and performance are a problem for workers in general, those who perform tasks, which demand high levels of cognition, are even more susceptible to the effects of fatigue. Fortunately, there are a number of management techniques, countermeasures, and interventions that will be discussed and defined to help mitigate these negative effects.

One aspect of the management technique is the education of staff on the difference between active errors, those events when individuals are in direct contact with physical evidence and latent errors, when the underlying support system comes into question. These topics have been part of the two year effort assigned to the Expert Working Group on Human Factors in Latent Print Analysis, an effort that has recently completed its task. Evaluating approaches to reducing errors in terms of their efficacy, appropriateness, cost, scientific basis, feasibility, associated risks, and quality of evidence have been documented and will be offered.

The class participation portion of the workshop provides an opportunity for the attendees to take the provided information and apply it to an actual criminal case. Applying lessons learned (when back at the crime scene, in the laboratory, or managing staff), work flow is increased when provided an opportunity to experience direct application. Part of the direct application will be segmenting levels of forensic work, using error coding techniques and assigning mitigation activities. A 1992 murder case, the conviction of Ray Krone and the associated bitemark evidence, will be used for this activity.

Therefore, this workshop has direct application to individuals who collect, handle, analyze, manage, and store forensic evidence. It introduces the concept of applying error mitigation techniques to the forensic science profession and provides established guidance learned in other professional fields. An actual criminal case will be presented allowing each attendee to gain experience in the actual error coding process and understand the underlying basis and resulting influence of errors.

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**Error, Cognitive Bias, Quality Assurance Quality Control**

## **W20 Flawed Forensics: Recognizing and Challenging Misleading Forensic Evidence and Disingenuous Expert Testimony**

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After attending this presentation, the attendees will be better acquainted with the current problems and challenges facing expert witnesses in various disciplines of forensic science. Attendees will learn how expert testimony or evidence is being challenged as unreliable, or misleading and disingenuous, what methods lawyers use to impeach such testimony or evidence, and the legal basis for excluding such evidence.

This presentation will impact the forensic science community by providing forensic scientists with examples of the problems and challenges they can face when testifying in court. All stakeholders in the administration of criminal or civil justice – experts, lawyers, and judges – will benefit from learning about scientific and evidentiary challenges to expert testimony, testimonial practices that experts should avoid, and the means by which lawyers challenge and judges exclude forensic expert testimony. This workshop will assist forensic scientists and lawyers in recognizing when the testimony of a particular expert witness is misleading and disingenuous and how to challenge and effectively impeach or preclude such testimony.

A multidisciplinary faculty will review actual testimony of forensic specialists in different disciplines. Discussion of cases and reported court decisions will illustrate the problems and ongoing challenges facing forensic

scientists testifying in criminal and civil cases. Critical knowledge about when and under what circumstances, testimony or evidence introduced by forensic scientists will be challenged as being misleading or disingenuous, and how such challenges take place in the adversarial process of our judicial system will be discussed.

The uses and limits of cross-examination to reveal weaknesses in the testimony of forensic scientists will be explored, and examples involving misleading and disingenuous testimony by toxicologists, pathologists, and other forensic scientists will be examined and discussed from a scientific and legal standpoint, and from the perspective of the forensic scientist, the trial lawyer, and the judge. Actual cases in which deficiencies in report writing and incomplete or misleading testimony by forensic practitioners contributed to wrongful convictions will be reviewed and analyzed, and recommended solutions designed to correct this problem will be discussed.

Workshop attendees will learn about the true meaning of the different types of casework “peer review” occurring within the forensic community, the methods lawyers can use to impeach disingenuous testimony concerning such peer reviews, and the legal basis for excluding peer review testimony by the actual reviewer.

High profile criminal defense attorneys will discuss cases involving flawed and fabricated scientific evidence. The lead defense attorney in the recent, highly-publicized capital murder prosecution of Casey Anthony, which resulted in not guilty verdicts on all charges involving the death of the defendant’s 2-year-old daughter, will discuss the challenges he faced before and during the lengthy jury trial, as well as the methods used to impeach the forensic evidence presented by the prosecution.

Attendees will become acquainted with the principles and pitfalls involving courtroom testimony in the wake of the National Academy of Sciences (NAS) Report and recent court decisions defining the parameters of forensic testimony. Specific examples of courtroom testimony will be utilized to illustrate problems in fingerprint comparison, tool mark identification, and bitemark analysis. Various aspects of testimonial evidence will be specifically targeted, such as the use of terminology in rendering a conclusion. The validity of subjective analysis and the intermingling of scientific principles will be addressed in the context of probability assertions. How the forensic practitioner handles the dual problems of explaining key forensic discipline definitions and producing appropriate back-up materials in buttressing validity will also be examined.

Finally, attendees will learn about the misleading use journal articles, treatises, and studies or experiments to substantiate or attack the basis for an expert opinion in the pre-trial and trial phases of a court proceeding, and the manner in which judges scrutinize and evaluate such evidence. Attendees will also learn about the ethical implications for a trial judge faced with disingenuous expert testimony and the nature of the ensuing judicial response.

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**Flawed Forensics, Misleading Evidence, Disingenuous Testimony**

## **W21 Innovation in Forensic Image and Video Analysis**

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After attending this presentation, attendees will understand how to validate forensic methods in image restoration and limitations and possibilities of forensic multimedia investigation.

This presentation will impact the forensic science community by providing an overview of the new developments and needs in forensic research.

During this workshop information will be provided on new developments of forensic investigation of (digital) images and video streams and the use of 3D computer modeling in forensic investigations. The workshop will be interactive and many examples of case material will be shown.

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), quality assurance, and validation aspects. During the last decades, the use of CCTV-camera systems and digital cameras on phones has become widespread. Typical questions concern the quality and the selection of images from a specific camera in a multi-camera-recording, and combining the information. 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. In addition, questions about image interpretation like facial recognition, body height, car speed (often in low resolution), time lapse, or compressed images have increased. A special discussion will be given on image analysis.

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? Gait analysis, 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 measurement in images are presented and discussed. Special attention will be given to measurement uncertainties of the results and the impact on the conclusions from these investigations.

Common sources of video streams and images are video recordings from handy cams, digital photo cameras, the 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 state-of-the-art image enhancement techniques such as contrast, stretching, and deblurring, as well as super resolution, stabilizing, and automatic tracking methods. It will also cover the issue of erased video files, and how to recover these when they are partly erased.

The state-of-the-art methods for camera comparison will also be presented, examples of comparing Photo Response Non Uniformity with software. With this method a camera can be linked to a camera. Also the cautions of identification and limitations of the technique are discussed, and solved with likelihood ratios.

The methods are discussed also in relation to forensic medical image analysis and possibilities within pathology, with many practical examples. Computing speed for some of the image analysis methods (especially for high volume of data), and the possibilities of fast parallel computing with GPU's and CUDA for forensic video applications are discussed.

Finally, an overview on the use of 3D computer modeling in forensic investigations will be provided because 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, the methods for visualizing uncertainties, and possibly erroneous suggestions coming from these visualizations. 3D video fusion for use in casework is demonstrated.

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**Multimedia, Video, Medical**



## A1 Footprint Ridge Density — A New Attribute for Sexual Dimorphism

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After attending this presentation, attendees will be able to recognize that the ridge density in footprints exhibit sexual dimorphism in Indian population.

This presentation will impact the forensic science community by recognizing the sexual dimorphism of the ridge density in footprints that may be used in identification of dismembered human remains in cases where an individual foot is recovered and brought for examination. It can give vital evidence in identification of the perpetrator of the crime in cases where the footprints are left behind at a crime scene.

Determination of sex has a vital role to play in forensic examinations. Footprints are often encountered at crime scenes especially belonging to murder and sexual assailants. From the available footprints at the crime scene, if the sex of the suspect is inferred, the burden of the investigating officer is reduced by half as the search of potential suspects will be restricted to a particular sex. Although researchers have attempted sex determination from fingerprint ridge density, the sex differences from the ridge density in footprints remain unreported. To the best of the authors' knowledge no systematic studies are available on the sex differences from ridge density in footprints worldwide. This preliminary study is done to evaluate the sex differences from ridge density in footprints and study its usefulness in discriminating sex in Indian population using statistical considerations.

The present prospective research was conducted on 106 young adults (56 males and 50 females) at the Department of Forensic Medicine, Kasturba Medical College, Mangalore, India. Healthy individuals aged 20 to 25 years were included in this study after taking informed consent. The subjects with any disease, deformity, injury, fracture, amputation, or history of any surgical procedures of the feet were excluded from the study. Each subject included in the study was asked to wash his/her foot clean with soap and water. A clean plain glass plate was uniformly smeared with black duplicating ink with the help of a roller. The subjects were asked to apply their feet on the smeared plate and then transfer them on to a white paper. Regular pressure was applied on the foot area to obtain the footprints. A 5mm x 5mm square was drawn on a transparent film and placed on the obtained footprint samples in the areas to be analyzed. In order to measure ridge density, the count was carried out diagonally on a square measuring 5mm x 5mm. The epidermal ridges were counted with the help of a hand lens. This count represents the number of ridges in 25mm square area and reflects the ridge density value. The ridge density value was similarly obtained individually from the four designated areas on the footprints that are commonly encountered at the crime scene. The four designated areas included: upper portion of the inner border of the great toe, the ball of the great toe, the ball of the 5<sup>th</sup> toe below the triradii point, and the central prominent part of the heel. The male female differences in ridge density were statistically analyzed individually for each of the designated areas from the footprints. Ridge density was compared between right and left sides for each of the designated areas. Statistical significance was defined at the standard 0.05 level.

Mean ridge density was significantly higher in females than males in the designated areas in both feet. No right-left differences were observed

among males and females. Statistically significant differences exist in the footprint ridge density between different areas in males and females in right and left feet. The likelihood ratio (LR) was calculated to obtain the probability inferences of sex, based on ridge density values. Posterior probabilities was calculated using Bayes' theorem and information obtained from both LR computations and posterior probabilities were used to show favored odds. The present research reveals that footprints in females demonstrate a greater ridge density, hence, finer ridge details than males in Indian population. Thus, the mean ridge densities can be used as a presumptive indicator of sex of an unknown footprint left at a crime scene.

**Forensic Science, Sexual Dimorphism, Foot Print Ridge Density**

## A2 Efficiency of Human DNA Isolation From Food Bitemarks

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After attending this presentation, attendees will consider the possibility of isolating human DNA from bitten foods.

This presentation will impact the forensic science community with a better understanding of using bitten foods found at crime scene in a criminalistic approach due to the possibility of human DNA isolation and comparison.

Typically, bitemark analysis is used for physical comparison between bitemarks present on physical evidence (human skin or bitten object) and the reference sample (such as suspect's teeth). However, if it is not possible to correctly identify the suspect using this method, it is important to recover salivary DNA. This evidence has been recovered and analyzed from bitten inorganic substrates like cigarette butts and human skin.

On the other hand, there are few studies in which it was possible to isolate human DNA from bitten foods. These attempts have not been successful due to food characteristics. Thus, finding and isolating human DNA from food bitemarks is extremely challenging.

The goal of this research was to analyze the efficiency of human DNA isolation from bitemarks in three different types of foods. The isolated DNA was characterized using PCR for two human housekeeper genes to ensure recovering and isolation of human DNA.

Three volunteers bit into three different foods: cheese, donuts, and apples. The food was kept at room temperature. After 15 hours, the food was placed in a plastic bag and frozen at -15°C. The day after, the food was submitted to the lab and saliva from each sample was collected using the double swab technique. The DNA was isolated using QIAamp DNA Mini Kit according to the manufacturer's protocol. The DNA was quantified using NanoDrop 2000c. PCR was performed to look for two human housekeeper genes, GAPDH (Glyceraldehyde 3-phosphate dehydrogenase, enzyme implicated in glycolysis) and RPL22 (human gene codifies 60S ribosomal protein L22). As positive controls, epithelial cells from two of the volunteers were used.

The results showed the differences in the quantity and quality of DNA isolation between foods. Although the highest DNA concentration was found in the apple and cheese samples, the ratio 260/280, which is commonly used to assess the purity of nucleic acids since proteins absorb light at 280nm, was lowest in apple samples and highest in cheese samples. The DNA concentration from donut samples was the lowest and the ratio 260/280 had an intermediate value. Positive controls showed a high DNA concentration and ratio 260/280.

PCR results were showed in 2.5% agarose gel. In the two positive controls, DNA from cheese samples and donut samples, 1µl DNA was

enough to amplify the housekeeper genes. However, DNA from cheese samples demonstrated highest amplification efficiency compare with DNA from donut samples. DNA from apple samples failed to amplify even when using a 5µl sample. These differences were related to the 260/280 ratio because apple samples had the lowest ratio which indicated that the samples were contaminated with proteins. This issue affected PCR efficiency, compromising the appropriate samples amplification.

The findings from this research provide the evidence that it is possible to recover and isolate human DNA from food bitemarks, although the quality of DNA depends on the type of food. These results show the potential importance of bitten food recovered from crime scenes.

**Food Bitemarks, DNA Isolation, PCR Efficiency**

### **A3 Genetic Analyses on Bone Remains: The University of Rome “Sapienza” Laboratory of Forensic Genetics’ Experience**

*Silvia Zoppis, MD\*, Manuela Rosini, MSc, and Carla Vecchiotti, PhD, Department of Legal Medicine, Viale Regina Elena 336, 00161, Rome, ITALY*

After attending this presentation, attendees will understand some principles of genetic analysis on bone remains. The challenges related to this kind of investigation, especially for what concerns criminal cases and the importance of the honesty of the forensic scientist when a certain and unequivocal interpretation of the DNA profile obtained cannot be provided.

This presentation will impact the forensic science community by highlighting the importance of bone remains as an evidentiary sample in forensic caseworks and the difficulties related to the genetic analysis of such samples due to degradation and/or inhibition factors. In these cases it is fundamental for the scientist to consider asserting that a complete and interpretable genetic profile not obtained from a sample (thus the sample cannot be considered useful for a comparison) does not mean a failure but, on the contrary, reveals scientific honesty and should stimulate the necessary progress in this field.

Genetic analyses on bone remains in the field of human identification represent one of the most stimulating and complex challenges for forensic geneticists. Unlike the analysis of biological materials such as blood, semen, saliva or urine that usually do not present any particular technical and operational difficulty so that personal identification can be achieved, the identification of bone remains forces the analysts to face multiple and complex variable factors (e.g., the degradation of genetic material and the environmental contamination of the samples) that can affect the success of obtaining a complete and interpretable STR profile. In such cases an accurate evaluation of the characteristics of the sample and the environmental conditions to which this finding has been exposed is extremely important.

This presentation describes the methods used in the Laboratory of Forensic Genetics of the Department S.A.I.M.L.A.L. of the University of Rome “Sapienza” for the analysis of bone remains for the purpose of either personal identification or the assessment of a parental relationship. A selection of twenty cases during the years 2007-2011 where genetic analyses were performed on different bone samples (femur, tibia, humerus, mandible) using different DNA extraction, amplification, and STR typing methods will be presented. The results obtained from each case will be compared in order to assess the specific role of three important variable factors: the age of the remains, the environmental conditions of storage/finding, and the cause of death.

Six out of the twenty cases showed interpretation problems related to the DNA degradation caused by environmental factors (for instance, the effects of high temperatures in case of charred remains and the acceleration of autolytic processes in case of hexumation of a corpse) and/or the presence of PCR inhibitors (e.g., calcium phosphate, humic acid) that likely were co-extracted with the DNA from the evidence sample. The results show that the possibility of obtaining a complete and interpretable genetic

profile depends largely on the three variable factors mentioned above, particularly with regard to the environmental conditions of storage/finding of the remains, thus confirming the need to optimize the analytical methods to minimize the effects of environmental inhibitors.

**Genetic Analyses, Personal Identification, Bone Remains**

### **A4 Standardization of Spermatozoa Identification in Sexual Assault Cases Using a Fluorescence-Based Assay**

*Anick De Moors, MSc\*, Royal Canadian Mounted Police, Forensic Science and Identification Services, 1200 Vanier Parkway, Ottawa, ON K1G 3M8, CANADA*

After attending this presentation, attendees will have an understanding of how a fluorescence-based staining assay was optimized and validated at the Royal Canadian Mounted Police (RCMP) using a large number of sexual assault type specimens.

This presentation will impact the forensic science community by enhancing the search and standardizing the identification of spermatozoa in sexual assault exhibits.

The detection of spermatozoa on swabs and suspected seminal stains on fabric provide strong confirmatory evidence in sexual offence cases. Currently, the identification of spermatozoa by the RCMP Evidence Recovery Unit Search Technologists is carried out using phase contrast microscopy. In case of specimens with little spermatozoa, the search may become labor-intensive and time-consuming.

The high specificity of the fluorescence-based staining assay selected in this study lies on the unique mouse monoclonal antibody specific to human sperm heads incorporated in the staining protocol. The validation studies performed by initial users of the fluorescence-based staining assay provided strong positive feedback which prompted the RCMP’s evaluation of this kit.

The optimization of the fluorescence-based staining assay involved testing different glass slides and coverslips, finding the optimal amount of 1M DTT pH 8.0 to use on vaginal and fecal slides for optimal staining, finding ways to preserve the fluorescence signals on slides, assessing the possibility of re-staining slides, finding optimal sample slide preparation (comparing a swab extract versus rolling a swab versus using a swab clipping), and performing some spermatozoa integrity tests.

The validation studies performed to evaluate the limitations of the fluorescence-based staining assay included: sensitivity, reproducibility, specificity and robustness using non-human semen, other human body fluids and contaminating yeasts, fibers and dyes, spermicides, sexual lubricants, condoms, medicated creams, and 24-year old semen stains. The practicality, sensitivity, and speed of the fluorescence-based protocol was compared to the current phase contrast protocol and examined in the context of an improved workflow in processing sexual assault cases.

Experiments carried out indicate that the fluorescence-based staining assay is: (1) highly specific and valid (no interference from semen obtained from various animals and absence of positive fluorescence signals in all control slides prepared from swabs with only blood, yeast, urine, vaginal epithelial cells, or fecal material); (2) sensitive and reliable (spermatozoa detected in vaginal swabs soaked in 1:1,000 and 1:10,000 semen dilutions; spermatozoa detected more effectively in real casework samples containing few spermatozoa compared to phase contrast microscopy); (3) fast (one minute versus an average of 10 minutes or more using phase contrast microscopy for <10 spermatozoa); (4) robust (spermatozoa detected from 24-year-old suspected semen stains, no interference from spermicides, lubricants, fluorescent and non-fluorescent condoms and antifungal creams); and, (5) simple to use for the detection of spermatozoa in a variety of sexual assault samples.

The swab clippings used to prepare the slides for spermatozoa identification produced very informative DNA profiles. The fluorescence-

based staining assay results were merged with duo female: male DNA quantification ratios and PCR amplification kits profiling outcomes. The following general correlations were found between the fluorescence-based staining assay results and STR male profile outcome: (1) vaginal swabs with 1:25 human semen dilution gave an average of 136 spermatozoa count from swab clippings (N=12), an average female:male DNA ratio of 0.24 and a major male profile using PCR amplification kit for which a match probability could be calculated; (2) vaginal swabs with 1:100 human semen dilution gave an average of 45 spermatozoa count, an average female:male DNA ratio of 1.3, a complete Yfiler profile and mixed profiles using PCR amplification kit with minor male components for which an inclusion probability estimate was calculated at 8-9 loci or a major male profile for which a match probability could be calculated; (3) vaginal swabs with 1:1000 human semen dilution gave an average of 4 spermatozoa count, an average female:male DNA ratio of 15.6, a complete Yfiler profile and mixed profiles with minor male components for which an inclusion probability estimate was calculated at 4, 5, or 8 loci. The optimized fluorescence-based staining assay combined with the use of 6mm circle slides could expedite and standardize the search for spermatozoa in specimens containing a limited number of spermatozoa.

**Spermatozoa, Sexual Assault Cases, Fluorescence**

## **A5 Katie's Law: A Threefold Increase in Database Samples Impacts a Team of Five**

*Megan Honderd, BS\*, and Carrie Thor, BS\*, Colorado Bureau of Investigation, 690 Kipling Street, Suite 4000, Denver, CO 80215*

After attending this presentation, attendees will understand the effects of implementing an All Arrestee law/bill based on the state of Colorado's legislative rules and guidelines.

This presentation will impact the forensic science community by giving other labs an insight into the Colorado Bureau of Investigation's (CBI) Database procedures and how the increased numbers of samples are processed.

Colorado is one of several states to implement an All Arrestee law/bill. The collection and use of DNA by Law Enforcement agencies has been a useful tool in solving and preventing crime. This bill was implemented to help prevent a significant number of violent crimes and to help assist in developing leads that may solve cold cases in the State of Colorado.

As a result of the new law, buccal samples are collected along with fingerprints at the time of booking for an adult arrested for a felony in the state of Colorado. Samples are submitted to the Colorado Bureau of Investigation, entered into the laboratory information management system, and held until the individual is charged with a felony. Once the individual has been charged, the sample is processed; a profile is developed and uploaded into CODIS.

The CBI Database has experienced no negative impact to the backlog status despite a threefold increase in the number of sample submissions. This has been accomplished with minimal changes to the Database Unit. No changes were made to the current instrumentation which consists of two semiautomated punches, a laboratory information management system, a robot for extraction, six thermal cyclers, and two genetic analyzers. The staff increased by four when the bill went into place which included the addition of one DNA analyst to help process the samples, one database technician to enter the samples, and two crime data specialists to research the samples for charges and process expungement requests. Before the bill went into effect, the Database Unit consisted of two DNA analysts, one CODIS administrator/analyst, and two technicians. This presentation will outline the system employed by the CBI Database laboratory in more detail.

Some of the challenges that the Database Unit had to overcome were interpretation of the bill, where to store the increased number of samples, how CBI would be notified when the charges are filed and a docket number is assigned, integration of data using the Colorado Crime Information Center (CCIC), and handling of expungement procedures.

Analysis efficiency will be further improved with the implementation of an expert system and a twenty-four capillary genetic analyzer in the near future. Overall efficiency in the future may be increased by working with agencies to update criminal history information and automate the charge information.

**Database or Databank, All Arrestee, Katie's Law**

## **A6 Human Body Fluid Identification by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry**

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After attending this presentation, attendees will have a better understanding of how Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF MS) was used for messenger ribonucleic acid (mRNA) profiling of blood, saliva, and semen.

This presentation will impact the forensic science community by introducing a new multiplex approach of mRNA profiling of body fluids of forensic interest.

It is now well established that different tissues have different genetic expression patterns. The discovery of mRNA biomarkers that are differentially expressed in three body fluids commonly found at crime scenes (blood, saliva, and semen) has contributed to the prospect of using mRNA as a tool for forensic investigation. The validation of these tissue-specific mRNA biomarkers by several research groups has paved the way for mRNA profiling as a potential supplemental method for human body fluid identification.

This presentation will introduce a new multiplex approach of mRNA profiling of body fluids of forensic interest. The most comprehensive methods for mRNA profiling tested to-date all share a common start which is total RNA extraction and complementary DNA (cDNA) synthesis. Diverging from this initial step are two frequently described and standard methods of mRNA profiling for forensic-like stains. The first one is qualitative in nature and combines end-point PCR and capillary electrophoresis (CE); whereas the second includes quantitative PCR chemistries. However, the drawbacks of these existing mRNA profiling methods are (a) the limited multiplex capabilities per reaction mainly due to a limited availability of fluorescent dyes/tags for both CE and qPCR, and (b) the use of internal sizing standards (CE). Sequenom's iPLEX® biochemistry on MALDI-TOF MS, commonly used for single nucleotide polymorphisms (SNPs) genotyping, was adapted for qualitative mRNA profiling. Briefly, an initial end-point PCR phase followed by a primer extension step was used with cDNA. A positive extension reaction was indicative of the presence of cDNA in the sample. This approach on MALDI-TOF MS has the ability to simultaneously determine the molecular weight of DNA fragments without the use of size standards, and thus provides multiple data points per experiment to enable analysis of high-level multiplex PCR reactions.

In this study, total RNA was extracted from mock forensic stains: blood (N=15), saliva (N = 13) and semen (N = 17), and converted into cDNAs. Multiplex primers (19 plexes composed of five blood, six saliva, four semen, and four housekeeping-specific primer sets) and corresponding extension primers/probes, were designed from the sequences of 12 mRNA markers. In general, the body-fluid specific primer sets correctly identified

the targeted body fluid. The range of specificity of the primer sets was 93-100% for blood and semen samples and 88-100% for saliva samples. A multidimensional scaling (MDS) plot was constructed in which the gene expression data (obtained as genotypes and converted into numeric values) were projected onto two viewable dimensions representing linear combinations of markers that provide variation in the data set. MDS analysis of the samples based on expression of all 19 PCR assays demonstrated that the gene expression profiles of the three human body fluids clustered uniquely and were distinct from each other. The results of this study suggest that MALDI-TOF MS has potential for use as a method for the identification human body fluids of forensic interest.

**mRNA Profiling, MALDI-TOF MS, Sequenom's iPLEX® Biochemistry**

## A7 Microwave Selective Heating in the Thermal Development of Latent Fingerprints on Porous Surfaces

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After attending this presentation, attendees will be informed about the potentialities of microwave heating technique as an innovative methodology in thermal development of latent fingerprints on porous surfaces.

This presentation will impact the forensic science community by providing application of microwave dielectric heating to the selective heat treatment of fingerprint residues on papers and other porous surfaces. Comparison with conventionally thermal developed fingerprints will be shown, highlighting the effects of peculiar characteristics of microwave dielectric heating on the visualization of fingerprints and on the obtained contrast with the surrounding porous surface.

Comparison with conventional thermal developed fingerprints will be shown, highlighting the effects of peculiar characteristics of microwave dielectric heating on the visualization of fingerprints and on the obtained contrast with the surrounding porous surface. There are several techniques which are currently used for the development of latent fingerprints on porous surfaces: optical methods and, more commonly, chemical treatment such as visualization with ninhydrin. However, there is always the need to develop new fingerprints detection techniques in order to increase the sensitivity, be readily deployed at crime scenes, be introduced in sequences of detection techniques, reduce the overall cost of fingerprint processing, and avoid the use of hazardous chemicals. Concerning all of these aspects, thermal development of latent fingerprint is of great potential since it is a simple, low-cost, and chemicals-free method. This is particularly true *in situations* where development might not otherwise be attempted for reasons of time and cost. It is widely recognized that thermal development of latent fingermarks on paper occurs following different stages. First, fluorescent marks are developed after rapid heating often resulting in the browning of marks which can become visible with longer heating times. Finally, fingermarks lose contrast as paper turns dark brown with further heating.

Several previous studies investigated different kinds of heating devices. Brown *et al.* performed experiments with a hot air gun, a direct contact heating method, a muffle furnace, and a GC oven. The results indicated that introducing the sample into a hot oven was the optimum technique for the thermal development of latent fingerprints on paper.<sup>1</sup> However, several impractical aspects of the method were evident. First, the introduction of the sample in the oven necessarily induced temperature fluctuations within the chamber that greatly influenced the degree of development of the sample. Moreover, the nature of most of the furnaces used dictated that the sample could not be monitored during treatment, making the technique difficult to execute optimally. More recently, Song *et al.* re-investigated the direct contact heating of the sample using less

thermally conductive surfaces.<sup>2</sup> In particular, the devices investigated as a thermal developer of fingerprints on porous surfaces included a commercial hair straightener with ceramic coated surfaces, a non-stick surface sandwich toaster, and heated glass. It was determined that the hair straightener was superior to the other devices due to the high control of temperature, the high portability of the device and the speed with which samples could be treated. These advantages made it more favorable than the heated furnace. Moreover, with the hair straightener the operator could easily monitor and control the progress of the development thus reducing the risk of destroying the evidence due to overheating of the sample. However, the technique is mainly limited since it is not possible to conventionally heat the fingerprint residues and the surrounding surface to a different extent.

The goal of this presentation is to propose microwave dielectric heating as an alternative thermal treatment technique for the development of latent fingerprints on porous surfaces. As it is well known, microwave heating is fundamentally different from conventional heating since heat is generated internally by means of electromagnetic wave-material interaction. Direct coupling of microwaves with matter leads to a rapid, volumetric and selective heating of the material which is impossible to reproduce with conventional heating sources. Microwave or dielectric heating occurs by energy transfer from the electromagnetic field to the material, rather than relying on heat transfer. Dielectric and magnetic properties are the basis of the material response to the applied electromagnetic field. Particularly for dielectric materials (such as paper), the dipolar loss mechanism, due to the re-orientation of dielectric polarization, is the most prominent loss mechanism at the microwave frequencies.<sup>3</sup> For this reason, microwave power absorption and the consequent heating are more effective for those materials with higher water- or, better, dipoles-content. Since the main chemical constituents of the glandular secretions (both eccrine and sebaceous one) constituting the fingermarks are mostly water and minor amounts of inorganic and organic compounds, selective absorption of microwaves can lead to the thermal visualization of latent fingerprints, with the possibility of enhanced contrast with respect to conventional thermal treatments.

A specifically (for paper and wood treatment) designed microwave single mode applicator was experimentally used to develop differently aged latent fingerprints, deposited on recycled copy paper and cardboards, from different donors. Output power level of approximately 500 W was directed from the magnetron generator to the sample, which was positioned in the centre of the WR-340 waveguide resulting in visible fingerprints in less than 60 seconds. Contrast with the underlying porous surface was also obtained with the conventional thermal development techniques.

As a matter of fact, the browning of the overall substrate was greatly reduced since the latent fingerprint residues selectively absorbed microwaves. Moreover, the overall time necessary to the visible browning of marks was decreased due to the high heating rates typical of microwave heating. The use of microwave multi-mode applicators was investigated as well, although it greatly increased the time necessary to visually detect the fingermarks. The results of all the experiments will be fully presented and the advantages, compared to the conventionally thermal developed fingerprints, will be thoroughly explained. Critical considerations concerning comparison with other development methods will be addressed during the presentation as well as the design of a dedicated and portable device.

### References:

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**Latent Fingerprints, Microwave Selective Heating, Thermal Development**

## **A8 Positive Human Identification of a Cold Case: Multidisciplinary Approach of Forensic Experts in Pathology, Anthropology, Odontology, and Genetics**

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After attending this presentation, attendees will have an understanding of issues arising from Italian legislation on exhumation of unidentified bodies and how the collaboration of forensic experts can greatly speed up the identification process.

This presentation will impact the forensic science community by assisting in improving the cooperation of experts in pathology, anthropology, Odontology, and genetics.

In Italy, the Presidential Decree 285/90 stipulates that bodies must be buried enclosed in wooden coffins and ultimately exhumed after a period of 10 years and the remains transferred into zinc boxes. In some regions of Southern Italy it is common for close relatives to be present during this procedure. This presentation concerns a case of an eighty-six year old male whose remains were exhumed without the authorization of the family along with several other exhumations. The cemetery operators placed the skeletonized and disjointed remains in galvanized containers and stacked them with a multitude of similar containers. Since a clear identification system was not in place, relatives were not able to recognize the specific box containing the remains of their kinsman.

The judicial authority launched an investigation, its primary objective being to establish the integrity and completeness of the remains and also the correspondence of the morphological and morphometric skeletal remains. Thereafter, it commissioned DNA profiles which were compared to those of samples of believed living relatives. The approach taken was similar to the one used to identify corpses after a mass disaster (Disaster Victim Identification - DVI). An interview was conducted with family members to collect antemortem detail. This provided vital information, namely that the deceased had an upper and lower mobile dental prosthesis and had surgical implants in the femur following a fracture two months prior to his death.

A preliminary survey of the various zinc containers was carried out in order to narrow down the number of potential possibilities. In only one of these were artifacts found which corresponded with the antemortem data of the subject, namely a mobile dental prosthesis and a titanium intramedullary implant associated with a comminuted fracture of the femoral neck and proximal diaphysis. These findings were combined with other data, such as an intact skull which clearly indicated a male of advanced years. The dental and anthropological assessment allowed the exclusion of the presence of remains from different burials — except for one clavicle — and confirmed that the bones belonged to a single Caucasian male subject whose age and osteobiographic characteristics were totally compatible with the antemortem data collected.

After the generic identification based on morphometric traits, a genetic analysis was carried out by comparing the DNA profile obtained from a femur fragment of the deceased with those obtained from close relatives. The genetic analysis confirmed the relationship.

This case represents an example of the practical application of multidisciplinary teamwork that follows the DVI procedures for the

identification of commingled human remains. The close collaboration between different disciplines such as anthropology, odontology, and genetics confirms that these individual areas of expertise should all be involved for the purpose of human identification following Interpol recommendations. More specifically, osteological and odontological assessment may assist the genetic analysis by narrowing the range of subjects for molecular comparison. This teamwork approach actively reduces both the time and costs of the investigation and raises the evidential value of the data obtained.

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**Forensic Science, Human Identification, Genetic Profile**

## **A9 A Study of Sex Differences in Fingerprint Ridge Density in a North Indian Population**

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After attending this presentation, attendees will understand the usefulness, importance and variability of fingerprint ridge density in distinguishing sex in forensic examinations as this is a recently developed area of research and the literature on this aspect has been scanty.

This presentation will impact the forensic science community by presenting new information on variability of fingerprint ridge density in an Indian population and its use in distinguishing sex of latent fingerprints found at the crime scene.

In the past, fingerprints collected from the crime scene and from the items of evidence of crime have been successfully used to identify suspects, victims, or any other persons who touched the surface in question. The thickness of epidermal ridges varies between individuals; females are supposed to have finer ridges than males and therefore a greater ridge density within a given area. The goal of this research was to test this hypothesis and attempt to infer sex from the fingerprint ridge density in an Indian population. One hundred ninety-four (194) individuals (97 males and 97 females) aged 18 and 25 years were included in the study. The fingerprints were taken from all the fingers. Thus, a total of 1,940 fingerprints were obtained and epidermal ridges were counted in the three defined areas (radial, ulnar, and lower) of each fingerprint. The fingerprint ridge density in the three defined areas and between sexes was compared using one way ANOVA and t-testing, respectively.

The distribution of fingerprint ridge density in radial and ulnar areas is similar with fingerprint ridge density ranging from 12-20 and 13-20 among males and 15-21 and 14-21 among females in the radial and ulnar areas, respectively. A considerably lower fingerprint ridge density is observed in the lower area (9-14 in males and 10-14 in females) than that observed in the radial and ulnar areas. Extent of overlapping was maximum in the lower area; 63.91% males (n=62) and 68.03% females (n=66) had a fingerprint ridge density of either 11 or 12 in the lower area. In the radial area, maximum overlapping was observed for the fingerprint ridge density value of 17, with 20 males and 21 females having a fingerprint ridge density of 17. Extent of overlapping in the male and female values for fingerprint ridge density was minimal in the ulnar area. It is evident that the mean fingerprint ridge density is maximum in the radial area (males=15.84, females=17.94), followed by ulnar area (males=15.51, females=17.11) and minimum in the lower area (males=11.29, females=12.05). Significant sex differences ( $p < 0.001$ ) were observed between the fingerprint ridge density values observed in the three areas analyzed. The sex differences are observed to be maximum in the radial area ( $t = -11.897$ ), followed by ulnar ( $t = -9.776$ ) and lower areas ( $t = -5.332$ ). Significant male-female differences ( $p < 0.001$ ) were observed in each of the three areas analyzed on each fingerprint.

When each finger was analyzed individually for sex differences in the fingerprint ridge density, it was observed that statistically significant sex differences ( $p < 0.001$ ) exist in the fingerprint ridge density of each individual finger in the three designated areas in both hands. It is further observed that fingerprint ridge density varies between different fingers in the three areas analyzed in the study. In the radial and ulnar areas, the fingerprint ridge density was minimum in the thumb and maximum in the ring finger followed by little finger in right and left hands among males and females. In the lower area, the fingerprint ridge density was maximal in the thumb in both hands among males and females whereas, minimum fingerprint ridge density was observed in the middle finger in the right hand and in the little finger in the left hand among males and females.

This study suggests that the fingerprint ridge density can be a relevant and useful parameter in estimating sex of a latent fingerprint of unknown origin from the scene of crime.

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**Personal Identification, Fingerprint Ridge Density, Sex Determination**

## **A10 Forensic Signatures of Laboratory Grown Bacillus Spores Based on Cellular Lipid Composition: Implications for the Analysis and Attribution of Microbial Evidence Collected From a Bio-Crime**

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After attending this presentation, attendees will become familiar with lipid composition of *Bacillus* cells, the effect that laboratory growth recipes have on the relative abundance of lipid biomarkers in cells, and multivariate signatures that may help provide investigative leads in a microbial forensic investigation.

This presentation will impact the forensic science community by establishing new biochemical markers that potentially can be used to analyze evidence collected at the scene of a biocrime and, consequently, can assist an investigation.

Lipids are dynamic features of the bacterial cell membrane. Synthesis and incorporation of different lipid structures can reflect the nutritional substrates available to an organism during growth. Within the laboratory, the particular recipe of growth nutrients used during batch culturing is often unique to the host facility or an individual scientist conducting the research. Previous studies have demonstrated that biochemical signatures are associated with certain growth recipe constituents (e.g., brain-heart infusion), but no study to date has systematically examined a comprehensive complement of complex nutrient additives and its effect on the forensic signatures of *Bacillus* spores.

In this study, lipid composition was investigated as an informative phenotype for the types of nutrients that were used during batch growth of *Bacillus cereus* T-strain (BcT) spores. BcT was chosen as a forensic surrogate because of its genetic, structural, and biochemical similarity to pathogenic strains of *Bacillus anthracis*. Five protein sources were examined: tryptone (enzymatic digest of casein protein), peptone (enzymatic digest of meat protein), soy protein, brain-heart infusion, and gelatin. BcT spores were grown in media containing the same base

formulation but supplemented with 8g/L of each of the five nutrient types. After growth, lipids were extracted from spore cultures using a saponification-hexane separation technique. Structural characterization and determination of 11 relative abundance for each lipid was determined with gas chromatography (GC). Composition was characterized by the relative abundances of the four structure classes of *Bacillus* fatty acids (iso-odd, iso-even, anteiso, and straight-chained) and the abundance of individual fatty acid methyl ester (FAME) biomarkers.

Results showed that spores grown on each of the nutrient sources possessed significant differences in the abundance of certain lipid markers. Specifically, spores grown on tryptone containing media were enriched in branched-odd (15:0 iso and 17:1  $\omega$ 10c) and depleted in straight-chained lipids (14:0, 16:0, 16:1  $\omega$ 7c). The converse was true for organisms grown on peptone-containing media. Spores grown on soy and gelatin additives showed lipid compositions intermediate between tryptone and peptone cultures. Spores harvested from brain-heart media showed proportions of branched-odd lipids that were similar to tryptone cultures, but were also enriched in one straight-chained lipid, 15:1  $\omega$ 5c suggesting that it may be an informative biomarker for brain-heart additive. Discriminant function analysis (DFA) was used to model the variation among each nutrient type and to generate a mathematical framework for systematically discriminating each of the five spore cultures based on lipid composition. DFA results showed excellent discrimination with linear equations based on eight lipid biomarkers. In addition, examination of the canonical variable coefficients identified promising stand alone biomarkers (14:0, 17:1  $\omega$ 10c) for nutrient source identification.

Historic research of bacterial fatty acids and spores led to the hypothesis that fatty acid content of bacterial spores could provide insight into the growth formulations and process by which the spores were produced. The results of the work reported here support the hypothesis. Thus, by comparing the fatty acid profiles of the evidence obtained at a biocrime with those of collected evidence from facilities under investigation, meaningful inclusions and exclusions might be made. While FAME analysis alone cannot constitute a forensic investigation, it can provide useful information regarding the provenance of a threat agent.

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**Fame, Microbial Forensics, Sample Provenance**

## **A11 Restoration of Partial Short Tandem Repeat Profiles Resulting From DNA Lesions Induced by Bleach and Hydrogen Peroxide Treatments**

*Maria Saeed, BS, 1201 Bon Air Crest Place, Richmond, VA 23235; Stephanie M. Rogers, PhD, Federal Bureau of Investigation, Counterterrorism Forensic Science Research Unit, 2501 Investigation Parkway, Building 12, Quantico, VA 22135; Shauna M. Dineen, MS, United States Army, 35 Apple Hill Lane, Lynnfield, MA 01940; Leah E. Willis, PhD, Federal Bureau of Investigation, Nuclear DNA Unit, 2501 Investigation Parkway, Quantico, VA 22135; and James M. Robertson, PhD\*, Federal Bureau of Investigation, Counterterrorism Forensic Science Research Unit, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA 22135*

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After attending this presentation, attendees will become familiar with the degradation of the Short Tandem Repeat (STR) profiles caused by treatment of DNA with either bleach or hydrogen peroxide ( $H_2O_2$ ) and the improvement of the profiles after treatment of the extract with a commercial kit containing a suite of enzymes designed to restore PCR amplification of damaged DNA to its normal level.

This presentation will impact the forensic science community by providing the analyst with the awareness that DNA from evidence that is either refractory in PCR amplification or that yields partial STR profiles may be restored either to yield full profiles or to increase its value as probative evidence.

Biological evidence collected at a crime scene or clothing from a victim may yield probative information about an incident. However, the evidence may have been exposed to the environment, or stains may have intentionally been treated with cleansing agents which typically contain bleach or H<sub>2</sub>O<sub>2</sub>. The DNA present in the evidence may be adversely affected by such environmental or chemical influences. Ultraviolet light, heat, humidity and freeze/thaw cycling are familiar environmental effects that are known to produce double-strand breaks, single-strand nicks, modified bases, and loss of nucleotide bases. Similarly, DNA lesions may be produced by bleach and H<sub>2</sub>O<sub>2</sub>. The lesions may prevent procession of DNA polymerase during the polymerase chain reaction (PCR), inhibiting the amplification of STR regions, and lead to full or partial loss of the DNA profile.

Living cells possess several DNA repair mechanisms to correct lesions produced during growth and exposure to various endogenous events and exogenous agents. The repair pathways use enzymes such as glycosylase to excise modified or mismatched bases, endonucleases to remove abasic residues, DNA polymerase to fill in the gaps, and DNA ligase to seal nicks. However, since evidence is composed of dead cells, these repair pathways no longer function. To repair damaged DNA from skeletal remains, forensic anthropologists resorted to using in-house formulations of several enzymes, and this work finally led to the development of a DNA repair kit by suppliers of molecular biology products.

In the work reported here, a commercial DNA repair kit was evaluated for the repair of damaged DNA presenting partial STR profiles. Either bleach or H<sub>2</sub>O<sub>2</sub> was applied to HL-60 cell line DNA, or blood and semen stains on cotton sheeting and non-porous surfaces using standard concentrations. The sterilization efficacy of the oxidizing agents on bacteria was examined in parallel experiments. Initially, DNA damage (oxidation) was assessed using a real-time PCR quantification assay. An increase in cycle threshold value for treated DNA compared to untreated DNA indicated DNA damage. Next, the DNA was amplified to obtain STR profiles. Samples with partial STR profiles were chosen to test the DNA repair kit and optimize the protocol with respect to reaction time, temperature, and enzyme mix volume.

Treatment with bleach or H<sub>2</sub>O<sub>2</sub> led to full or partial STR profile loss, depending on the severity of the process. Incubation at 37° C with the repair enzymes resulted in allele recovery from the partially damaged DNA within three hours, but the best recovery often required an overnight time period. Generally, treatment with the enzyme mix resulted in increased peak heights for most all of the alleles, particularly for the larger STR loci that had peak heights below the detection (50 rfu) and/or stochastic thresholds (200 rfu) before repair. No allele drop-ins were observed. Currently, the emphasis is on optimizing the reaction conditions to reduce the time required to achieve full profile restoration from partially damaged DNA.

The use of the commercial repair kit may provide a means to obtain a full STR profile from environmentally exposed evidence or from evidence obtained after oxidizing treatments with bleach or H<sub>2</sub>O<sub>2</sub> that would otherwise be refractory or result in partial profiles. The repair does not involve multiple, time-consuming steps. It can be easily incorporated into the current STR analysis procedure and should work on a liquid-handling robotic system.

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**Bleach and Peroxide, STR Analysis, DNA Repair**

## **A12 Fire Debris Software**

*Mary R. Williams, MS\*, and Michael Sigman, PhD, University of Central Florida, National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2367*

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After attending this presentation, attendees will be introduced to newly developed software for the analysis of fire debris.

This presentation will impact the forensic science community by utilizing the newly developed software for the analysis of fire debris. The software combines current data analysis methods along with newer

methodology. Features of the software include some data analysis methods described in ASTM E1618-10 and automated searching procedure based on the total ion spectrum from gas chromatography/mass spectrometry (GC/MS) of libraries of liquid residues and substrate materials.

In fire debris analysis, a residue is extracted from the fire debris and analyzed by GC/MS. Currently, data analysis methods include total ion chromatograph, extracted ion chromatograph and target compound chromatograph visual pattern recognition of the residue against a known reference liquid. One or all of these methods are used to characterize the liquid residue into one of seven major classes based on the chemical composition of the residue and then further sub-classified by its carbon range. An extracted fire debris residue can be compared to a reference liquid by visual comparison of their stacked total ion chromatograms within in the software. In cases where the analyses have been performed with different GC/MS methods, the total ion chromatogram plots can be aligned within the windows of the software for better visualization. Extracted ion chromatograms for the residue and the reference liquid based on characteristic ions of the same functional groups can be aligned and plotted. The relative contribution of the characteristic ion groups can be calculated and plotted within the software. These tools will support and facilitate current methodologies of fire debris data analysis.

A newer data analysis method is utilized in the software to automate searching a library or libraries of reference liquids and substrate materials. The search is performed on the total ion spectrum of the GC/MS data which is the sum of the intensity of each m/z across the chromatographic time range. A result from the search is a list of the reference liquids from the library in rank order from most similar to the least similar compared to the residue. The libraries are obtained from the Ignitable Liquids Reference Collection and Substrate Databases, which are joint projects between the National Center for Forensic Science and the Technical/Scientific Working Group for Fire and Explosions. A sample reference number, product name, and classification are given for the selected library reference liquid or substrate material. In a click of a button the software will connect the user (on an internet accessible computer) to the entry in the database for that selected reference liquid or substrate material to obtain more information. A subsequent result from the library search is a posterior probability of class assignment based on Bayesian Decision Theory.

The software developed for fire debris analysis will provide information in a suitable format for fire debris analysts to perform data analysis by current methods. New methods incorporated into the software will analyze the data in a complimentary fashion with the ability to rapidly search an extensive library of reference materials and provide a posterior probability for the assigning a classification.

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**Fire Debris, Ignitable Liquids, Software**

## **A13 Evaluation of Novel DNA Extraction Methods and Modifications to Existing Methods**

*Kelly M. Elkins, PhD\*, Metropolitan State College of Denver, Chemistry Department, PO Box 173362, CB 52, Denver, CO 80217*

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After attending this presentation, attendees will learn about new methods for extracting DNA from biological samples containing cellular material.

This presentation will impact the forensic science community by providing systematic data that can be used by the analyst in selecting a method for extracting DNA from crime scene or reference samples especially when considering time and cost.

DNA extraction is a method of purifying DNA from other cell contents including proteins, enzymes, and membranes. DNA extraction is employed routinely by crime labs to recover DNA from biological samples containing cellular material. While a number of methods for extracting DNA from crime scene evidence are available to the practitioner, research results are presented in which new methods have been evaluated and older methods have been modified to reduce the time needed to recover the DNA. The extracted DNA is used for modern DNA typing analyses including autosomal, Y-chromosome and mitochondrial DNA typing using STRs, SNPs, and sequence comparison. The DNA extraction methods used in crime laboratories including Chelex<sup>®</sup> 100, Phenol-Chloroform-Isoamyl Alcohol, dialysis, and commercial kits (e.g., Promega's DNA IQ<sup>™</sup> and Qiagen's QIAamp<sup>™</sup>) vary widely in terms of cost and extraction time. The 5% Chelex<sup>®</sup> method is the cheapest method but it is time-consuming due to a recommended six to eight hour, 56 °C incubation step performed prior to vortex mixing, boiling, and centrifugation steps. The Chelex<sup>®</sup> beads chelate metal ions, including magnesium, known to be essential for DNase nuclease activity. Inactivating nuclease enzymes that digest DNA reduces DNA degradation in the extraction process. In this study, modifications to the Chelex<sup>®</sup> method were evaluated including reducing the reagent concentration and incubation time in order to decrease the overall cost and time involved in using this method for casework and other samples. In addition, extractions with Silicycle<sup>®</sup>, EDTA, and citrate were performed to determine if these methods would also yield amplifiable DNA. Like Chelex<sup>®</sup>, Silicycle<sup>®</sup>, EDTA, and citrate reagents also chelate metal ions. The extraction efficiency with consideration to DNA yield, cost and time was tabulated for all of the methods.

In this study, the Chelex<sup>®</sup> method was evaluated for DNA recovery and amplifiability using reduced incubation times (thirty to ninety minutes) and overnight without Proteinase K. The results showed that any incubation time over sixty minutes and the procedure from which Proteinase K was omitted produced results similar to those produced using the standard method. Omitting the Proteinase K that digests proteins significantly reduces the cost of employing this method. In addition, the concentration of Chelex<sup>®</sup> was varied from 1% to 5% solutions. Reduced Chelex<sup>®</sup> concentrations yielded amplifiable DNA at a reduced cost. In addition, extractions with similar concentrations of Silicycle<sup>®</sup>, EDTA, and citrate were evaluated for use in DNA extraction using varying incubation times for comparison to the Chelex<sup>®</sup> method. The Silicycle<sup>®</sup> method was observed to extract successfully amplifiable DNA as evaluated by real time-PCR using TPOX primers validated for DNA quantitation and post-PCR agarose gel electrophoresis. EDTA and citrate are very inexpensive chelating compounds that could be useful to the forensic community as DNA extraction agents.

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#### **DNA Extraction, Real-Time PCR, Chelex**

### **A14 Single Channel Simultaneous Analysis of DNA and MicroRNA**

*Graham Williams, MSc\*, University of Huddersfield, Forensic and Analytical Research Centre, School of Applied Sciences, Huddersfield, HD1 3DH, UNITED KINGDOM*

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After attending this presentation, attendees will be aware of a new proposed protocol that will analyse DNA and miRNA simultaneously.

This presentation will impact the forensic science community by exhibiting a new protocol that will expand the capability of forensic science laboratories with minimal modifications and expenses.

It is often necessary to establish the identity of the body fluid from which a DNA profile was obtained. This issue is frequently encountered in sexual offences where it can be necessary to distinguish between saliva and vaginal material or where there is a very small amount of the body fluid present, such as trace amounts of blood on a dark surface. This issue is

currently being resolved by the use of mRNA and microRNA (miRNA) analysis utilising a co-isolation technique to separate out the DNA and the miRNA phases for subsequent analysis.

This study presents an alternative protocol in that the DNA and miRNA are co-isolated without physical separation. All downstream processes are carried out simultaneously through a single channel process resulting in a single electropherogram indicating both the DNA profile obtained from the body fluid stain and the identity of the body fluid.

A number of blood and saliva samples were collected from volunteers (with informed consent). Blood was collected using the finger prick method and depositing blood onto a filter paper. Saliva was collected using buccal swabs. Dual extraction was carried out using the QIAamp DNA Mini Kit with no modifications. miRNA was present in the eluents due to their small size and abundance within each cell. The DNA/miRNA sample then underwent cDNA synthesis by carrying out stem-loop reverse transcription PCR, which reverse transcribes and lengthens the miRNA marker prior to amplification.

Amplification was carried out using a modified version of the Amp/STR NGMSElect kit (ABI). The modification was the incorporation of labelled miRNA primers. The primers used are complementary to miR-205 and miR-451 markers which are specific to saliva and blood, respectively. No other modifications to the amplification protocol were made.

Following capillary electrophoretic separation on the ABI Prism 310 genetic analyser, full DNA profiles were obtained matching the volunteer DNA profiles. In all blood samples, a peak indicating the presence of miR-451 was generated and in all saliva samples, a peak indicating the presence of miR-205 was generated indicating a 100% correct identification rate (n=15). Small peaks were observed in the miR-205 bins for blood and vice versa; however, such peaks were considerably smaller than the expected peaks and in many cases were less than 150 rfu.

Reverse transcription negative controls were carried out by using sterile water in place of the MMLV reverse transcriptase. In all cases, no discernable peaks were obtained within the miRNA bin sets. The DNA profiles were unaffected.

In all cases, the correct DNA profiles and the correct body fluid identity were obtained, thus demonstrating the proof of principle in that a single stream simultaneous analysis of DNA and miRNA is possible and robust. The advantages of this technique are that only minor modifications are required to install the BFID capability into a forensic science laboratory. The single work stream element means that there are reduced opportunities for contamination, and it is cost-effective. Finally, this single stream simultaneous process means that it may be possible to definitively associate a DNA profile with a particular body fluid.

Future work will involve the identification and characterization of further body fluid markers with a view to developing a full BFID panel multiplexed with a DNA profiling system.

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#### **DNA, miRNA, Co-isolation**

### **A15 Internal Validation of AmpFISTR<sup>®</sup> Identifier<sup>®</sup> Plus Amplification Chemistry on Applied Biosystems' ABI PRISM<sup>®</sup> 310 Genetic Analyzer**

*Tiffany D. Bard, BS\*, 611 22nd Street, Apartment 322B, Huntington, WV 25703; and Cara E. Lupino, MS, Rhode Island Department of Health, 50 Orms Street, Providence, RI 02904*

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After attending this presentation, attendees will have acquired a deeper understanding of how well Identifier<sup>®</sup> Plus amplification kit advances the interpretation process. Nine studies reveal the superiority of this amplification kit when run on a 310 genetic analyzer.

This presentation will impact the forensic science community with a greater understanding of the results Identifier<sup>®</sup> Plus can bring to the

laboratory. Identifiler® Plus achieves greater sensitivity, faster amplification time, and increased interpretation of mixtures.

An internal validation of the Identifiler® Plus amplification kit was conducted to assist the Rhode Island Department of Health Forensic Science Laboratory's transition from a two kit amplification system (Profiler® Plus /COfiler®) to a one kit system increasing turnaround time and decreasing consumable usage. Since this is a small laboratory with strict budgeting, transitioning to Identifiler® Plus will increase throughput, save money by reducing consumable usage, and assist in relieving the backlog of cases.

The Identifiler® Plus amplification kit includes the 13 core CODIS markers, Amelogenin, and an additional two markers to increase the level of discrimination of the final profile as compared to Profiler® Plus/COfiler®. The new chemistry of this kit combines the Ampli®Taq Gold DNA Polymerase with a Master Mix reagent and reduces the overall kit package by two tubes and one box. Identifiler® Plus also provides this laboratory with a 5-dye amplification system and faster amplification time. The new improvements to the PCR thermal protocol remove about an hour of the amplification time. The combination of a one kit system paired with the new PCR protocol increases the laboratory's turn-around time and allows cases to be processed faster and more efficiently.

Samples were amplified with Identifiler® Plus on a GeneAmp® PCR System 9700 Thermal Cycler using a 29 cycle thermal protocol. Nine studies including sensitivity, precision/reproducibility, concordance, mixture, known and non-probative evidence samples, match criteria, contamination, denature, and stutter percentage were performed on two Applied Biosystems PRISM® 310 Genetic Analyzers and generated data was analyzed using GeneMapper ID v3.1.0.

The sensitivity study assessed the range of DNA concentrations that could produce reliable typing results. A precision/reproducibility study was performed to determine if accurate and reliable genotypes could be reproduced over consecutive days. All alleles were evaluated to determine if they fell within the recommended  $\pm 0.5\text{bp}$  window. The concordance study assessed the ability of Identifiler® Plus to accurately reproduce the NIST SRM 2391b reference samples. A mixture study was performed to examine the behavior of a sample containing two contributors comparing the resulting profiles to previous ones typed in Profiler® Plus/COfiler®. Match criteria and contamination studies were completed to determine if the positive and negative controls were working properly; positive controls had expected profiles and negative controls contained no contamination. A denature study was evaluated to determine if there was a significant difference between denaturing/chilling the samples before placement on a genetic analyzer and not denaturing/chilling. Lastly, an intra-laboratory stutter study was performed to evaluate if stutter remained under the 15% - 20% threshold.

Sensitivity study results indicated 0.5ng was the ideal input of target DNA to amplify at 29 PCR cycles with Identifiler® Plus. The sizing precision for each allele demonstrated the accuracy and reliability of sizing the Identifiler® Plus amplicons, even when several maximum/minimum values exceeded the recommended  $\pm 0.5\text{bp}$  window. Results obtained during the concordance study demonstrated intra-laboratory concordance when NIST SRM samples are amplified with Identifiler® Plus on multiple genetic analyzers. Mixture results showed a major contributor could be extracted at every marker with Identifiler® Plus when amplifying a target input of 0.3ng in samples containing a mixture of two individuals' DNA at ratios 10:1 and 1:10.

The methods utilized in the known and non-probative casework samples study were successful in the recovery of at least a partial DNA profile in concordance with Profiler® Plus/COfiler®. With proper lab technique and appropriate DNA target amount, extraction positives and amplification positives produced the correct profile with few extraneous peaks, and no contamination of any negative sample occurred during the validation. For the denature study, the step of 95°C denaturation and a snap-cooling step on an ice block prior to placing the samples on the instrument

for separation caused the reduction of peak heights as compared to samples that were not denatured and snap-cooled prior to run.

**Internal Validation, AmpFISTR® Identifiler® Plus Amplification Kit, ABI PRISM® 310 Genetic Analyzer**

## A16 Validation of the PowerPlex®18D Direct Amplification System

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After attending this presentation, attendees will better understand the capabilities of direct PCR amplification for processing forensic DNA profiles.

This presentation will impact the forensic science community by showing the reliability of a new technology that can potentially save a significant amount of time and money during the production of forensic DNA profiles. It will also provide other laboratories that are considering adopting direct amplification with a general internal validation scheme.

As the role of forensic DNA analysis has grown, there has been a significant increase in the number of samples that forensic DNA laboratories receive for analysis. Many states are moving toward, or have already adopted, legislation that would require a DNA sample to be collected from all arrestees, drastically increasing the throughput requirements of many laboratories. In order to maintain efficiency and prevent a sample backlog, it is imperative to reduce the time and cost associated with forensic DNA testing.

Direct PCR amplification significantly reduces the time required for DNA analysis by eliminating both DNA extraction and quantification on single source blood or buccal stain samples. Promega's direct PCR amplification chemistry, PowerPlex® 18D (PP18D), further decreases the time required to produce a final profile by using shorter thermal cycling - approximately one-half required by traditional amplification chemistries. With this kit, database or reference samples can be processed, reviewed, and uploaded into CODIS in as little as one day.

Due to the lack of a purification step when using PP18D with FTA® punches, the chemistry has been designed to overcome most types of inhibition that would commonly be encountered with this sample type. An additional advantage to the PP18D kit is the amplification of two non-CODIS, highly discriminating penta-nucleotide repeat unit markers that can increase the level of discrimination associated with profiles generated using this amplification system.

This study sought to validate the PowerPlex® 18D direct amplification kit for use with single-source FTA® card samples. Samples were collected on the Whatman® EasiCollect™ device. One 1.2mm punch was taken using a Harris manual punch and amplified for 27 cycles on an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler. Half reaction volumes (7.5µL water, 2.5µL 5X Master Mix, 2.5µL 5X Primer Pair Mix) were used to further reduce the cost associated per sample. Fragment separation was performed using Applied Biosystems 3130xl Genetic Analyzers. Data generated was analyzed using the Applied Biosystems GeneMapper® ID-X software v1.1.1. When applicable, quantification was performed using the Quantifiler® Human DNA Quantification kit and the Applied Biosystems 7500 Real-Time PCR System.

PP18D was determined to yield full profiles from samples with concentrations as low as 0.4ng/µL. The kit was shown to be precise at all 18 loci with 99.7% certainty. Known/non-probative samples that were collected from employees were compared to known profiles, and all samples (85 of 85) were concordant. Seven samples were amplified and

run on different instruments and different days to test reproducibility and were found to be concordant. Other common sample types (cotton swabs, Omni® swabs, and extracted DNA) were also tested and yielded full profiles.

Based on the findings of this study, PP18D for processing database samples, as well as troubleshooting single-source casework samples, will be incorporated. Future studies will be done to determine the feasibility and reliability of performing direct PCR amplification on non-FTA® card samples once the lysis buffer from Promega is released for commercial use.

**Direct Amplification, Validation, Efficiency**

## **A17 Volume Reduction Solid Phase Extraction of Forensic Samples on a Plastic Microfluidic Device**

*Briony C. Strachan, MSci\*, 409 McCormick Road, Chemistry Building, Charlottesville, VA 22904; and James P. Landers, PhD, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will gain an understanding of progress towards disposable plastic microdevices that can facilitate the volume reduction of large volume, dilute biological samples for downstream processing in human identification via STR analysis.

This presentation will impact the forensic science community by introducing a disposable plastic microdevice applicable for DNA extraction and purification from dilute biological evidence collected in forensic investigations, enabling higher throughput of samples than previous glass devices. This work is a step towards the use of a micro-total analysis system for forensic genetic analysis.

Microfluidic devices present numerous advantages to current forensic analyses, including low reagent consumption and reduction in analysis time, combined with the ability to deliver a closed sample in-answer out multi-process system within a single device.<sup>1</sup> Additionally, these devices require less sample template, enabling preservation of sample for further analysis when there is limited quantity during forensic casework.

Successful utilization of solid phase extraction (SPE) within microfluidic devices has been established through numerous publications from our lab, demonstrating silica-based phases capable of highly efficient and reproducible DNA purification.<sup>2</sup> Although successful, a challenging forensic sample type for microfluidic platforms are those collected from surfaces or stains, which may involve large volume (milliliters) of a dilute sample for processing. The challenge of macro-to-micro interfacing has been previously addressed by the development of volume reduction solid phase extraction (vrSPE), where a large SPE phase was designed to accept sample volumes 10-fold larger than traditional SPE devices.<sup>3,4</sup> Such microdevices have been fabricated in glass: an expensive, time-consuming process that utilizes hazardous chemicals (e.g., HF), however, the use of plastic substrates (like poly (methyl methacrylate); PMMA) circumvents these issues. Volume reduction SPE microdevices can be created from PMMA by micromilling processes for pennies per chip using laser ablation, with an 8-fold reduction in fabrication time. The presented research adapts the vrSPE technology to a plastic device, allowing for simple, inexpensive extraction and concentration of DNA from dilute biological samples, ready for PCR and subsequent STR analysis.

The ease of design and fabrication of vrSPE PMMA devices enables creation of multiple geometries to tailor a device to a specific purpose. Several new designs will be presented including those that allow for a 4-fold increase in the sample volume (from 500µL to 2mL) to be accepted onto the device, or conversely, a 4-fold reduction in time (from 30 minutes to ~8 minutes) to load a 0.5mL sample. Furthermore, multiplexing the vrSPE method will be shown for the simultaneous tetraplex extraction of four whole blood samples from four different individuals, with each 16-plex STR profile reported. Each design provides a significant improvement for both analysis time and throughput compared to traditional glass vrSPE.

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### **Volume Reduction, PMMA Microchip, DNA Extraction**

## **A18 Do You Really Know What Your Robot is Doing?: The Importance of Paying Attention to Liquid Handling Details**

*Mark Pietras, BA, John T. Bradshaw, PhD, and Keith J. Albert, PhD\*, Artel, 25 Bradley Drive, Westbrook, ME 04092*

After attending this presentation, attendees will understand that liquid handler performance, and behavior, can and will affect assay work. Uncovering liquid handler errors can help optimize and/or troubleshoot assay work.

This presentation will impact the forensic science community by exposing the importance of really understanding liquid handling devices, specifically, automated robots. As more forensic bench work turns to automation, it is extremely important that the automated volume transfer steps are known (verified) so the methods can be tweaked and optimized for the automated assays.

The introduction of automation into forensic science, biology, and chemistry labs has arguably led to significant advances in testing capabilities over the past 20+ years. Automation has certainly led to increased numbers of experiments, as compared to manual testing, particularly for pipetting operations. Because of this advantage, liquid handling robots have become commonplace even in small laboratories. However, in spite of all the advantages that a liquid handling robot brings to a laboratory, it also brings a different set of commonly overlooked challenges such as ensuring quality.

The focus of this presentation is to highlight the need of ensuring quality in important assays performed with automated liquid handlers. Nearly all assays performed within a laboratory are volume-dependent. In turn, all concentrations of biological and chemical components in these assays, as well as the associated dilution protocols, are volume-dependent. Because analyte concentration is volume-dependent, an assay's results might be falsely interpreted if liquid handler variability and inaccuracies are unknown or if the system(s) goes unchecked for a long period. If liquid handlers are properly employed (with the right methods/materials for the specific assay), and they are regularly assessed for performance, they can be powerful systems for lowering costs, increasing throughput and avoiding errors associated with manually-pipetted methods. It is imperative, therefore, to quantify the volumes transferred with an automated liquid handler, especially for the specific automated methods that are used to perform assays. Measuring and knowing the exact volumes transferred for specific and/or routine methods will inherently lead to confidence in the

experiment. Knowing an assay's exact volume and component concentrations is critical to properly interpreting the results.

It may be argued that the largest challenge presented by using a liquid handling robot is the potentially incorrect assumption that the robot is doing what it is should be doing. The robot may in fact be doing exactly what the user told it to do, but is that really what the user wanted? One might say that the real question is, do you *really* know how your robot is behaving, and particularly, do you *really* know how your robot is performing for your assay work?

This presentation discusses real case studies of how liquid handlers were performing, or rather misperforming, for certain test procedures. Herein, examples of the importance of monitoring various commonly employed tasks will be presented which are likely considered mundane and often assumed to have little bearing on overall robot performance. Specific examples showing how liquid handler performance can be altered based on: (1) pre-wetting disposable pipette tips; (2) running identical methods on identical robots; (3) protocol differences between high volume and low volume dispenses; and (4) effect of volume transfer mode (reverse or waste mode vs. forward mode). The examples presented will help users to think more about the specific tasks they are asking their robots to perform, and hopefully uncover certain steps that, if observed and controlled, will result in optimized liquid handler performance to ensure the highest quality work possible.

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**Automated Liquid Handlers, Liquid Handler Behavior, Optimizing Liquid Delivery**

## **A19 Evaluation of Lyophilized Reagents for STR Analysis**

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The goal of this presentation is to demonstrate a proof of concept experiment in which PCR reagents were prepared in and subjected to non-ideal temperature conditions.

This presentation will impact the forensic science community by providing a springboard for further exploration of lyophilized reagents designed for use in non-ideal forensic environments.

Recent literature indicates growing interest in the use of DNA as a biometric tool for applications in both the lab and the field. A factor which must be considered for field applications is the ability to maintain reagents in non-ideal conditions. Commonly used typing kits recommend storage of primers and reaction mix at 2 to 8°C and polymerase at -15 to -25°C. This would be problematic, for example, in a situation where a battlefield lab is deployed in a location such as Afghanistan. Suppliers may have difficulty reaching such locations and reagents would be exposed to a non-ideal, high temperature environment during shipping and at the field laboratory. An understanding of how PCR reagents will be affected by their environment is therefore necessary.

The purpose of this study was to evaluate the use of dried primers and stored at various non-ideal temperatures, along with commercially available lyophilized polymerases for use in STR analysis. Five temperatures were chosen for evaluation: room temperature, 4°C, 37°C, 50°C, 65°C, and 80°C. Several commercially available lyophilized polymerases already containing reaction mix were evaluated as well. Primers were dried down utilizing a speed vac and stored at their respective temperature for one week along with the lyophilized polymerase/reaction mix. A PCR amplification protocol was employed followed by capillary electrophoresis analysis. In addition, the effect of the high temperature environment on the dried primers was tested by using polymerase and reaction mix which had not been exposed to the non-ideal conditions. The latter reagents were stored according to manufacturer recommendations until amplification.

Full or partial profiles were obtained for temperatures closest to recommended storage conditions and some amplification was observed for several loci at temperatures up to 65°C. Artifacts such as incomplete adenylation and peak imbalance were observed and suggest that protocol improvements are necessary. Full profiles could be obtained with primers stored at temperatures up to 80°C when polymerase and reaction mix which had not been exposed to the non-ideal, high temperature environment were used for the PCR amplification. This suggests that the reagents which were most affected by the higher temperatures were the polymerase and reaction mix (in particular dNTPs). It is important to note that the polymerases which were chosen, although lyophilized, were not specifically designed for STR analysis in mind. Some reagents were tailored towards real time PCR applications, which likely contain non-ideal amounts of components such as MgCl<sub>2</sub> for end point PCR applications. This is suggested in these experiments by the presence of artifacts such as incomplete adenylation and non-specific PCR products even when reagents were stored in the conditions which were recommended by the manufacturer.

Results observed at higher temperatures believed to be incompatible with PCR reagents indicate that with optimization, there is potential for future development of lyophilized reagents for use in PCR field kits. If a product could be designed and tailored to STR analysis and the forensic practitioner's objectives, it is likely that the results obtained here could be improved in terms of profile quality.

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**Battlefield Forensics, STR Analysis, PCR**

## **A20 Who's on Your Shoes? Investigating DNA Profiles From New and Worn Shoes**

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The goal of this presentation is to demonstrate to the forensic community that multiple DNA profiles can be recovered from the soles of three types of shoes before being worn outside of the store and for some shoe types, the wearer can become the primary DNA contributor after just several hours of wear.

This presentation will impact the forensic science community by presenting a pilot study which successfully recovered DNA from the soles of all three shoe types. It will demonstrate that all three varieties of shoes produced mixed profiles when initially purchased, and will document at which point a major profile, consistent with the wearer, could be generated.

The recovery of DNA that can generate a DNA profile from crime scene evidence is an important aspect of forensic investigations. Shoes represent an item that could potentially be recovered from a crime scene and provide valuable information about the suspect or victim. Shoe impressions may be used to link a suspect with a crime scene and the shoes themselves can later be seized for additional laboratory analyses. Such additional investigations often involve the collection of DNA from the soles and heels of the shoes. A potentially complicating factor to the generation of a DNA profile is the possibility that DNA is deposited on the shoe when being tried on in the store. This could be a possible form of contamination and contribute to the generation of a mixed DNA profile. To date, no previous research has been discovered that investigates whether DNA is present on shoes when they are purchased. The goal of this research is to conduct a pilot study to determine if DNA is present on the soles of three types of new shoes before being worn outside of the store, if DNA is present before being worn, and at what time does the wearer become the primary contributor on the sole.

Three shoe types: flip-flops, close-toed casual shoes (commonly referred to as "flats"), and sneakers were chosen for this study because each

has a different level of exposure to the environment and different levels of contact with the sole of the foot. The bare foot comes in direct contact with the flip-flops and the flats; however, sneakers are commonly worn with socks which could potentially reduce the chance of epithelial cells being deposited from the foot onto the sole of the shoe. In addition, the flats and sneakers represent a more closed environment while the flipflops expose the epithelial cell DNA to environmental factors. The sole of each shoe was swabbed before being tried on by the wearer after purchase. Then, the shoes were worn and swabbed again at four, eight, and twelve hour intervals of wear. Because suspects may throw their shoes away after a crime, or dispose of the shoes from their victims, the investigators were also interested in testing whether the placement of the shoes in an outdoor garbage receptacle would influence the ability to generate a DNA profile. Therefore, after the twelve hour wear interval, the shoes were placed in the garbage for six days and the soles were swabbed again after removal. All sampling was conducted using sterile swabs and the DNA was extracted using a common commercial DNA extraction kit. The DNA was quantified via quantitative PCR, and DNA profiles were generated to establish whether the DNA samples represented a mixture of multiple donors or if a major contributor could be established.

DNA of high enough quantity for subsequent DNA typing was recovered from 67% of the samples collected from the flip-flops, 67% of the samples collected from the flats, and 33% of the samples collected from the sneakers worn with socks. The quantification data suggests that some shoe types are better reservoirs for DNA than others. In addition, all three varieties of shoes produced mixed DNA profiles when initially purchased. Samples typed from both the flats and the sneakers suggest the wearer can become the primary DNA contributor after just several hours of wear, while samples taken from the flip-flops consistently generated mixed or incomplete profiles. The DNA typing data suggest that some shoe types will better reflect the actual wearer of the shoe, which may be a crucial link in a forensic investigation.

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#### **DNA Profile, Shoes, Forensic Investigation**

### **A21 A Systematic Approach to the Analysis of DNA From Earrings in Varying Conditions**

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The goal of this presentation is to demonstrate the possibility of generating a usable DNA profile from samples collected from stud earrings, to investigate whether or not the raw material used to construct the stud earrings can influence the quantity of DNA recovered from the jewelry surface, and to examine the effects of outdoor environmental conditions on both DNA quality and quantity.

This presentation will impact the forensic science community by investigating a DNA-bearing source that could produce a DNA profile when more commonly used sources, such as body fluids or tissue, are not left at the crime scene, or are not in an analyzable condition.

A variety of evidence can be collected from crime scenes and includes items such as hair, fingerprints, bodily fluids, personal effects, and weapons. From these materials, investigators attempt to isolate information pertinent to both victim and perpetrator identification. Although new research is constantly being conducted to maximize the amount of trace DNA that can be collected from evidence and to incorporate new DNA-bearing sources, it is still lacking in several areas. Specifically, there are no discovered studies, to date, that systematically investigate the potential for jewelry and other personal effects commonly found at crime scenes to be

acknowledged as options of items bearing viable DNA. Because earrings are commonly worn by both sexes in our society, they represent good potential sources for the collection of DNA samples when more commonly analyzed sources are not available. Personal effects found in association with the crime scene may serve as circumstantial evidence to link a victim or suspect to that location. DNA collected from such items may also be useful when known comparison samples are available to assist in identifying missing persons or other unknown decedents. This presentation will benefit the forensic community by investigating a DNA-bearing source that could produce a DNA profile when more commonly used sources, such as body fluids or tissue, are not left at the crime scene, or are not in an analyzable condition. For example, jewelry can be accidentally removed from the body during a struggle, or taken by a perpetrator as a trophy during a robbery or assault. The ability to link a potential suspect or victim to an area where an assault previously occurred would be beneficial to crime scene investigators. In addition, a body may be discovered in a condition that is not conducive to positive identification via more traditional means such as fingerprint, dental, or DNA typing of the body itself. In such a situation, the ability to generate a DNA profile from associated personal effects would be valuable to the investigation.

The goal of this project is to determine whether amplifiable DNA can be collected from epithelial cells deposited on earrings composed of three common metals: gold, silver, and surgical steel, and whether environmental exposure could compromise the ability to generate a DNA profile. The gold, silver, and surgical steel studs were worn by participants for varying amounts of time ranging from one to five hours. The earrings were then removed from the participants' ears and immediately swabbed along the posts and earring backs with sterile cotton swabs. The earrings were returned to the participants and worn again for at least one hour. The studs were once again removed from the participants' ears, placed in mesh containers, and positioned in an outside location where they were exposed to the ambient environment. The earrings remained outside for four days and were exposed to rainstorms, high winds, and ultraviolet radiation from the sun. The studs were removed from the mesh containers and swabbed once again on the posts and earring backer.

The DNA collected from the swabs was extracted using a common commercial kit and all samples were quantified using real-time PCR. All earring materials yielded quantifiable DNA of varying amounts suggesting earrings made from gold, silver, and surgical steel are potentially valuable personal effects for the generation of a DNA profile. In addition, DNA typing of the samples suggests that forensically significant profiles can be generated from earrings made of common metals, even when exposed to adverse weather conditions.

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#### **DNA, Suboptimal, Earrings**

### **A22 Analysis of Synthetic Polymers Using MALDI-TOF**

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The goal of this presentation is to demonstrate the need for objective identification of synthetic polymers in the trace evidence realm of forensic science and to propose the analysis of the fragmentation patterns of these polymers obtained from MALDI-TOF mass spectra to be used for fiber identification.

This presentation will impact the forensic science community by providing an objective method for trace evidence analysis and identification of synthetic fibers.

Synthetic fibers, or polymers, are created through addition or condensation reactions that combine monomers. Each manufacturer may introduce various chemical additives, which affect the chemical and physical properties of the material. Such additive categories include: flame retardance; dyes, pigments; UV absorbers and antioxidants; plasticizers;

surfactants; and lubricants. Each manufacturer is at liberty to distinguish their product with various additive combinations. However, these additive combinations are not able to be used for identification purposes with the current analysis techniques for synthetic fibers. Current methods of analysis include: visual comparison and microscopy, which are both subjective identification techniques, and Fourier transform infrared spectroscopy (FTIR), which identifies the polymer into a general type. Fiber evidence collected from a crime scene can provide valuable information in the investigation of the crime. However, it is often not a critical piece of evidence due to the lack of objective identification of fiber source. Trace fiber evidence can come from many different sources that often appear identical through the above mentioned analyses. This makes it nearly impossible to determine a source of origin. A synthetic fiber database classified by polymer type and manufacturer additives could objectify fiber analysis by narrowing and confirming the fiber's source of origin. This creates a need for an analytical technique that can ultimately identify the fiber type and manufacturer source. Matrix-assisted laser desorption ionization (MALDI) is a mass spectrometry analytical technique that utilizes a laser beam to ionize the analyte, in this case the fiber. The absorbing matrix is mixed with the sample, allowing the analyte and the matrix to co-crystallize on the target plate. The matrix uses the energy provided from the laser to desorb itself from the target, pulling analyte particles with it into the gas phase as ions. In ion form, the analyte particles are then carried to the mass spectrometer detector, the time-of-flight (TOF) mass spectrometer. The TOF works by accelerating the ions toward the drift region where all ions have the same kinetic energy but different masses, thereby separating based solely on particle size. The goal of this research is to provide an objective identification procedure through the use of MALDI-TOF analysis that can distinguish various synthetic polymers based on the individual manufacturers' additives. MALDI-TOF was used to determine the polymer additive composition for comparison of similar polymer types. Optimized MALDI parameters were obtained for nylon, olefin, and polypropylene to produce mass spectra with high resolution and complete peak separation. This was obtained by spotting the target with a salt solution comprised of trifluoroacetic, sodium salt in tetrahydrofuran (THF) followed by a matrix solution, comprised of  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) in THF. The optimized parameters were then used to obtain two sets of mass spectra for four differently manufactured nylon fibers using the matrices, CHCA and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB). These same fibers were also analyzed using FTIR as a comparison. The FTIR results confirmed the fiber identity as nylon, but could not distinguish any differences between the different manufactured nylon fibers. The MALDI-TOF mass spectra were analyzed to distinguish between matrix peaks, polymer peaks, and chemical additive peaks. When comparing spectra obtained for nylon against the spectra for the matrices CHCA and DCTB, there were similar peaks present that were not attributed to the matrix. Further analysis is required to detect spectral differences due to additive composition of differently manufactured polymers. However, when compared to FTIR analysis, this method provides a possible successful objective method for synthetic fiber analysis and identification.

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#### **Synthetic Polymer, Matrix-Assisted Laser Desorption Ionization, Manufacturer Additives of Synthetic Polymers**

## **A23 Quality Control: How Sterile Are Your Laboratory Examination Gloves?**

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The goal of this presentation is to inform participants of the potential for cross-contamination of DNA from nitrile examination gloves used in the DNA laboratory setting by investigating potential manufacturer contamination and potential contamination by analysts, and the possible implications for such cross-contamination in interpreting forensic DNA casework.

This presentation will impact the forensic science community by demonstrating the difficulty of depositing contaminating DNA on nitrile examination gloves by the brief and casual contact required to remove gloves from their boxes. However, it also demonstrates the possibility of obtaining DNA of a high enough quantity to potentially complicate forensic DNA analyses.

Quality control is an essential component of the forensic laboratory as it is crucial in obtaining accurate analytical results and maintaining the integrity of the testing. The use of latex or nitrile examination gloves is an essential quality control measure against cross-contamination from a DNA analyst's hands to the sample they are processing in a forensic DNA laboratory. The gloves create a barrier between the epithelial cells that can potentially be shed from the analyst's hands and the sterile laboratory environment. However, opened boxes of gloves are often left in arbitrary spaces until the supply is depleted and analysts often retrieve gloves from the boxes with their bare hands. The objective of this research is to systematically test whether such laboratory procedures could potentially introduce contaminating DNA into the laboratory setting.

Sterile swabs were used to collect samples from nitrile examination gloves and the boxes in which they were housed. Both previously opened and previously unopened boxes of gloves were tested. The previously opened boxes of gloves were randomly chosen from a university laboratory in which a genetics course was being conducted. The internal box surface, rim of the box, and the exposed surface of the top glove were swabbed for possible epithelial cells deposited by an individual reaching into the box to retrieve a pair of gloves. In addition, one glove was removed from the box by a gloved researcher and the exposed surface of the next glove was swabbed. This procedure was repeated with the removal of a second glove. Previously unopened boxes of nitrile examination gloves were opened by an ungloved analyst. The internal box surface, rim of the box, and the exposed surface of the top glove were swabbed for possible DNA deposited by the analyst. The analyst removed three gloves with bare hands and the exposed surface of the next glove was swabbed. This procedure was repeated with the removal of another three gloves. In addition, previously unopened boxes of nitrile examination gloves were opened by a gloved analyst. The internal box surface, rim of the box, and the exposed surface of the top glove were swabbed for any DNA that may have been deposited in the box during packaging by the manufacturer. The gloved analyst removed ten gloves and the exposed surface of the next glove was swabbed. This procedure was repeated an additional two times with the removal of twenty additional gloves.

The DNA was extracted from the swabs using a common commercial kit and quantified via quantitative PCR. One sample taken from a previously opened box of gloves contained DNA that was of high enough quantity for further analysis. All other samples produced a no DNA after quantitative PCR. The sample containing DNA was amplified and did not generate a successful DNA profile.

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#### **DNA Cross-Contamination, Nitrile Examination Gloves, Forensic DNA**

## **A24 Ricin Bioavailability: Poor Binding to Human Serum Proteins and Toxicity Facilitation**

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The goal of this presentation is to review how glycoproteins in human blood and serum are able to bind to ricin which is a highly toxic carbohydrate-binding protein. It was also an objective to show the capabilities of lectin blotting of electrophoresis gels in providing information on the characteristics of proteins found in human blood.

This presentation will impact the forensic science community by providing some insight into the extent of binding of serum glycoproteins by the toxin ricin. The poor binding of serum glycoproteins by ricin suggests that the glycoproteins in this body fluid may not effectively compete with cell surface proteins for ricin binding. This suggests that serum may not ameliorate ricin toxicity as might have been predicted because it contains glycoproteins that bind to the lectin RCA-I which has similar carbohydrate binding specificity to that of ricin.

It has been proposed that binding of ricin to proteins in human blood may affect its bioavailability, and, hence, its capacity to kill cells. Ricin binds to galactose residues in terminal beta glycosidic linkages on glycoproteins. Its binding to cell surface proteins and lipids which bear these structures allows ricin uptake by cells subsequently causing cell death.

Samples of 1mg/ml of the purified serum proteins, alpha 2 HS glycoprotein, transferrin, and immunoglobulin G were mixed with an equal volume of SDS gel electrophoresis buffer which contained 10mM dithiothreitol and denatured by boiling. Six different human serum samples were diluted 20-fold in SDS gel electrophoresis buffer which contained the reducing agent dithiothreitol and the proteins were also denatured by boiling. Sample volumes of 20ul were electrophoresed in 4-20% gradient polyacrylamide gels and electrotransferred to nitrocellulose. Blots were probed with the following biotinylated lectins: ricin; ricinus communis agglutinin-II; sambucus nigra agglutinin; and, concanavalin A. The blots were developed by using streptavidin-alkaline phosphatase which was followed by the chromogenic blot substrate combination of 5-bromo-4-chloro-3 indolyl phosphate and nitroblue tetrazolium.

Six serum samples and the purified human proteins, transferrin, immunoglobulin G and alpha2 HS glycoprotein were separated by SDS gel electrophoresis. Total protein staining of the electrophoresis gel with Coomassie Brilliant Blue dye revealed the typical pattern of protein bands for the serum samples. A predominant protein band of approximately 66,000 Daltons corresponding to serum albumin was present in all serum samples. Strong protein bands at 50,000 and 25,000 Daltons (immunoglobulin G) and 75,000 Daltons (transferrin) were also present in the serum samples by comparison with the purified proteins which were electrophoresed on the same gel. Alpha2 HS glycoprotein is heavily glycosylated and does not stain well with Coomassie Blue but was detected in the sample of the purified protein as bands at a position of 50,000 Daltons. The carbohydrate structures present on the purified proteins have been studied in detail by other investigators and those on transferrin and immunoglobulin G would be expected to be able to bind to ricin by blotting electrophoresis gels. Other lectins of known carbohydrate-binding specificity were used in this study as controls to show that the lectin blotting technique was working properly. Concanavalin A (Con A), sambucus nigra agglutinin (SNA), and ricinus communis agglutinin have binding specificities that allowed prediction of their binding to the purified proteins and could be used to help interpret the results with ricin.

As expected, Con A bound to purified transferrin and the 50,000

Dalton polypeptide of immunoglobulin G which contains high mannose and biantennary structures but poorly to alpha2 HS glycoprotein. SNA binds to carbohydrates that contain alpha 2-6 linked sialic acid and to purified transferrin and alpha2 HS glycoprotein but not to immunoglobulin G which usually contains little or no sialic acid. Sialic acid molecules are sometimes linked to galactose on proteins and would prevent binding of ricin to such glycoproteins. From these results, it will be possible to later perform experiments involving enzymatic removal of sialic acid to see how ricin binding is affected and to use SNA as a control. RCA-I is a lectin that binds terminal beta-linked galactose on glycoproteins and might be expected to bind the same glycoproteins as ricin. RCA-I bound strongly to many serum proteins and also to transferrin and to the 50,000 Dalton polypeptide of immunoglobulin G, both of which have been characterized as containing terminal beta-linked galactose. RCA-I did not bind to alpha2 HS glycoprotein which instead is known to contain large amounts of sialic acid. Surprisingly, ricin exhibited comparatively weak binding to immunoglobulin G and transferrin and serum proteins and no binding to alpha2 HS glycoprotein.

From these results it was concluded that ricin showed poor binding to serum proteins whereas the related protein RCA-I, which comes from the same plant, bound strongly to serum proteins. The poor serum protein binding by ricin could help explain its extreme toxicity because proteins in blood would compete poorly with ricin for binding to cells.

**Ricin, Serum, Blood**

## **A25 How Does Sampling Strategy and Phone Type Influence the Generation of a DNA Profile Collected From Cell Phones?**

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The goal of this presentation is to demonstrate the differences in DNA quantity and quality collected from different areas of cell phones and the influence of environmental exposure on the generation of a DNA profile by collecting samples from cell phones recovered after an extended period of time in an outdoor context.

This presentation will impact the forensic science community by demonstrating effective sampling strategies when cell phones are found at crime scenes.

DNA analysis represents an important investigative tool for crime scene investigations. DNA can be collected from a variety of objects and analyzed in order to aid in human identification or link an individual to a crime scene. For example, published case reports have demonstrated that DNA profiles can be recovered from items such as steering wheels, pens, contact lenses, and even food products. Cell phones represent a common item that is used by a large portion of society and thus likely to be recovered as evidence from crime scenes. Cell phones are objects that are extensively handled due to frequent use by an individual and therefore represent a good surface for collecting a DNA sample. A 2007 study investigated the ability to obtain DNA profiles from flip phones; however, no studies have been conducted that systematically investigate the ability to obtain DNA profiles from other types of cell phones.

Different areas of cell phones are subject to different types of contact with the human body. For example, the keyboard mostly comes in contact with the human fingers, the backplate mostly comes in contact with the palm of the hand, and the screen mostly comes in contact with the ear, cheek, and mouth regions of the face. Different degrees of handling and different avenues of DNA deposit, namely sloughed skin cells, sweat, or

saliva, suggest that there may be an optimal location for collecting DNA samples from a cell phone. Furthermore, different areas of the cell phone are subject to different types of environmental exposures with some areas being more vulnerable to environmental variables or contamination from extraneous DNA.

This pilot study investigates the best sampling strategy for obtaining analyzable DNA from several locations on three types of cell phones and explores the possibility of obtaining amplifiable DNA from cell phones that have been exposed to the environment for an extended period. The three cell phone types chosen for this study include handheld, flip phone, and touch screen cell phone models. Samples were taken from the keypad, backplate, and screen of each cell phone after 24-hours of use and again after placing the cell phones in an outdoor location that left them vulnerable to temperature fluctuations, rainfall, and sunlight (UV light). The samples were collected using sterile swabs. DNA was extracted using a common commercial DNA extraction kit, and quantified via quantitative PCR. DNA profiles were generated to establish whether the DNA samples represented a mixture of multiple donors or if a major contributor could be established and whether environmental exposure interfered with the DNA typing process.

DNA was collected from all samples taken from the handheld and flip phones, and 67% of the samples collected from the touch screen phones contained DNA of high enough quantity for DNA typing. The data suggest that optimal sampling locations vary by cell phone type and that samples collected from cell phones can produce complete profiles although exposure to environmental elements resulted in some loss of alleles. Single contributor profiles consistent with the user were produced from samples collected after 24-hours of use from the keyboard of the touch screen and flip phone models, and from the backplate of the handheld model. After seven days of environmental exposure, all DNA profiles consisted of a mixture or partial profile in which some of the user's alleles were detected, suggesting that environmental exposure for even a short amount of time can negatively influence DNA typing results.

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#### **DNA, Cell Phones, Sampling**

## **A26 Assessing the Degree of Similarity Between Accidental Patterns on Shoeprints Associated With Wearers That Participate in Shared and Independent Activities**

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After attending this presentation, attendees will understand the degree of similarity between accidental characteristics based on the context in which footwear is used, how accidental characteristics change with wear over time, and how imaging science and semi-automated numerical methods can assist with comparisons.

This presentation will impact the forensic science community in three ways. First, it will comment on how rapidly, and to what extent, wear-derived random accidental characteristics develop on the outsoles of individuals that participate in shared versus independent activities. Second, it will assess the divergence of an accidental pattern with continued wear, taking into account the fact that new accidental characteristics may randomly develop, while existing accidental characteristics may randomly erode. Third, this research illustrates the utility and reliability of using imaging science and semi-automated numerical methods to quantify the similarity in accidental patterns that vary with continued use and wearer-context.

The goal of this research is to address the similarity and rate at which wear-derived random accidental characteristics develop on the outsoles of

individuals that participate in shared versus independent activities. To carry out this research, two groups of volunteers were solicited and provided with new, approved footwear. The first group was asked to wear the footwear while repeatedly participating in shared group activities over a three-month period of time; the second group was permitted to wear the approved footwear while carrying out daily independent activities. At predetermined step-intervals, participants submitted their footwear for analysis which consisted of data: (a.) acquisition; (b.) registration; (c.) segmentation; (d.) processing; and finally, (e.) comparison.

The actual data in this experiment consisted of 600 pixel-per-inch (PPI) scans of footwear outsoles and impressions acquired using an established Magna brush method. Once acquired, the digital scans were registered to a common two-dimensional coordinate system, removing translational and rotational variations from the footwear impressions that cannot be avoided during the data acquisition step. Once registered, the digital images were examined for the presence of individualizing characteristics that represent the accidental pattern associated with the footwear. Once identified, these individualizing characteristics were segmented from the background invariant class characteristics that are shared by all footwear in this study. Next, each image was divided into small pixel blocks to generate a one-dimensional feature vector. Using the position and area associated with each identified accidental characteristic in the segmentation image, a feature vector representative of each shoe was automatically populated. The resulting accidental pattern was then compared to itself, thereby generating a known-match distribution. Each accidental pattern was also compared to all other impressions generating a known non-match distribution. The known-match and known non-match distributions generated by wearers that participated in shared versus independent activities were compared to determine how wearer-context impacts the degree of similarity in accidental patterns. The within and between group known-match and known non-match distributions associated with a given step-interval were also compared with each other and over time to investigate the rate at which the similarity in accidental patterns diverge with continued wear, including an assessment of the rate at which accidental characteristics randomly develop and erode.

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#### **Footwear, Correlation, Semi-Automated**

## **A27 A Geometric Morphometric Approach to Fingerprint Analysis**

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After attending this presentation, attendees will learn about efforts utilizing geometric morphometric tools for the spatial analysis of fingerprints to determine fingerprint uniqueness.

This presentation will impact the forensic science community by adding an assessment of this spatial analytical approach and the establishment of a statistical basis for determining fingerprint uniqueness.

Geometric morphometrics, a spatial statistical modeling method, is useful for quantitatively studying biological shape variation. Geometric morphometric analyses include methods from multivariate statistics, non-Euclidean geometry, biometrics, and digital graphics that preserve biomathematical aspects of the objects being analyzed. Furthermore, morphometrics includes a collection of readily available statistical tools (e.g., NTSYSpC and R). Within the forensic science community, forensic anthropology has led the way in exploring the applicability of geometric

morphometric techniques. Examples include analyzing mandibular morphology and craniofacial landmarks, performing a three-dimensional virtual reconstruction of a fragmented cranium, and estimating pediatric skeletal age.

For this study, a sample of fingerprints taken from the Oregon population have been utilized for geometric morphometric analyses including characterization, pattern recognition, and probabilistic modeling (i.e., determining the areas where differences are concentrated among friction ridge contours) in an effort to fully ascertain the relevance and efficacy of this spatial approach to resolving fingerprint uniqueness. Procedures for the selection of landmarks (e.g., specific minutiae primarily associated with deltas and cores), the superimposition of fingerprint images (i.e., Procrustes), the visualization of shape change (i.e., thin-plate spline), the ordination of superimposition data (i.e., principle components analysis), and the application of multivariate statistics were established.

A comparison of typical approaches for conducting geometric morphometrics with employment of a geographic information system (GIS) to emulate morphometric techniques is also presented. GIS is a collection of hardware and software components that integrate digital map elements with relational database functionality. The strength of GIS lies in its inherent ability to integrate, store, edit, and analyze spatial features and relationships, as well as the query and display of spatial information. These systems include traditional mapping capabilities (e.g., land surveying and aerial photography) and provide users with tools to interactively search and analyze any spatial information, including fingerprint space. GIS is increasingly being applied to the analysis of the positions, patterns, and relationships between objects located in a defined space. These objects include discrete entities expressed as points, lines, or polygons. Collections of objects in a defined space may be linked or associated with one another geometrically or by functional associations. GIS-based spatial analysis is very similar in concept and scope to graph theory in discrete mathematics. Techniques in spatial analyses include data modeling, image processing, grid algebra, surface analysis, and network analysis and visualization. The GIS-based tools available for spatial analysis have grown exponentially in recent years, all driven by the practical need to understand, predict, and model relationships between objects located in space. Our geometric morphometric procedures were emulated in GIS which was initially developed for meeting other objectives associated with ongoing fingerprint research being conducted in our laboratory. The impetus for this comparison of geometric morphometric techniques using a GIS platform included minimizing data handling and increasing overall efficiency in spatial analysis.

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**Fingerprints, Geomorphometric Morphometrics, GIS**

## **A28 Forensic Science Ethics and Criminal Prosecutions: Missing Pieces**

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After attending this presentation, attendees will better understand the interconnection between professional conduct and the “Brady” obligation of prosecutors.

This presentation will impact the forensic science community by discussing the importance of communication between government laboratories and prosecutors.

This presentation will examine the role of ethics in forensic science. First-rate Codes of Ethics already exist in a variety of sources: scientific societies; accreditation bodies; certification associations; and within parent organizations that house forensic science laboratories. Codes of ethics

serve to help practitioners understand their obligations to the criminal justice system and to their profession. However, failure to abide by codes of ethics and even sanctions resulting from breaches of ethics, may not affect a trial in which an expert is a witness.

The situation is complicated because there is no clear legal mandate for an employer to turn over information of ethical misconduct to the prosecution. In addition, it is uncertain whether a government body employing an individual sanctioned for ethical wrongdoing by a professional organization would even know of such that sanctions were imposed. Ethical sanctions are often considered confidential matters. Further, there is no requirement for government experts to be members of professional associations having Codes of Ethics. Thus, an expert who engages in unethical practices, but who is not a member of a professional society, is not bound by that society’s code.

Under Brady, prosecutors have a legal duty to disclose information and to inform the defendant of information deemed to be material that could influence the outcome of a trial or could impeach the integrity of an expert witness. Impeachable information would likely include ethical sanctions. Presumably, ethical misconduct, and especially sanctions from a professional society, would be considered Brady material. But how would a prosecutor become aware of an ethical breach of ethics?

Here is a hypothetical: A member of a professional association, e.g., the American Academy of Forensic Sciences, violates ethical standards and is censured for that conduct. Let’s say he or she is removed from the organization. Would such information be routinely provided to the individual’s employer that the person was censured? If a censured individual worked for a public forensic science laboratory, that information may be considered “Brady material” by the prosecution. But how does the prosecutor who calls that individual know of the censure? It could be argued that the information should be turned over to the defense. As it stands, the public lab administrator may or may not be unaware of the ethical breach. If the results of a professional society’s ethics investigation are turned over to the individual’s employer, does the lab have a legal duty to advise the prosecution? Does the professional association have a responsibility to advise the employer, the courts, the public, or the prosecution of such matters?

A solution to this problem is likely some form of government oversight of forensic science to establish rules of ethical conduct within the profession. Simply establishing a uniform Code of Ethics as the National Academy of Sciences report on forensic science recommends does not fully address the current situation. In addition, courts at some point need to define what the Brady obligation means, not only for the prosecution but to the Government’s forensic science delivery system.

**Forensic Science Ethics, Brady Material, Discovery**

## **A29 Legal vs. Scientific Proof: And Never the Twain Shall Meet?**

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After attending this presentation, attendees will: (1) understand the definition of legal proof and scientific proof; (2) understand the difference

between the two kinds of proof; (3) understand the criteria for proof that forensic scientists need in court; and, (4) understand the criteria for proof that attorneys need to establish in court.

This presentation will impact the forensic science community by providing an opportunity for dialogue between the forensic science and legal communities so that they can have a better understanding of each other's criteria for proof in court.

In a criminal case, the goal of the prosecutor is to prove that the accused is guilty of the crime(s) charged, including all of the elements of each crime. The prosecutor must prove his case "*beyond a reasonable doubt*." If, in a murder trial, a gun possessed by the accused was suspected of firing the fatal bullet, the prosecutor's task is to prove that the fatal bullet was fired from the weapon. If the accused deposited blood at the scene of the murder, the prosecutor must try to prove that the blood came from him. This trial is going on in real time and the evidentiary questions must be answered with today's knowledge, now. Naturally, this begs the question: what constitutes proof beyond a reasonable doubt?

Science, including forensic science, doesn't see things in the same way as the law. Science is a constantly moving target. As scientists do research, more data is collected and conclusions may change. As science advances, so does the ability of forensic scientists to associate items of evidence with people or objects. What was true 25 years ago may not be true now. For example, back then, there was no way to reliably associate blood evidence with one particular individual. Today, DNA typing has made that possible. What will happen 25 years hence? For many years, it has been accepted, almost without question, that a bullet can be traced to a particular rifled weapon. Now, these conclusions are being questioned. All of this means that the concept of scientific proof changes as advances in science change, but the concept of legal proof doesn't change. All of the elements of a crime must still be proven beyond a reasonable doubt. Obviously, the particular elements that must be proven are unique to each case.

This program will examine the issues of legal and scientific proof. Is there a clear understanding of the two types of proof among the actors in the adjudicative process including the scientists? What are the best ways of reconciling the problem of legal proof being an immediate, static set of requirements, whereas the elements of scientific proof of a given concept change with time? How does this impact the appeal of adjudicated cases?

To get at these and related questions, we are convening a panel of distinguished experts in the legal field and in forensic science. They will be given a series of questions that will hopefully get at the issues of proof. Time permitting, questions will also be accepted from the audience.

**Legal Proof, Scientific Proof, Attorney**

### **A30 A Philosophical View of Forensic Science: Native and Non-Native Principles That Form the Foundation of the Discipline**

*Max M. Houck, PhD\*, Analytic Services, Incorporated, 2900 South Quincy Street, Suite 800, Arlington, VA 22206*

After attending this presentation, attendees will gain insights into the philosophical foundations of forensic science and enhance their perspectives on the basic theories and principles that may guide this discipline.

This presentation will impact the forensic science community by influencing how forensic scientists think about their discipline and profession and may guide thinking on basic research that is needed and has been called for by the forensic community, national groups, and critics.

This presentation offers a philosophical view of forensic science that provides at least some of the necessary underpinnings to conduct its work and research. In this view, forensic science is seen to have three native principles that form a philosophical basis: other principles or concepts; borrowed from related historical disciplines; and fill in necessary working modes.

The first native principle is classification. Any collection of objects, real or imagined, is a set; set theory is the branch of mathematics that studies these collections. Basic set theory involves categorization and organization of objects and involves operations such as set union and set intersection. Advanced topics, including cardinality, are standard in undergraduate mathematics courses. The notion of set is undefined; the objects constituting (its members or elements) the set define it. The members of a set may be real or imagined but membership criteria for a set should be definite and accountable. Forensic science's taxonomies are additive to the taxonomies of nature and the manufacturers, although are initially based on them. Forensic science has developed an enhanced appreciation for discernment between otherwise similar objects but hasn't exploited them to their benefit. Thus, although classification is an inextricable part of forensic science, forensic science does not necessarily truck in fixed taxonomies.

The second native principle is uniqueness. Uniqueness comes with assumptions that affect the resolution of analyses. The first assumption is that all things are unique in space and, thus, their properties are non-overlapping. The second assumption is that properties are constant with time. The assumption of uniqueness of space is considered axiomatic and, therefore, an inherently non-provable proposition: The population size of "all things that might be evidence" is simply too large to account. A statistical analysis is therefore warranted when uncertainty, either of accounting or veracity, exists.

The third native principle that guides forensic science is the exchange principle, which posits that when two items come into contact, information may be exchanged; the results of such a transfer would be proxy data. Because forensic science demonstrates associations between people, places, and things through the analysis of proxy data, essentially all evidence is transfer evidence.

Principles from other sciences, mainly geology, apply to forensic science. The first is Uniformitarianism, which states that natural phenomena do not change in scope, intensity, or effect with time. Paraphrased as "the present is the key to the past," the principle implies that a volcano that erupts today acts like volcanoes did thousands or millions of years ago; geologists can interpret proxy data from past events through current effects. Likewise, in forensic science, bullets test-fired in the laboratory today are comparable to bullets fired during the commission of a past crime. The same is true of any analysis in forensic science that requires a replication or reconstruction of processes in play during the crime's commission.

Three additional principles from geology applicable to forensic science are of

- Superposition, in a physical distribution, older materials are below younger materials unless a subsequent action alters this arrangement;
- Lateral Continuity, disassociated but similar strata (layers) can be assumed to be from the same depositional period; and
- Chronology, the notion of absolute dates in a quantitative mode and relative dates in a relational mode, that is, older or younger.

A forensic example of the principle of superposition would be the packing of different soils in a tire tread, the most recent being the outermost. A good case of lateral continuity would be the cross-transfer of fibers in an assault, given that the chances of independent transfer and persistence prior to the time of the incident would be improbable. An example of absolute chronology in forensic science would be the simple example of a purchase receipt from a retail store with a time-date stamp on it; examples of relative chronology abound but can be thought of as a layer cake, with the older layers at the bottom.

**Philosophy, Foundations, Principles**

## A31 Alternative Models Promote the Self-Regulation of the Forensic Enterprise

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The goal of this presentation is to identify alternatives for integrating key organizations and stakeholders into coordinating, guiding, and regulating aspects of the forensic science profession to enhance its effectiveness, reputation, and professionalism.

This presentation will impact the forensic science community by stimulating a discussion and debate among its stakeholders about oversight and compliance alternatives for forensic science based on critical precepts needed to strengthen the profession.

There is little fundamental disagreement about the areas of forensic science needing improvement and standardization when forensic services are considered globally. Studies conducted periodically over the last 25 years by government-funded working groups, committees, and organizations, have identified similar concerns about shortcomings in forensic science, from fundamental education to consistent standardization in analysis methods to continuing need for research. The National Academies of Science National Research Council's *Strengthening Forensic Science in the United States: A Path Forward* is only the latest study to echo these needs and enjoys the greatest current visibility.

As the *NAS Report* reminds us, forensic science has no overarching structure or unifying entity able to provide direction, guidance, or oversight to the profession. The solution proposed by the *NAS Report* is the creation of a federally operated regulatory body, the National Institute of Forensic Science. This is not the only viable model to fill this gap.

Many laudable organizations originated to improve distinct aspects of forensic science, including accreditation, certification, sharing professional information and research results, and educational curricula. All have contributed significantly to advancing the profession. Much of the work is voluntary because most of the organizations have little to no professional staff, or their staff function is limited to operations that focus on the organization. These aggregate organizations represent the main professional aspects of the forensic enterprise and their activities need to shape the forensic profession. What is lacking is a strategic architecture and processes to coordinate and synchronize these aspects, and more, of the forensic science profession. Without such a strategic approach, professional progress will continue to lag behind expectations and need.

A National Forensic Science Board (NFSB) could coordinate forensic programs and activities nationwide. The US forensic science industry has many of the resources in place needed to lead and significantly advance all aspects the profession. What we are lacking are consistent coordinated efforts. The NFSB concept proposes to fill that gap.

Several models are available for forensic science to use as a starting point to build this strategic architecture. They include the National Safety Transportation Board (NTSB), a government entity, and the National Fire Protection Association (NFPA), a private, nonprofit. What both organizations have in common is that they have no regulatory authority and yet, both are the undisputed authoritative voice and promulgator of standards in their respective areas. Through proven deliverables meeting public need, each organization has gained respect and built trust. This has given them the authority they need to positively guide, impact, and strengthen their industries.

The NFSB model takes a similar approach. It proposes to integrate and synchronize the on-going efforts leveraging the subject matter expertise that has been developed by each of the recognized forensic professional organizations. It would also ensure that all the stakeholders that have

labored so diligently to identify needs and propose solutions are heard, including the legal community, victim advocates, wrongfully accused, community needs, laboratory management, and individual examiners. With non-regulatory oversight, a collaborative, holistic approach can be developed to gain an accurate understanding of capabilities, resource gaps, and profession requirements and to address the profession's needs.

Some key concepts for how such a Board might be organized and operate include:

- Serving as an independent advisory body for the Executive, Legislative, and Judicial Branches;
- Providing consistent leadership and oversight in the furtherance of excellence and reliability in the forensic sciences by issuing recommendations on topics that include, but are not limited to:
  - Proper analytical procedures and processes,
  - Appropriate formal education,
  - Initial and ongoing training requirements for forensic practitioners to prepare for and maintain analytical competency,
  - Appropriate training for forensic laboratory managers,
  - Methods for ensuring competency;
- Acting as an investigative body for and issuing recommendations on acute forensic science issues, including:
  - Receiving, investigating, and addressing stakeholder concerns and complaints,
  - Evaluation of laboratory failures,
  - Appropriate use and limitations of forensic analyses or procedures,
  - Current research needs for forensic disciplines;
- Providing consistent direction and support to forensic science discipline groups, that includes:
  - Recommending certification and accreditation changes,
  - Defining the purpose, responsibilities, and expectations of practitioners,
  - Coordinating, defining, and prioritizing the research needs,
  - Reviewing and promulgating work product as authoritative forensic guidance,
  - Supporting professional staff.

There are several significant barriers to creating and operating a model like a NFSB, all of which can be overcome. Financial resources are not the primary hurdle; the costs to start and sustain such a Board with professional staff are anticipated to be modest. Three major challenges to launching this concept are:

1. Territoriality: Forensic science has demonstrated a tremendous capacity for volunteerism. Until the profession can move beyond immediate, operationally-discrete functions (with their perceived political threats, concerns, and challenges) and trust in a larger process that will encompass, recognizes, and addresses our collective concerns, significant collective progress will continue to be stymied and limited to individual initiative and its necessarily limited scope of success.
2. Inertia: Any undertaking of this magnitude and scope requires managing expectations and building relationships that will transcend the individual needs of any one forensic organization.
3. Fear: Any proposal to accomplish a model like what is outlined for the Council will have naysayers; no plan will be quite right for all parties. Leadership will be required to stem inevitable territoriality, to lead people to act on their better natures, and to provide a consistent vision of goals and outcomes.

The forensic community needs to decide its fate actively, rather than respond to sniping, attacks, and political pressures; however, this includes considering and possibly embracing stakeholders' and critics' perspectives. Failure to coordinate and synchronize the forensic profession's collaborative efforts will result in continued misunderstandings among stakeholders and critics, resulting in an increasing loss of faith in forensic science and, ultimately, the criminal justice system.

This presentation will address alternatives to implementing and operating a National Forensic Science Board.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army, the Department of Defense, or the Los Angeles County Sheriff's Department.

**Forensic Science Management, Operational Policy Oversight, Forensic Science Board**

## **A32 A Market History of the United Kingdom's Forensic Science Service**

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After attending this presentation, attendees will provide the audience with a historical review of the origins of the UK's Forensic Science Service, its development from regional laboratories into a centralized national agency to its scheduled demise as a government-owned company in 2012. The participants will gain a better understanding of the internal and external forces that created, shaped, and debilitated this internationally-recognized forensic leader.

This presentation will impact the forensic science community by presenting the "lessons learned" aspect of one of the core issues facing forensic science: how to provision, allocate, and manage scarce resources. The Forensic Science Service has become an unintended case study in the on-going discussion of the best methods of providing forensic services to a criminal justice system. This review will help inform the community of the base facts that led to the current situation and provide insights for future forensic policy worldwide.

*"You know why there are so many whitefish in the Yellowstone River? Because the Fish and Game people have never done anything to help them." —Russell Chatham, Silent Seasons, 1978*

The Forensic Science Service had a long and distinguished history. Originating from proposals to the Home Office in 1929 for the establishment of a national police college with scientific laboratories to support police investigations and to undertake research, it coalesced in the 1930s and 1940s with the combining, relocating, and creation of laboratories for casework and research around the UK. Throughout the 1970s, the Home Office Forensic Science Service (FSS) created six purpose-built regional laboratories to replace the pre-war facilities. The Metropolitan Police (London) laboratory was merged with the FSS in 1996.

Up to 1990, the FSS was the only significant forensic provider in England and Wales. Funded by government grants and proportional fees paid by regional police agencies, the FSS was a wholly-governmental entity. Between 1979 and 1987, the staffing levels in the FSS remained fairly constant and all of the staff were civil servants. The fee structure system was not transparent and the true costs of the forensic support was hidden amongst the myriad of other government services the police 'bought'; additionally, large forces that were only occasional users of forensic services paid dearly. Nevertheless, through 1987, the demands on laboratory services grew rapidly as the number of operational police officers deployed increased, recorded crime increased, significant changes in the forensic science 'toolkit' (such as DNA) were introduced, and quality assurance and standardization across the regional laboratories increased their administrative burden. This led to a growing dissatisfaction amongst the police users about the FSS's timeliness; from the FSS's point of view, police were unable to forecast their demand accurately.

The Thatcher government sought mechanisms that identified elements of the civil service which would benefit from being given more autonomy and managerial and budgetary control. These "Executive Agencies" emerged from civil service reforms and were reorganized as discrete 'business units' within central government departments to deliver their services directly. Executive Agency was seen as the appropriate vehicle for

change in the FSS to regulate the demand and supply of its service. In this mode, the FSS would become more flexible, responsive, and provide "value for money." In 1991, the FSS became an Executive Agency of the Home Office. At the same time, the FSS introduced a "product-based" charging model for services, each product having a unit charge or a time charge. All of the FSS costs had to be supported by earned revenue but, as an Executive Agency, the FSS was a non-profit making organization. Monies earned over and above costs were returned to the police; any shortfall was supported by the Government as a short-term loan and had to be repaid in the following year.

While the FSS had 100% share of the available forensic market, other entities had seen the opportunity to enter this potentially lucrative field: Executive Agency had opened the market. Client police forces shared FSS price lists with competitors and asked for quotes, particularly in "commodity" market sectors, where the police were buying "tests" rather than "services." For the police, the increasing competition to FSS meant they began to have choices in forensic science supplier and the provision of some services, like DNA profiling, became very price sensitive.

In 1999, the legal position of the FSS changed from that of Executive Agency to that of Trading Fund status. The FSS remained part of the Home Office, its parent government department, but the advantage of this changed legal status was that it could retain its income from operating activities and use this to meet its expenditure. In effect, the FSS had to operate like any other commercial business and the organization was required to recover its full costs and earn the appropriate return on capital employed. However, the Local Government Act (1999) required public bodies, including Police Authorities, to obtain "Best Value" in procuring their services. This required Police Authorities to challenge suppliers and to competitively tender request for services, including the procurement of forensic services; this policy directly affected the FSS's market share. The legislative aim was to encourage a more competitive market and no single supplier would win the entire contract regardless of how competitive a bid was made. No matter how competitive FSS made their bids they could only lose work to the competitor organizations.

Increased competition led to the FSS faltering and it was recommended that a new structure, that of a Government-owned company (GovCo), would help the FSS adapt; the goal was to eventually change the FSS into a Public Private Partnership (PPP or fully privatized) with the transition lasting no more than 12-18 months. The FSS became a GovCo in October 2005; however the staff, Unions, and clients for the FSS were fiercely opposed to the goal of a fully privatized company. The Government dithered and the momentum to create a PPP was lost. In 2005, the House of Commons Select Committee on Science and Technology (7<sup>th</sup> Report) was highly critical of the government delay in moving FSS Ltd from GovCo to PPP status. New police procurement legislation in 2008 split operational forensic science into a series of work packages and, for each work package, specified the services to be delivered, the quality standards to be met, and created a standard user specification. Under this procurement scheme, the forensic suppliers could only compete on price; innovation and improved customer service levels counted little. Financially on the ropes, the FSS closed three laboratories by March 2011, letting go 500 operational scientists in the process.

**Forensic Science Service, Market, Economics**

## **A33 Does the "Scientific Method" Apply to Forensic Science? Should It?**

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The purpose of this presentation is to dispel claims that there is a unitary scientific method and that this scientific method should apply to forensic science. The presentation will demonstrate that different sciences have their own methods for formulating problem to investigate and for gathering evidence. The presentation will also show that forensic science

is properly classified as a historical science like archaeology rather than an experimental science like chemistry. Finally, the presentation will explore the implications of such a classification for forensic science education.

This presentation will impact the forensic science community by understanding the distinction between “experimental” sciences and “historical” sciences and will also understand the differences in the methodologies employed by these two categories of science. They will also understand why forensic science is properly classified as a ‘historical’ science rather than an experimental science.

The National Research Council’s recent report *Strengthening Forensic Science in the United States: A Path Forward* (National Academies Press: 2009) in discussing forensic science education stated: “Forensic examiners must understand the principles, practices, and contexts of science, including the scientific method.” Many scientists and philosophers of science maintain that there is a unitary ‘scientific method’ that is applicable to all sciences. Some of these scholars believe that all science can be reduced to physics and that the research methods of physics should be applied across-the-board to all sciences. On the other hand, other scholars in the field of science studies (most notably Dean Henry Bauer in his *Scientific Literacy and the Myth of the Scientific Method* (University of Illinois Press: 1994) have argued that there is no unitary scientific method that is applicable to all sciences. For many of these commentators, this view is primarily a pragmatic recognition that different sciences deal with different subject matter and make use of different standards for selection of research problems and for the evaluation of evidence. Psychologists Hilary Putnam and Jerry Fodor have argued that there are logical grounds for dismissing the claim that all science can be reduced to physics. If such is the case, the different branches of science should have distinct logical structures and would have their own “scientific methods.”

Professor Carol Cleland has stressed the critical distinction between “experimental” sciences such as physics and chemistry and “historical” sciences such as astronomy, geology, paleontology, archaeology and evolutionary biology. “Experimental” sciences seek to formulate laws that predict the outcomes of future experiments, while “historical” sciences seek to reconstruct past events from traces left behind. “Experimental” sciences infer effects from causes; “historical” sciences infer causes from effects. While “historical” sciences may conduct experiments that superficially resemble those of “experimental” science, these experiments seek to find traces of past events predicted by specific reconstructions of those events.

Forensic science clearly belongs to the “historical” sciences rather than the “experimental” sciences. All the evidence examined in forensic science laboratories is the result of past events. Forensic examinations are conducted so that investigators can infer what crimes have been committed, who committed them, and how they were committed. In order to accomplish these goals, forensic scientists must sift through the myriad pieces of evidence found at the typical crime scene and determine the forensically relevant items of evidence and the appropriate examinations to perform on them. Such choices are guided by the forensic scientist’s understanding of the capability of specific items of evidence to distinguish between various possible reconstructions of the crime.

The classification of forensic science as a ‘historical’ science has important implications for forensic science education programs. While some forensic scientists (such as forensic anthropologists) have been educated in fields where reconstruction of the past is the norm, most forensic chemists and many forensic biologists come from educational backgrounds that stress the ‘experimental’ sciences. Consequently, forensic science education programs should place stronger emphasis on historical, reconstructive reasoning. This should be done through the use of simulated or mock crimes which students are called upon to investigate. The mock crime scenarios should include relevant as well as irrelevant evidence and the students should be required to identify the relevant items of evidence, select the most forensically useful methods of analysis, carry out examinations of the evidence, and weigh the likelihoods of different crime reconstructions.

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**Forensic Science, Scientific Method, Historical Science**

## **A34 The Forensic Science Laboratory in Kosovo**

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After attending this presentation, attendees will be introduced to the facilities and operation of the forensic laboratory in Kosovo.

This presentation will impact the forensic science community by describing the current capabilities of the Kosovo forensic science laboratory as well as future initiatives.

Kosovo is located in Southeast Europe with a population of 2.2 million people. A forensic science laboratory in Kosovo has never been organized at the level of most modern forensic science laboratories. This was made possible after the war of 1999 with the help and support from the United States. The United States Government/ICITAP provided 9.5 million dollars to support this project. Ideas and supporting materials for the construction of the forensic laboratory in Kosovo by the United States have greatly benefitted law enforcement in Kosovo. This poster presentation is an elaboration of areas of expertise that the laboratory offers as well as the current status and need for expansion. In particular, future areas of expertise are needed in order to achieve accreditation under ISO 17025 international standards.

The laboratory is 1000 square meters and employs 38 experts. Forensic laboratories in Kosovo are crucial to criminal justice system. Forensic scientists in Kosovo laboratories provide valuable information that aids in the investigation and prosecution of crime through the scientific examination of physical evidence. Their efforts carried out to the highest standards of scientific objectivity, integrity, and quality, give voice to the silent witnesses of physical evidence and contribute to the cause of justice. The addition of a LIMS (Laboratory Information Management System) has improved the laboratory’s ability to track the internal flow of evidence and case analysis. This system is used to track cases, analytical results, and evidence as it is processed through the laboratory. The LIMS system assists with the assignment of cases, case flow, and backlog control. LIMS can also generate and maintain case reports and allow for statistical analysis of the types of cases, number of cases, turnaround times, and other management data.

The Kosovo forensic laboratory also has an intensive quality assurance program which covers the following areas: staff qualifications, training, proficiency testing, administrative policies, technical procedures, security and evidence integrity, chemical reagent quality control, equipment maintenance and performance verification, documentation of laboratory analysis, and the review of casework, reports, and testimony.

The forensic laboratory in Kosovo is a full service laboratory offering a wide variety of services. Included is the analysis of controlled substances, toxicology, latent prints comparisons, firearms and tool mark identification, trace evidence examination, DNA analysis, and questioned document examination.

Technological equipment of all sectors and photos from the Automated Biometric Identification and Automated Fingerprint Identification Systems will be presented in order to stimulate a discussion and encourage the exchange of information. The forensic laboratory in Kosovo has significant needs that must be met in order for forensic services to continue to improve and meet the demands of ISO 17025. The forensic laboratory scientific staff is encouraged to participate in seminars and scientific conferences in the United States and in Europe in order to adopt techniques and newer methods in various forensic disciplines. Each sector in the forensic laboratory already has staff who are Associate Members in organizations such as European Network of Forensic Science Institutes and the International Association of Identification, and has members who participate in working groups within these organizations.

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**Laboratory, Forensic, ISO17025**

### **A35 Development of Optimized Recovery and DNA Typing Methodologies for the Analysis of “Touch and Contact DNA” Samples**

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The goal of this presentation is to provide an understanding of new methodology for the collection and DNA typing of epithelial cells collected from touch and contact DNA samples.

This presentation will impact the forensic science community by providing a characterization of the biological material in touch or contact DNA evidence and providing strategies for DNA recovery and analysis from isolated cells.

In forensic casework analysis, it is sometimes necessary to obtain genetic profiles from increasingly smaller amounts of biological material left behind by persons involved in criminal offenses. The ability to obtain DNA profiles from trace biological evidence is routinely demonstrated with so-called “touch DNA evidence” (generally perceived to be the result of DNA obtained from shed skin cells transferred from donor to an object or person during physical contact). While these studies clearly demonstrate the ability to obtain genetic profiles from trace biological evidence (e.g., paper and documents, keyboards, bedding and fabrics, shoe insoles, firearms and cartridge cases and drinking containers), they often employ a “blind-swabbing” approach and therefore fail to include an evaluation of the type of cellular or genetic material that may be present in such samples. This often results in the presumption that the DNA profiles are obtained from shed skin cells as opposed to, for example, saliva traces without any scientific basis for this assertion. This possible misrepresentation of the source of biological evidence could place undue weight to a given piece of evidence. It is therefore essential that methodologies for the analysis of biological material from touch and contact DNA samples allow for a skin vs. non-skin tissue source identification and allow for a demonstration of a direct link between the biological material and recovery of DNA profiles from it.

The goal of this work was focused on the development of collection strategies for the recovery of biological “particles” from contact DNA samples and also the recovery of DNA profiles from the collected particles. For initial method development, buccal epithelial cells were used as they would be expected to be more stable than shed biological “particles.” Buccal smears were prepared directly on a low retention polymer material. Individual or multiple buccal cells (five and ten cells) were then collected using a water soluble adhesive and immediately placed into a tube containing reagents for direct cellular lysis. Amplification of the lysates was then performed using LTDNA protocols. Using the developed collection and typing methodologies, STR profiles were obtained from as little as one buccal epithelial cell. No inhibitory effects from varying cell staining reagents were observed. With the success of the developed collection and typing methodologies developed, these methods were applied to the analysis of contact and touch samples. Contact samples were prepared by placing the low retention polymer material in direct contact with worn clothing items (shirts, pants, and hats) or human skin. The structural nature and quantity of the biological material presence was then determined with the aid of various staining techniques, with a possible nucleus observed in only a few particles within each sample. In order to obtain STR profiles from the contact samples, increased numbers of “particles” needed to be collected (e.g., 100 “particles” in some cases). The collection of only “nucleated” particles, if identified, may result in improved recovery of genetic profiles. Therefore, additional work will be needed in order to further characterize the nature of the biological

“particles” present in contact and touch samples in order to determine the most suitable type and quantity of “particles” needed for analysis. Despite the need for additional work, the results of this initial work provide an indication that it will be possible to perform a more comprehensive molecular-based approach to the characterization, analysis, and interpretation of trace biological material recovered from touch and contact samples.

**Touch/Contact DNA, Epithelial Cells, DNA Analysis**

### **A36 Internal Validation of GeneMapper®ID-X for Use in Forensic Casework**

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After attending this presentation, attendees will understand the need for an efficient software system to help streamline data analysis in laboratories experiencing a bottleneck of forensic DNA interpretation.

This presentation will impact the forensic community by demonstrating the effectiveness of GeneMapper®ID-X for analysis of both casework samples as well as an expert system for casework standards. Moreover, mixture analysis tools from GeneMapper®ID-X and ArnedXpert™ will be presented and compared.

Due to the implementation of robotic equipment to extract, quantitate, amplify, and detect forensic DNA samples, the bottleneck of forensic DNA analysis has shifted to data interpretation. There is now a need for computer software that maximizes efficiency and encompasses the resources needed for DNA analysis. Applied Biosystems’ GeneMapper®ID-X is one of the software systems capable of reducing this bottleneck and providing a suite of tools to assist in single source and DNA mixture interpretation.

The Palm Beach County Sheriff’s Office Forensic Biology Unit (FBU) DNA validation data was originally created, collected, and analyzed using Promega’s PowerPlex®16 amplification kit, two AB®3130xl platforms, and GeneMapper®ID v3.2.1 (GMID). The FBU recently purchased GeneMapper®ID-X v1.2 (GMID-X) for use with questioned casework samples and plans to utilize the expert system capabilities for streamlining the interpretation and review of known casework samples. The validation of GMID-X for use with casework included the following studies: known samples and stutter, NIST, non-probative, reproducibility, precision, contamination and analytical threshold, sensitivity and stochastic thresholds, and mixtures. The studies were completed according to the Scientific Working Group for DNA Analysis (SWGDM) guidelines. Original GMID data was analyzed with GMID-X and the results were compared.

The GMID-X software was able to produce accurate, reliable, reproducible, and concordant results between data obtained with GMID. Concordant allele calls, basepair, and RFUs were obtained between GMID-X and the originally analyzed data using GMID. The validation data demonstrates that GMID-X is suitable for use with forensic casework using PowerPlex®16 and the FBU’s two AB®3130xl platforms.

Upon completion of the validation of GMID-X for typical forensic samples, the validation of GMID-X as an expert system was initiated. GMID-X can automatically review allelic ladders, controls, and samples using user defined thresholds, and with color-coded process quality value flags (PQVs), it can assist in the manual review of single source samples. For the validation of GMID-X as an expert system, known single source samples were run through the software to determine the applicable peak quality values for the FBU laboratory. A workflow with different analysis methods and table settings distinct from those used by the FBU to analyze and interpret unknown casework samples was created.

GMID-X also contains a new Mixture Analysis Tool that is meant to assist analysts in deconvoluting two-person mixtures by performing

mixture calculations and helping provide analysts with a common platform from which to interpret DNA mixtures. Another mixture analysis tool that can be used in conjunction with *GMID-X* is *ArmedXpert™*. A mixture study was conducted with two mixture sets on both software systems in order to determine ease of use and establish a workflow for each mixture analysis tool. *ArmedXpert™* requires *GMID* or *GMID-X* to correctly size and generate DNA profiles prior to importing them into *ArmedXpert™* for analysis. Once a profile is imported, it can be compared to staff profiles, compared to other samples run in the same batch, compared to known standards, or sent to the mixture interpretation section of the software for up to three-person DNA mixture deconvolution. *ArmedXpert™* and *GMID-X* can also perform random match probability, combined probability of inclusion/exclusion, and likelihood ratio statistics.

Overall, *GMID-X* is one of several software systems available to reduce the forensic data review bottleneck. The implementation of an expert system into a laboratory's workflow will reduce the need for manual review of known samples by streamlining the analysis of typical forensic casework samples. *GMID-X* has been validated for use with casework samples and the foundation for its validation for use as an expert system for known single source samples has been established. *GMID-X* and *ArmedXpert™* are important tools available for DNA analysts to assist with the often time-consuming and complicated data interpretation of single source and mixture profiles. The generation of statistically appropriate calculations is another important advantage of these programs.

**GeneMapper®ID-X, ArmedXpert™, Validation**

### **A37 Evaluation of SampleMatrix® for DNA Storage**

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After attending this presentation, attendees will understand the benefits of using a non-freezing alternative for long term storage and transportation of DNA samples. Freezing is currently the most commonly used method for storing extracted DNA in many laboratories. Preserving good quality DNA for perpetuity is obligatory and mandatory by many forensic laboratory protocols in order to facilitate prospective and retrospective analyses. Storage requires a large number of freezers, maintenance and power costs, and back-up generators. An alternative to low temperature storage is the *SampleMatrix®*, commercially available as a synthetic polymer. Storage technology using *SampleMatrix®* mimics the natural molecular principles of anhydrobiosis by forming a thermo-stable barrier around the biological sample to protect it from degradation. The *SampleMatrix®* is widely used in academic institutions and is gaining acceptance by forensic laboratories.

This presentation will impact the forensic science community as the results of this study may provide a more environmental friendly and cost effective solution for storing aliquots of DNA samples. Previous studies that evaluated the efficacy of the *SampleMatrix®* to protect DNA extracts have utilized standard Combined DNA Index System (CODIS) Short Tandem Repeat polymorphisms (STR) primer sets to monitor DNA degradation. The original commercial multiplex CODIS STR kits amplified amplicon sizes ranging from 97-464 base pairs (bp).

Newer CODIS STR kits target even smaller amplicons (<300bp). Since DNA degradation is characterized by the fragmentation of larger DNA regions into smaller ones, it was hypothesized that monitoring for a decrease in the number of larger DNA fragments would be more informative while being easier than examining STRs. To test the hypothesis, DNA degradation was induced by storage of extracts in sealed

tubes at high temperature and DNA decay was monitored by SYBR Green qPCR utilizing four primer sets designed to amplify amplicons of 92, 250, 500 and 970 base pairs in size. The samples were tested from day one through 135 at a weekly interval for the first month and every three weeks for the remainder of the time period. The DNA samples (1, 5, and 20ng/μL) with and without the *SampleMatrix®* were heated to 37°C (equivalent to 1.046 years at room temperature for 135 days) or 50°C (equivalent to 2.575 years at room temperature for 135 days) for fast aging of the DNA and to determine the efficacy of the *SampleMatrix®*. This experimental procedure is designed to mimic exposure of the purified DNA samples to high temperatures such as during transportation, which according to according to FedEx shipping guidelines can occur at temperatures as high as 60°C, depending on the time of the year. Controls were maintained at -20 °C for an accurate comparison.

Preliminary results indicate that the *SampleMatrix®* is useful in preventing the degradation of DNA at low concentrations (1 ng/μL). When using the 1ng/μL DNA samples, significant differences in the qPCR cycle threshold (Ct) values between the control and experimental samples were seen (F-Statistics,  $p < 0.009$ , for all primer sets and both temperatures). Even when held at 50°C for 30 days, there were no significant (F-Statistics,  $p > 0.05$ ) differences in the Ct values of the control versus the experimental samples at 5 and 20 ng/μL for all primer sets in this study.

Additional experiments include another high-heat study using lower concentrations of DNA (<1 ng/uL), similar to what is expected to be recovered as "touch DNA" evidence. In summary, the *SampleMatrix®* may be useful in forensic laboratories, since it may guarantee good quality DNA for cold cases by eliminating DNA degradation caused by repeated freeze thaw cycles and the need to handle DNA on ice during processing.

**Long-Term DNA Storage, SampleMatrix®, DNastable®**

### **A38 The Role of DNA Stained Currency in the 2008 Lancashire Gang Robbery**

*Breanna Mead, MS\*, James Hayward, PhD, Benjamin Liang, PhD, Lawrence Jung, BS, Stephane Shu Kin So, PhD, Dominick Avento III, BS, and MeiLin Wan, MS, Applied DNA Sciences, Incorporated, 25 Health Sciences Drive, Stony Brook, NY 11790; and Simon Scott, and Paul Broxson, Blackburn Police Station, Whitebirk Drive, Accrington, Lancashire BB1 3, UNITED KINGDOM*

After attending this presentation, attendees will better understand the frequency of armed robbery of Cashand-Valuables-In-Transit (CViT) as well as the problems that these types of robberies are posing to the global economy. The history of the case solved by the Lancashire Police Department (United Kingdom) in 2008 dealing with a gang robbery will also be presented. This particular robbery resulted in over 70,000 British Pounds being stolen, and a cash transport guard being shot and wounded. Attendees will also learn about the role that DNA stained currency played in assisting detectives solve this particular case.

This presentation will impact the forensic community and/or humanity in demonstrating that CViT robberies have become a costly problem. Over £19.4million were stolen in 2008 in the United Kingdom alone. The transportation of cash and valuable items between financial institutions has long been a target of robberies in the UK. Regulations in the UK dictate that security guards protecting the cash and valuables are not allowed to carry weapons. Furthermore, vehicles utilized to transport valuable goods are also manufactured to be smaller than their US counterparts in order to fit onto much more narrow streets making them an easy target for thieves.

This has posed a great problem in the CViT field requiring an unconventional approach that can help in linking stolen cash to criminal activities. Conventional staining ink systems could not provide the information necessary for establishing traceability of the recovered evidence to the crime as they did not have an acceptable forensic tagging method. A taskforce was established that involved the Metropolitan Police Service (Scotland Yard), security companies, and experts in the field to

utilize forensic level tagging in cash staining dyes for the purpose of apprehending suspects and collecting forensic evidence. In order to face this issue, botanically derived DNA is combined into an exploding ink cartridge (also called a degradation unit) then placed into a cash transportation box. This cash box is then filled with cash or valuables that are to be transported. Should the box be tampered with in any way, the exploding ink cartridge will deploy and spray all of the items within the box with a botanical DNA-marked ink mixture.

In the Lancashire case, police apprehended a suspect as he was trying to use stained currency. The evidence was screened using gel electrophoresis for the presence of specific inks that are utilized in cash-in-transit boxes. After the initial screening process demonstrated that the essential inks were present, any DNA present on the currency was extracted. Primers specific for the botanically derived DNA utilized in cash-in-transit boxes were used to amplify any DNA present on the evidence. Once amplified, DNA was separated using capillary electrophoresis and analyzed using genetic software. Once the specific genetic combination was discovered from the stained notes, the combination was tracked back to the cash transportation box that the DNA was issued to which, in turn, had been reported as being stolen. With the help of cash-staining ink marked with botanically derived DNA, robberies dropped by 50% from 2009-2010, and as a result, the number of convictions from this novel technology is currently on the rise.

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**Cash-and-Valuables in Transit, Armed Robbery, Botanically Derived DNA**

### **A39 Comparison of Genetic Markers and Developmental Validation of the Multicopy LINE-1 Marker for Use in a Sensitive Real-Time Quantification Method**

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After attending this presentation, attendees will understand how to apply the Scientific Working Group on DNA Analysis Methods (SWGDM) criteria in a developmental validation of a new marker for forensic DNA quantitation of low copy number and degraded samples and the comparison with different genetic markers in use.

This presentation will impact the forensic science community by giving information on a new marker that can be used as an alternative tool for the quantification of degraded and low copy number samples.

Three different genetic markers were studied for use in a sensitive real-time PCR quantification method using a SYBR® Green detection system. The markers studied were: a long interspersed nuclear element (LINE-1), a multi-copy short interspersed nuclear element (*Alu*), and a reduced size short tandem repeat marker (mini TH01). The markers were compared on target specificity, sensitivity, linearity, accuracy, and precision. The LINE-1 and *Alu* methods were the most sensitive systems with the ability to detect down to approximately 1pg/μl. However, the LINE-1 method was able to remain linear up to 50ng/μl compared to *Alu* which experienced a loss in linearity at 10ng/μl. The LINE-1 method displayed more accuracy and precision than the other methods at three different concentrations of a known DNA standard (10, 5 and 1ng/μl). LINE-1 displayed the following values (mean ± standard deviation) for each sample: 8.6±0.23 for 10ng/μl, 2.7±0.06 for 5ng/μl, and 0.42±0.03 for 1ng/μl. Although the LINE-1 method consistently estimated values lower than expected, the system performed similar to Quantifiler® human DNA quantitation kit for the same samples. The LINE-1 and mini TH01 primers displayed better target specificity than *Alu* according to the melt curves generated by each.

In addition to these comparative studies, the LINE-1 method was tested on species specificity, population, stability, inhibition, and mock case work samples. The LINE-1 method performance met all the SWGDAM criteria. With the exception of primates, the LINE-1 primers do not amplify other species. The samples tested from individuals of known ethnic origin were all positive. The stability of the system was tested by analyzing DNA that was artificially degraded with the DNase I enzyme. As expected, the system indicated that the amount of quantifiable DNA present in the samples decreases as the amount of degradation increases. The system was also tested in the presence of common PCR inhibitors with and without the addition of bovine serum albumin (BSA) to the system. Finally, the system was tested on several common mock case work type samples including touch DNA samples and samples that are considered to be low copy number or degraded. Utilizing the LINE-1 marker appears to provide an adequate screening and quantification method for the analysis of forensic case work samples, specifically low copy number or degraded samples.

It is recommended that forensic DNA analysts become familiar with the developmental validation SWGDAM criteria and its application.

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**Real-Time PCR, Low Copy Number, LINE-1**

### **A40 Differential Extraction of Mixtures in Sexual Assault Casework Using Pressure Cycling Technology (PCT)**

*Deepthi Nori, MFS\*, Florida International University, 1340 Southwest 102 Court, Miami, FL 33174; and Bruce R. McCord, PhD, Florida International University, Department of Chemistry, University Park, Miami, FL 33199*

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After attending this presentation, attendees will understand the principles of pressure cycling technology and its applicability in analyzing mixtures from sexual assault casework.

This presentation will impact the forensic science community by providing a better understanding of how this novel technology can be used to perform selective lysis of a specific type of cells and decrease the analysis time by simplifying the extraction procedure.

Forensic DNA analysis has elevated the degree of confidence in the analysis and interpretation of evidence but the bottle neck that plagues the crime labs across the country is the tedious, time consuming protocols that require practice and expertise in analyzing mixtures. Organic differential extraction is the most commonly used method to isolate sperm DNA from sexual assault evidence. This two-step extraction procedure involves selective digestion of epithelial cells in the first step followed by isolation and digestion of the sperm cell pellet. The major disadvantages of this technique are incomplete separation of sperm and non-sperm fractions, particularly in samples that are overwhelmed by large numbers of female epithelial cells relative to sperm cells and the time-consuming nature of the process.

Pressure cycling technology sample preparation system (PCT SPS) involves the use of pressure pulses to disrupt tissues, cells, and cellular structures enabling the recovery of their components. Barocycler® NEP2320, a commercially available instrument from Pressure Biosciences Inc, is equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 45000 psi resulting in the lysis of cells. A working pressure range of 5- 45 kpsi, number of programmable cycles (1-99), duration of holding time at ambient pressure and at high pressure are the four parameters that can be controlled to achieve this objective. This can be used in conjunction with mechanical homogenization, temperature control by an external water bath, or commercially available extraction kits.

The current study involves the application of pressure cycling technology in the selective digestion of sperm cells from evidence mixtures collected from different substrates with an emphasis on the role of buffer

composition on sperm DNA yields. The cells were extracted in 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to 45000 psi pressure for 60 cycles. Samples were placed in specially designed PULSE™ tubes and introduced into the pressure chamber. This pressure treatment was followed by phenol/chloroform/isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with Promega Plexor® HY system.

According to previous studies, high selectivity and improved recovery with the reducing agent, Tris (2-carboxyethyl) phosphine (TCEP) indicated the potential for highly selective detection of sperm cells in comparison to the addition of detergents or changes in temperature. These observations were applied to mixture studies of evidence obtained from various substrates such as swab and fabric. Preliminary data indicates that pressure cycling technology has application in differential extractions indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells in the presence of appropriate buffers.

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### **Pressure, Sexual Assault, Differential Extraction**

## **A41 Increasing Efficiency in a DNA Unit Using Lean Six Sigma**

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The goal of the presentation is to show how implementation of Lean Six Sigma can lead to a decrease in backlog and a better working environment for analysts.

This presentation will impact the forensic science community by showing how the use of Lean Six Sigma can dramatically impact the workflow and throughput of forensic cases.

The number of DNA requests received in many public crime laboratories has increased such that backlogs exist and turnaround-times are extended. The Louisiana State Police Crime Laboratory (LSPCL) saw a 22% increase from 2006 to 2007 in the number of DNA requests submitted. The completion rate was not increasing at a rate to compensate for the increased submittals nor could the completion rate eliminate the backlog of requests that had accumulated. Turn-around-times exceeded a calendar year.

The Efficiency Improvement Grant provided the tools needed for LSPCL to change the technical workflow of DNA forensic analysis which allowed the backlog to be greatly reduced, case turn-around-time to be reduced, and productivity increased such that LSPCL can complete 100% of the DNA requests that are received each month. Management of the administrative work within the DNA Unit has also been changed to ensure that the capacity of the laboratory is maintained and no new backlogs are created.

LSPCL hired external consultants and engaged in several projects aimed at solving the current state problems and changing processes to ensure continued success. Through the development of Lean Six Sigma (LSS) methodology, these projects achieved all goals set forth and has led to a culture that is client driven, quality focused, and efficiency minded. LSS is the unique combination of Lean thinking and Six Sigma process improvement to form a thorough and comprehensive approach to quality improvement, process improvement, and the elimination of waste to produce a remarkably efficient and quality driven product. The combination of Lean thinking and Six Sigma variation reduction, when merged together, forms seven guiding principles. The principles are: (1) focus on the customer; (2) identify and understand how the work gets done; (3) manage, improve, and smooth the process flow; (4) remove non-value

added steps and waste; (5) manage by fact and reduce variation; (6) involve and equip the people in the process; and, (7) undertake improvement activity in a systematic way. DMAIC is the acronym that describes the seventh principal. It is a systematic improvement framework, and it is the framework that the LSPCL and consulting teams follow to make dramatic efficiency improvements. DMAIC stands for Define, Measure, Analyze, Improve, and Control. During the define phase of this project, a process map of the standard operating procedure was developed in which the twelve major steps of the process and the major functions under each step were identified. Additionally, a Value Stream Map (VSM) was developed that depicted the flow of cases and information through the laboratory. During the measure phase, current processes were measured to establish a baseline performance. Current state of cases and data for concepts such as turnaround time, total queue time (waiting to be worked), total process time, value added time (amount of time spent performing actual tasks), non-value added time, and the number of cases in progress (WIP) were measured. State spaghetti charts were created to graphically illustrate the physical movement of people and evidence throughout the laboratory. Next, the analyze phase focused on analyzing the data collected during the measure phase and investigating the causes of the problems, bottlenecks, backlogs, and defects uncovered during the previous two phases. Daily production meetings were implemented to discuss daily progress and workflow. Construction of a Level Load Chart was developed to help create flow throughout the laboratory. The improve phase involved three distinct segments: (a) generate ideas about possible solutions; (b) select the most appropriate solution; and, (c) plan and test the solution.

Laboratory processes and equipment were relocated to reduce motion waste, and standards of work were implemented. Unnecessary procedural steps were removed and a master hour-by-hour schedule was implemented along with a three week schedule of a proposed casework cycle. Teams of three DNA analysts performing DNA analysis on batches of 8-10 cases in a five day production cycle were created. The final phase, control, enabled the laboratory to put in place a Management System to continually monitor its output and adjust operations when the data indicated or when the customer's requirements changed. Visualization of current process and performance measurements were displayed. Data such as samples processed, cases received and completed, backlog, turnaround time, and status of unassigned priority cases are tracked visually on a daily, weekly, and monthly basis and analyzed for a constant, smooth output. LSS can be applied to any process or any industry. LSPCL used this methodology to enhance the technical scientific process.

As of June 2011, LSPCL has decreased its turnaround time from an average of 291 days in May 2008 to an average of 31 days with 95% of DNA requests completed within 30 days. LSPCL has tripled the productivity from 50 cases per month to 160 cases per month. The backlog has decreased from approximately 1,400 cases in May 2009 to 120. Total queue time decreased from 181 days in May 2010 to five days. Additionally, the number of samples completed per month has increased from 312 to 979 with an average number of cases completed per analyst per month increasing from 1.9 to 11.5 cases.

LSS methodology gives the user the necessary tools and a process that provides rapid and sustained improvements for technical or administrative processes. LSS was, and continues to be, a powerful management tool in the DNA forensic operations of the LSPCL. With turn-around-times of <30 days, a workflow that allows a DNA forensic request to be completed within 6 days, the capacity to work all cases submitted each month, and a backlog that is eliminated each month, LSS has proven its ability in the forensic laboratory. The methodology allowed LSPCL to meet all project goals and has afforded increased operational efficiency and increased quality and service to the agencies served. The real-time support provided to investigations is helping solve crimes and is thereby making the citizens of Louisiana safer.

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### **Increasing, Laboratory, Efficiency**

## A42 DNAgard®Tissue as a Room Temperature Microbial Preservation System for Forensic Soil Samples

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After attending this presentation, attendees will become aware of the different storage techniques used for soil evidence; DNAgard®Tissue (Biomatrica) as a biological soil sample preservation system; and microbial community profiling (MCP) as a forensic tool.

This presentation will impact the forensic science community by evaluating a novel use for DNAgard®Tissue as an in-lab or on-scene forensic sample storage/preservation medium.

DNAgard® consists of a specialized liquid solution that allows for the immediate stabilization of the DNA from cells and tissues. According to Biomatrica, this method allows for samples to be stored at room temperature for up to six months. In addition, this presentation will compare commonly used and novel storage techniques, e.g., length in storage per storage condition, as well as its impact on DNA quality and quantity. This will provide a basis for future research in microbial community profiling as a forensic tool and, in particular, how microbial DNA profiles may be used for evaluation of soil as evidence. The soil environment is dominated mainly by prokaryotes whose diversity can vary greatly. Bacteria are the most dominant form of these microbial communities found in soil whereby bacterial DNA profiling may provide an opportunity for linking soil samples with one another. MCP can easily be performed in forensic laboratories as many, if not all, crime laboratories have the required capillary electrophoresis genetic analyzers and PCR equipment to perform this analysis. Collection and storage of soil samples is a critical aspect in preparation for microbial DNA analysis. Traditional storage techniques used by forensic laboratories include either storing soil samples at -20°C or at room temperature. Traditional soil analysis often involves drying and sieving of samples prior to evaluation. While this may be essential for chemical and microscopic analysis of soil components and profiles, such procedures may have lethal and destructive effects on microbial communities.

The goal of this study was to determine the best method for storing soil samples for microbial DNA analysis. Seven storage conditions were compared: (1) room temperature; (2) -20°C freezer; (3) DNAgard®Tissue at room temperature; (4) -20°C freezer then air-dried; (5) -20°C freezer then oven-dried; (6) room temperature then air-dried; and, (7) room temperature then oven-dried. Each week, for a total of 5 weeks, soil samples were removed from storage and tested in triplicate. Microbial DNA was extracted using PowerLyzer™ PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.), a commercial kit chosen for its reported ability to extract good quality DNA from difficult environmental samples. This method is also reported to remove all humic substances and other PCR inhibitors. Following extraction, the microbial DNA was analyzed using a Nanodrop 2000c Spectrophotometer (Thermo Scientific) where the A260/280 ratio and sample concentration were determined. Preliminary testing revealed that storing soil samples in DNAgard®Tissue for up to five weeks resulted in extracted DNA of higher quality and yielded more consistent results when compared to other storage conditions. This study provides fundamental information to proceed to the next phase of testing which is microbial community profiling performed on an Applied Biosystems (AB) GeneAmp® PCR System 9700 with amplified products separated on the AB 3130xl Genetic Analyzer. The data generated will be analyzed using AB GeneMapper® ID Software v3.2.1.

**Soil, Microbial, DNAgard**

## A43 Developing an Empirically Based Ranking Order for Bone Sampling: Examining the Differential DNA Yield Rates Between Human Skeletal Elements — Phase I

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After attending this presentation, attendees will learn the order in which skeletal elements, from a relatively short post mortem interval, are most likely to produce DNA profiles suitable for identification.

This project will impact the forensic science community by replacing intuition with empirical data to establish a comprehensive ranking according to each bone's potential to provide usable genetic material for DNA identification, thus providing investigators with a clear bone sampling strategy. These results are particularly applicable for identification projects following mass fatality events with high fragmentation, which often rely heavily on DNA to identify and reassociate disparate fragments.

DNA may be the only method available for positive identification when access to ante-mortem information is limited; when readily identifiable features, such as fingerprints, have been compromised; or when elements are fragmented as in a plane crash. Large-scale identification efforts focused primarily on osseous material including the World Trade Center disaster and the post-conflict identifications conducted in the former Yugoslavia have led to major advances in understanding genetic material used for individual DNA identifications. It has been noted that bones and teeth yield higher levels of DNA than muscle and are often the only surviving tissue available to establish identification. However, bones differ in their structure, function, and composition leading to possible differences in DNA yield. Existing research into specific bone selection for DNA identification is inadequate.

Current selection of skeletal elements for DNA testing is based on the collective wisdom of forensic practitioners who typically request dense cortical bone such as the femoral shaft. This preference is bolstered by retrospective studies measuring success rates between skeletal elements, focusing on both mitochondrial (Edson et al. 2004, Leney 2006) and nuclear DNA (Milos et al. 2007). These studies found weight bearing long bones to be most successful, however, smaller elements such as patellae or phalanges were often not tested. A more recent examination of DNA success rates by skeletal element found that several smaller elements not typically sampled, such as the patellae and foot phalanges, were more successful than dense cortical bones (Mundorff et al. 2009).

Phase I of this project involved DNA testing each skeletal element and tooth type from three recently skeletonized individuals from the donated collection at the University of Tennessee Forensic Anthropology Center. The same bones (n=56), from each of three skeletons were tested (total n=168). To minimize destruction during sampling, a 3/8-inch circular hole was drilled in the bone instead of cutting out a window or wedge. This sampling approach resulted in the collection of ~0.20g of bone powder from both small and large bones. Following extraction and quantification, samples were normalized to 2ng when possible, and amplified with AmpliF/STR Identifier™.

Both the quantity and quality of the DNA from each sample was analyzed to determine which bone types consistently yielded complete DNA profiles, defined as 15 loci and Amelogenin, from all three skeletons. Triplicate data from each bone type was combined to determine the average relative fluorescent unit per locus by element type. The quantification value along with the quality of the resulting profile provides a guide to establishing a comprehensive ranking according to the potential of each element type to yield a complete DNA profile.

Results demonstrate that the quantity and quality of DNA obtained from different skeletal elements is highly variable. Many atypical DNA sample choices, such as cancellous tarsal bones (ankle), the patella (kneecap) and the distal hand phalanx (finger tip), outperformed more traditionally sampled dense cortical bone such as the femur, tibia, and humerus.

These results supplement traditional DNA sampling protocols by ranking skeletal elements according to their ability to produce high quality DNA profiles. Topping the list are smaller elements that can be removed intact, reducing potential contamination, and can also be removed with a disposable scalpel, reducing the need for a bone saw (and electricity, labor, and expensive equipment), allowing for easier and more efficient sampling in the field.

Phase 2 of this project (in progress) will determine whether these results hold true for skeletonized remains from longer post mortem intervals (0-3 years, 4-10 years, 11-20 years, and 21-50 years) and will document DNA degradation over time.

The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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**Human Identification, DNA Sampling, Skeletal Element**

## **A44 Epigenetic Markers: A Forensic Tool for the Determination of Biofluids Present at Sexual Assaults and Other Crime Scenes**

*Tania Madi, BSc\*, 90 Southwest 3rd Street, Apartment 1414, Miami, FL 33130*

The goal of this presentation is to demonstrate to attendees a new method to identify the originating tissue source of DNA.

This presentation will impact the forensic science community by providing a novel tool that may be useful in the differentiation of biofluids and cell types such as saliva, blood, sperm, and epithelial cells.

Often in forensic cases, knowledge of the originating source of the DNA found at crime scenes is required. Although certain chemical and microscopic tests exist in tissue type identification, they are mainly presumptive with varying sensitivity and specificity. Therefore, the development of a method to identify the source of DNA found at crime scenes is imperative. This study explores the possible use of epigenetic markers in identifying DNA sources.

Epigenetic modification of mammalian DNA is a naturally occurring mechanism crucial for the function of the genome and its transcriptional regulation. Although the DNA sequence in each individual cell is identical, its epigenetic profile is not. Epigenetic modifications include methylation at CpG islands and histone deacetylation. These modifications play a role in regulating the transcription and expression of genes allowing cells to differentiate into functionally and metabolically specialized cell types. The study of epigenetics as a useful tool in forensic tissue identification has proven to be promising. In the current study, researchers present a set of epigenetic markers found to be differentially methylated in four common bio-fluids found at crime scenes: saliva, blood, sperm, and epithelial cells.

After PCR amplification, any methylation information is lost. It is therefore necessary to subject DNA to bisulfite conversion, a chemical treatment that converts all unmethylated cytosine bases in the DNA to uracil and subsequently to thymine bases during PCR. These base changes are then detected in downstream analysis such as PCR or sequencing. The detection of a thymine base at a CpG site indicates that the modification process was successful in converting the cytosine base and that the site was not methylated. In contrast, if a cytosine base persists during the modification process and a thymine base is not detected, the CpG site is likely methylated.

Samples of each tested biofluid were first taken from ten individuals. The DNA was extracted and the entire genome subjected to bisulfite conversion. Modified DNA was PCR amplified at several specific loci that

are believed to be differentially expressed in these biofluids. Loci chosen contain a minimum of five CpG sites. The resulting PCR products were then sequenced using a Pyromark (Qiagen) pyrosequencer. Pyrogram peaks displaying the sequence and the percent methylation level at each CpG site were then analyzed and compared between the four cell types for methylation pattern differences. These profiles showed hyper or hypomethylation of one cell type relative to the other cell types. Using this method, epigenetic markers were identified to differentiate saliva, blood, sperm, and epithelial skin cells. The results of this study indicate the potential of a novel tool in identifying biofluids found at crime scenes.

**Epigenetic, Pyrosequencing, DNA**

## **A45 Optimizing Human Semen Stain Detection Using Fluorescence**

*Kelly Conroy, BS\*, San Jose State University, Forensic Science Program, 1 Washington Square, 508 MacQuarrie Hall, San Jose, CA 95192; and Steven B. Lee, PhD, San Jose State University, 1 Washington Square, MacQuarrie Hall 521, San Jose, CA 95192*

After attending this presentation, attendees will become more familiar with the abilities and limitations of current forensic alternate light source methods and filter glasses currently used to screen sexual assault evidence samples as a presumptive test for semen stains. The attendees will then gain additional knowledge on the fluorescent excitation and emission wavelength combinations that have been newly developed that have shown potential in enhancing visual detection of semen stains on different fabrics.

This presentation will impact the forensic science community by increasing viewer's knowledge of semen stain detection. In addition, the results of the study indicate the need to conduct further research in semen stain detection. The combinations of new excitation wavelengths with emission and blocking filters may impact the forensic community by enhancing the detection of semen stains and thus increasing the ability to process sexual assault evidence.

Human semen fluorescence has been observed for many years and is currently used as a presumptive screening test in forensic laboratories. The purpose of this project is to improve the visual detection of semen stains on different types and colors of fabrics with new combinations of different wavelengths of fluorescence using a standard forensic alternate light source (ALS), coupled with new fluorescence filters. As the detection of semen stains is one of the first steps in sexual assault evidence processing, the ability to locate these stains is important in order to obtain more probative evidence.

To establish a baseline for the project, a four year old positive control sample of semen deposited on a white tissue was examined under the Spectrum 9000, a forensic ALS, at six different discrete excitation filter settings. The stain was then viewed through various long pass, short pass, and band pass filters covering a range of wavelengths as well as yellow and orange goggles (480nm long pass, 545nm long pass respectively). The samples were photographed and observations were made on the visibility of the stain, taking into account amount of stain visible, relative brightness of the stain, contrast of the stain with the background, and the ability of the camera to record the stain.

Photographic documentation and visible qualitative evaluation of preliminary results indicate excitation wavelengths include 570nm, whereas previous reports listed the excitation range of semen as being from 300-500nm. Fluorescence emission filters in the 510-590nm range allow the stain to be easily detected by the eye of the observer. Since there was fluorescence observable at lower wavelengths that are blocked by the orange goggles commonly used in forensic laboratories, there is potential for capturing more of the visible fluorescence by the use of unique band pass filters. The results establish a baseline for a white substrate for the project, and the photos taken of the stain through the various filters will be analyzed using image analysis software to determine quantitatively if the

unique combination of fluorescence and discrete filters selected increase the ability to detect semen stains as compared to methods currently employed in forensic biology laboratories.

Future areas for research include utilizing the band pass filters that have been identified during the baseline tests to evaluate semen stains of various amounts deposited on various substrates after varying time intervals of storage to determine the effectiveness of the newly identified filters with relatively fresh stains versus those stored over time. Preliminary work highlights the appearance of a broader than previously thought excitation range for human semen.

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**Semen Stains, Alternate Light Source, Fluorescence**

## **A46 Evaluation of Automated Systems in a Mitochondrial DNA Unit**

*Andrea C. Phares, BS\*, 13126 Ashford Park Drive, Raleigh, NC 27613; Katie M. Horsman-Hall, PhD, 700 North 5th Street, Richmond, VA 23219; Stephen E. Rodgers, MS, and Brian Shannon, MS, Virginia Department of Forensic Science, 2nd Floor, Mitochondrial DNA, 700 North 5th Street, Richmond, VA 23219; and Brad Jenkins, MS, Virginia Department of Forensic Science, 700 North 5th Street, Richmond, VA 23219*

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After attending this presentation, attendees will learn how implementation of two automated systems, the Qiagen EZ1® Advanced and Agilent 2100 Bioanalyzer, will benefit forensic mitochondrial DNA units by limiting user handling, decreasing lab time, and allowing for prediction of length heteroplasmy prior to sequencing.

This presentation will impact the forensic science community by understanding how implementing these automated systems into a mitochondrial DNA unit allows for faster analysis by the analyst. Limited user handling of these systems results in decreased risk of contamination, while elimination of analyst interpretation of agarose gel bands allows for more accurate quantitation. Contamination thresholds were set and heteroplasmy may be screened to better prepare for downstream data.

Mitochondrial DNA (mtDNA) analysis is a prominent tool in forensic laboratories for missing persons, unidentified remains, and analysis of samples from which nuclear DNA (nuDNA) is difficult. mtDNA analysis is both time consuming and sensitive to contamination and would therefore benefit greatly from automated systems. The goal of this research was to evaluate two automated robotic platforms, the Qiagen EZ1® Advanced and the Agilent 2100 Bioanalyzer for their suitability for implementation into a mitochondrial DNA unit. The Qiagen EZ1® Advanced would allow for faster DNA extraction with no detectable contamination with limited analyst intervention. The Agilent 2100 Bioanalyzer would allow for more accurate quantitation than currently implemented gel electrophoretic methods as well as indication of heteroplasmy and contamination.

The Qiagen EZ1® Advanced robotic platform allows for the automated extraction and purification of six samples simultaneously. This system was evaluated for concordance by comparison to known mtDNA profiles from both buccal swabs and blood cards as well as resistance to cross-contamination between samples and runs. The ability for the Qiagen EZ1® Advanced to sufficiently extract mtDNA for sequencing from alternate knowns (toothbrush bristles and razors) and nuDNA from all samples used above was demonstrated. The Qiagen EZ1® Advanced DNA extracts were concordant with known mtDNA profiles. Cross-contamination was not observed in studies running alternating samples and blanks. The Qiagen EZ1® Advanced was able to produce full mtDNA and nuDNA profiles from all samples.

The Agilent 2100 Bioanalyzer allows for automated DNA quantitation through separation of nucleic acids into microfabricated channels with fluorescent detection of post-PCR products. This system was evaluated for detection sensitivity with a dilution series of a 200bp fragment, known buccal swabs, and HL60 DNA. Data was compared to determine the optimal amount of DNA required for sequencing. Reproducibility was also

shown with these dilution series by running the samples in triplicate on three chips. The 200bp fragment was quantitated with agarose gel electrophoresis to evaluate accuracy between the two methods. The Agilent 2100 Bioanalyzer was also run with known samples containing length heteroplasmy, to determine whether these length variants could be detected prior to sequencing. A contamination threshold, similar to that set by local FBI laboratories, was also determined by examining low concentration samples where DNA was detected but did not sequence. The Agilent 2100 Bioanalyzer has been shown to accurately quantitate samples below 20ng/μl. The Agilent 2100 Bioanalyzer was able to reproduce quantitation values on three separate chips. The instrument was able to correctly predict length heteroplasmy before sequencing by observance of split peaks within the electropherogram.

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**Qiagen EZ1® Advanced, Agilent 2100 Bioanalyzer, Mitochondrial DNA**

## **A47 Towards a Multi-Chamber Plastic Microdevice for Simultaneous Amplification of Multiple DNA Samples**

*Jenny A. Lounsbury, MSFS\*, Daniel C. Miranian, and James P. Landers, PhD, University of Virginia, Department of Chemistry, 409 McCormick Road, Charlottesville, VA 22904*

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After attending this presentation, attendees will have gained an understanding of the progress being made towards the development of a multi-sample microfluidic PCR platform for the simultaneous amplification of multiple DNA samples.

This presentation will impact the forensic science community by demonstrating a multi-chamber microdevice capable of amplifying up to seven DNA samples simultaneously, increasing sample throughput as compared to other PCR microdevices and demonstrating the applicability of a microfluidic platform for forensic analyses.

The polymerase chain reaction (PCR) is a key step in the processing of forensic biological samples. Typically, it is the most lengthy step (typically 2.5-3.5 hours) contributing to a time-consuming analytical process on the order of eight to ten hours. However, it has the advantage of high sample throughput, amplifying up to 96 samples at one time with conventional thermal cyclers. Microfluidic platforms for PCR have demonstrated numerous advantages over conventional PCR, including reducing the time required to complete the reaction as well as reducing sample and reagent consumption. Although PCR has been successfully adapted to a microdevice,<sup>1</sup> the majority of devices are limited in that they can only amplify one to two samples at a time. In order for a PCR microchip to be a generally accepted platform, the development of a device capable of amplifying multiple samples at once is clearly important.

Concurrently, the trend in microfluidics is to move towards alternative substrates for microchip fabrication to reduce fabrication and device costs. Typically, microdevices are made from glass, but are time-consuming and laborious to fabricate, often requiring the use of hazardous chemicals. Microdevices from polymeric substrates, such as poly(methyl methacrylate) (PMMA), can be fabricated using simpler techniques, including laser ablation and hot embossing.<sup>2</sup> In addition, PMMA is inexpensive, so devices can be disposed of after a single use and, as a result, this eliminates the risk of contamination between samples.

Previous work has demonstrated the use of infrared-mediated PCR (IR-PCR)<sup>3</sup> which utilizes a halogen lamp and a fan to heat and cool a sample in a PCR microdevice, respectively. IR-PCR provides increased heating and cooling rates compared to conventional block thermal cyclers, therefore reducing the time required for PCR. IR-PCR amplification of short tandem repeat (STR) regions of the human genome in a single chamber PMMA microdevice has been demonstrated using a combination of commercially-available fast polymerases, yielding a full STR profile (16 of 16 loci) in 33 minutes<sup>4</sup> – a ~5-fold reduction from average conventional amplification times.

Multi-chamber PCR microdevices were fabricated in PMMA using a CO<sub>2</sub> laser system, with eight PCR chambers arranged in a circular pattern within a 1cm diameter focal spot of the halogen lamp. The temperature in each chamber was monitored using miniature type-T thermocouples and the inter-chamber temperatures were found to vary up to 4°C, likely due to variation in the emitted radiation from the halogen lamp. Results show that, even though there were significant differences in the peak height of the amplified products (due to the temperature variation), suggesting slight differences in the power delivered to each chamber, STR amplification was successfully performed in the multi-chamber device, yielding full STR profiles (seven of seven loci) in all seven chambers in 42 minutes. With modifications to provide a more homogenous delivery of power from the lamp (i.e., the use of a diffuser), the temperature variations will be minimized, which, in turn, will minimize peak height variations. Furthermore, these results indicate that a polymeric multi-chamber microdevice for simultaneous sample amplification has the potential to increase throughput on a microfluidic platform while reducing overall analysis time as well as cost-per analysis.

#### References:

1. Easley, CJ, Karlinsey, JM, Bienvenue, JM, Legendre, LA, Roper, MG, Feldman, SH, Hughes, MA, Hewlett, EL, Merkel, TJ, Ferrance, JP, Landers, JP. *PNAS*. 103(51):19272-19277.
2. Sun, Y, Kwok, YC and Nguyen, NT. *J Micromech Microeng*. 16(8):1681-1688.
3. Roper, MG, Easley, CJ, Legendre, LA, Humphrey, JAC and Landers, JP. *Anal Chem*. 79(4):1294-1300.
4. Lounsbury, JA, and Landers, JP. *in preparation*.

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#### Multi-Chamber PCR, STR Typing, PMMA

### A48 Strategies for the Enrichment of Low Copy Number DNA Templates

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After attending this presentation, attendees will understand a novel genome amplification protocol, useful with forensic-type samples. They will be presented with the steps taken that were necessary to optimize the reaction, minimizing typical low copy number artifacts.

This presentation will impact the forensic science community by introducing a novel technique useful in the analysis of limited DNA samples and describing the experimental steps taken for its optimization.

In recent years, whole genome amplification (WGA) techniques have been used to enrich the genomic material in a sample so that low-level DNA can be successfully amplified. Using a WGA protocol, the entire genome is pre-amplified using multiple random start points for the molecular xeroxing process, providing an enriched full-genomic template for a second polymerase chain reaction in which the specific DNA fragments of interest can be amplified. Therefore, as it has been applied in human forensics, WGA is essentially the first step in a genome-wide nested PCR protocol. Due to the particulars of the forensic sample, (e.g., the use of loci containing repetitive DNA, the possibility of heterozygote imbalance, stutter and other PCR artifacts) no currently available WGA protocol completely addresses the needs of the forensic scientist.

In recent years, whole genome amplification (WGA) techniques have been used to enrich the genomic material in a sample so that low-level DNA can be successfully amplified. Using a WGA protocol, the entire genome is pre-amplified using multiple random start points for the molecular xeroxing process, providing an enriched full genomic template for a second polymerase chain reaction in which the specific DNA fragments of interest can be amplified. Therefore, as it has been applied in human forensics, WGA is essentially the first step in a genome-wide nested PCR protocol. Due to the particulars of the forensic sample, (e.g., the use of loci containing repetitive DNA, the possibility of heterozygote imbalance, stutter and other

PCR artifacts), no currently available WGA protocol completely addresses the needs of the forensic scientist.

The premise for an improved WGA technique is simple. Standard WGA unnecessarily complicates a sample by enriching for total DNA when, in fact, there are a limited number of well-defined loci of interest for forensic profiling. A more judicious strategy would be to target only the regions of interest for pre-amplification, providing them as an enriched template for the follow-on profiling of the specific loci of interest. This modified WGA has been termed Targeted Genome Amplification (TGA).

Briefly, TGA primers are designed to contain 5' and 3' invariable tails that are noncomplementary to, and therefore do not bind, any site in the human genome. Rather than enriching the sample for total DNA, only the regions containing the loci of interest, i.e., regions about 400 base pairs in length surrounding each STR locus are pre-amplified. In the same TGA reaction, primers complementary to these invariable TGA primer tails are included. They bind the tails and amplify the intervening sequences. The goal of this step is a balanced amplification of all loci maintaining the relative ratios of mixtures.

The technique has been optimized as a 3µl TGA reaction requiring no sample transfer between tubes. A fast PCR protocol is employed for the TGA. The reaction can be completed in less than 30 minutes. Subsequently, the STR multiplex PCR mix is added directly to the completed TGA reaction. Using this technique, PCR artifacts commonly observed with low copy number profiling such as peak height imbalance and stutter have been reduced to permit correct genotyping. This study successfully generated six-locus STR profiles from as little as 25pg DNA and three locus profiles from as little as 15pg DNA. The technique has been applied with some success to forensic-type samples. Results of the experiments will be presented and the course of future studies will be discussed.

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#### Whole Genome Amplification, DNA Damage, DNA Repair

### A49 Human Autosomal SNP Profiling Using Fully-Automated Electrospray Ionization Time of Flight Mass Spectrometry

Christine V. Marzan, PhD\*, Ibis Biosciences, 2251 Faraday Avenue, Suite 150, Carlsbad, CA 92008; Sandy D. Kirkpatrick, MS, and John V. Planz, PhD, University of North Texas, Health Science Center, 3500 Camp Bowie Boulevard, EAD 310E, Fort Worth, TX 76107; and Steven A. Hofstadler, PhD, and Thomas A. Hall, PhD, Ibis Biosciences Incorporated, 2251 Faraday Avenue, Suite 150, Carlsbad, CA 92008

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The goal of this presentation is to demonstrate the use of electrospray-ionization mass spectrometry (ESI-MS) system in the automated analysis of forensically-relevant human autosomal SNP markers.

This presentation will impact the forensic science community by using an automated assay with sensitivity, reliability, convenience, and ease of use/analysis suitable for forensics applications has been developed for the Ibis PLEX-IDTM and developmentally validated.

Single nucleotide polymorphisms (SNPs) represent a simple yet powerful tool for individual identification. Efforts by Pakstis and Kidd<sup>1</sup> to produce an ideal panel of genetically unlinked binary SNPs with high heterozygosity, low population bias, and uniform distribution over global populations have resulted in a 40-SNP panel analyzed across 40 global populations. A fully-automated PCR/electrospray ionization mass spectrometry (ESI-MS) assay capable of genotyping these 40 SNP markers has been developed for the Ibis PLEX-ID™ platform and has been developmentally validated.

The 40-SNP assay consists of a pre-fabricated kit containing all components necessary to analyze a sample except for template DNA. Eight five-plex PCR reactions per sample are arranged in columns of a 96-well assay plate such that up to 12 samples may be analyzed on a single assay plate. After adding DNA template to the appropriate wells of an assay plate

and thermal cycling, all downstream analytical steps through data processing are fully automated. A novel ESI-MS deconvolution algorithm deconvolves multiplexed PCR product charge state distributions into individual masses while retaining relative output signal amplitude estimates in proportion to relative input DNA concentrations, allowing evaluation of inter- and intra-locus product balance to aid interpretation of potential mixtures/contaminated profiles. Forward and reverse strands of each PCR product are measured with high mass accuracy, such that base compositions (A, G, C and T nucleotide counts) can be directly calculated for each product, allowing accurate assignment of nucleotide identity at the SNP position. The average mass measurement deviation for 31,030 independent DNA strand assignments was  $12.4 \pm 11.4$  parts per million (ppm), corresponding to  $\sim 0.2$  Da for PCR products of the average size amplified in the assay.

The 40-SNP assay has been characterized for concordance to existing methodology, sensitivity, reproducibility, species specificity, and the ability to detect when genotyping results indicate a pure sample or a mixture/contaminated sample. Concordance has been demonstrated for all loci using individual TaqMan<sup>3</sup> assays for each locus for 20 samples and comparing results to those obtained with the Ibis SNP assay. All loci displayed 100% concordance to TaqMan<sup>3</sup> results. Dilution studies suggest that reliable genotyping results can be obtained at template input levels close to 125pg of DNA per reaction.

Species specificity analysis indicated no interference using a 10-fold excess of exogenous cat, dog, *E. coli*, *S. aureus*, *C. albicans* or *A. oryzae* genomic DNA. Non-primate DNA did not produce PCR products recognizable as SNP locus products, and did not interfere with correct typing results when in excess in the presence of human DNA. Bacterial DNA at a 100-fold excess over human DNA did not interfere with correct genotyping results. Primate DNA (African green monkey, squirrel monkey, rhesus macaque, and marmoset monkey) did produce products and interfered with genotyping results to different degrees.

Fully-automated data analysis produced 5998/6000 correct genotype assignments (99.97%) in reproducibility studies utilizing three samples each analyzed 50 consecutive times at a constant DNA template input. A convenient software interface has been developed for visual review of data analyses. The Ibis PLEX-ID<sup>TM</sup> ESI-MS platform is capable of typing the full spectrum of forensically-relevant markers, Y-STR, autosomal STR, mitochondrial DNA, and SNPs on a single instrument within the same automated run.

#### Reference:

<sup>1</sup> Pakstis, *et al.* *Hum. Genet.* 121: 305-317.

#### Autosomal SNPs, Single Nucleotide Polymorphisms, Mass Spectrometry

## A50 DNA Preservation in Partially Decomposed Soft Muscle Tissue Samples Using Different Preservative Solutions

Muhammad S. Nazir, MSc\*, School of Forensic & Investigative Sciences, University of Central Lancashire, Preston, Lancashire PR1 2HE, UNITED KINGDOM

After attending this presentation, attendees will better understand how to choose different preservative solutions for better preservation of DNA in partially decomposed soft muscle tissues under field conditions where no cold storage is available.

This presentation will impact the forensic science community by providing new ideas to preserve DNA under field conditions for successful profiling. The solutions mentioned in presentation are easy to handle under field conditions, and DNA can be preserved for a period of six months successfully at room temperature using these preservatives.

Following mass fatality incidents, DNA profiling is essential for identification and reassociation of fragmented, burnt, or decomposed

corpses that would be very difficult or impossible by traditional means such as fingerprinting and odontology. However, successful DNA recovery depends on the collection and preservation of biological material obtained from deceased individuals and the availability of reference samples (Graham *et al.*, 2008).<sup>1</sup> Inefficient preservation methods can cause destruction of intact DNA to such an extent that data is not always available for victim identification (Bing and Bieber, 2001).<sup>2</sup>

In order to assess the efficiency of different preservative solutions (10% buffered formalin, 96% ethanol and cell lysis solution - with and without sodium azide), partially decomposed soft muscle tissue samples were collected at different accumulated degree-days (ADD) from pig (0, 70 and 150 ADD) and rabbit (0 and 70 ADD) carcasses and were placed in preservative solutions.

DNA extraction was performed using DNeasy<sup>®</sup> Blood and Tissue kit according to the manufacturer's instructions at different time points (1 month and 6 months). DNA quantification was performed using agarose gel electrophoresis (2%) and Quant-iT<sup>™</sup> PicoGreen<sup>®</sup> dsDNA Reagent from Invitrogen<sup>™</sup>, UK. DNA fragment analysis was performed using Applied Biosystems<sup>™</sup> 310 and 3500 genetic analyzers.

In order to perform DNA fragment analysis from postmortem soft muscle tissue samples of the model organisms chosen, two nuclear genes, Connexin 43 and RAG-1, were aligned to identify conserved regions. Primers were designed to amplify 70 bp, 194 bp, 305 bp, and 384 bp amplicons. The primers were also designed to amplify human DNA which allowed the use of commercially purchased DNA standards to be used as controls. Following DNA extraction, PCR analysis was performed using the four primers sets in a multiplex (4-plex) and was optimized so that it worked over a wide range of template amounts (0.1ng to 75.83ng). The multiplex (4-plex) PCR was found to work efficiently in triplicate samples of all three species down to 0.3ng of DNA template.

The results showed that the 96% ethanol and cell lysis solution, with and without 1% sodium azide, are better solutions for DNA preservation in both fresh (0 ADD) and partially decomposed tissues (0, 70 and 150 ADD) for a period of six months, whereas 10% buffered formalin is a poor source of DNA preservation causing high DNA degradation.

Future work will include: DNA extraction from preserved samples after one year, development of real-time PCR quantification assays, DNA fragment analysis using ABI 310 and 3500 genetic analysers, and analysis of results using appropriate methods such as ANOVA, ANCOVA, and regression analysis.

#### References:

<sup>1</sup> Graham, EAM, Turk, EE and Ruttly GN. Room temperature DNA preservation of soft tissue for rapid DNA extraction: An addition to the disaster victim identification investigators toolkit? *Forensic Science International: Genetics*, 2, 29-34.

<sup>2</sup> Bing, DH and Bieber, FR. (2001). Collecting and Handling Samples for Parentage and Forensics DNA-Based Genetic Testing, *John Wiley & Sons, Inc.*

#### ADD, RAG-1, Multiplex (4-plex)

## A51 The Effect of a Harsh Environment and Indirect Human Activity on the Perseverance of DNA

Sarah Cavanaugh, MSFS\*, Bode Technology, Incorporated, 10430 Furnace Road, Suite 107, Lorton, VA 22079

After attending this presentation, attendees will have gained insight into the possibilities for recovering DNA from evidentiary samples subjected to human and environmental exposure. They will be made aware of the current theories for locating reliable evidence for DNA and given quantitative data regarding those theories.

This presentation will impact the forensic science community by demonstrating that low template DNA samples can be collected and

analyzed after eighteen months by first considering the ambient environment and substrate on which the sample may have been deposited. This presentation will provide scientists with information to evaluate the currently implemented methods and adopt or modify their current protocols.

Locating reliable evidentiary samples for DNA analysis becomes more complicated as time between deposition of a biological sample and collection of sample increases. Exposure to environmental factors can also decrease the chances of obtaining a DNA profile. Certain factors such as temperature, humidity, and substrate are known to negatively affect DNA recovery, and a number of theories have been used by forensic scientists to help locate areas from which the best possible biological sample can be collected. However, a comprehensive and quantitative study regarding environmental impact and the effect of contaminating human traffic and activity on DNA profile recovery has not yet been published.

The research presented will show a comprehensive longitudinal study to determine the optimum locations at a crime scene that is best suited for biological evidence collection and to assess sample reliability for DNA analysis after eighteen months. This effort consisted of two concurrent studies: Study 1 examined DNA sample recovery by taking into account biological sample type, human traffic flow through a room, evidence placement within the room, substrate, and time; Study 2 examined DNA recovery by taking into account biological sample type, ambient environment (hot and humid or room temperature), substrate, and time.

For both studies, six replicate sets of mock evidence fluid samples: blood, semen, saliva, urine, oily fingerprints, and regular fingerprints were deposited on substrates and exposed to the environment in question for 18 months time. Study 1 had two different substrates available, and Study 2 used nine different substrates. Sample sets were collected, extracted, and analyzed after 0, 3, 6, 9, 12, and 18 months. Any DNA profile obtained was compared to the sample's known profile to calculate percent profile recovery and contamination (designated by the number of additional, unassociated alleles).

JMP®, a powerful Design of Experiments (DOE) statistics program, was used to create the test plan of both studies as well as analyze all the data and make correlations between the ability to recover the correct profile and the various factors (time, environment, and substrate) being evaluated. The JMP® software is able to analyze a response based on the effects of multiple factors. This type of experimental design and statistics capability is novel for the forensic field and allows the user to interpret a smaller data set with fewer replicates while still determining strong correlations.

After 18 months, the majority of samples were highly degraded and/or contaminated, as would be expected. However, more partial profiles were recovered from blood and oily fingerprints after the extended time period than the other sample types. This study also provides quantitative data for the best substrates from which to recover evidentiary samples, the environments most likely to maintain viable DNA sources, and relative contamination amounts for both. Lastly, this presentation demonstrates the power of JMP® Design of Experiments software, a novel tool for large scale studies within forensic-based research.

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#### **DNA Recovery, Environmental Effects, Time Study**

## **A52 Improved Isolation of DNA From Forensic Dental Specimens**

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After attending this presentation, attendees will learn a different less destructive technique to obtain DNA from forensic dental specimens versus the more traditional crush and grind method of obtaining DNA from the pulp.

This presentation will impact the forensic science community by giving forensic biologists and those that also work on mass disasters, a technique of obtaining DNA from a dental specimen while preserving it for possible return to family members especially if it is the only sample that remains from an individual.

The DNA Missing Persons Unit within the Department of Forensic Biology at the New York City Office of Chief Medical Examiner performs nuclear and mitochondrial DNA testing for the purposes of identification, re-association of body parts, and upload into the Combined DNA Index System (CODIS). While success rates for postmortem samples such as blood, tissue, and bone were as expected, dental specimens failed to give consistent results. This may have been caused by exposure to detrimental environmental conditions. These environments may vary from bodies found in water, to remains buried in soil, or encased in cement. The goals of this study are to evaluate how exposure to the following conditions - water, soil, and cement - affects the ability to extract nuclear DNA from dental samples and to determine what improvements can be made to the examination and extraction protocols.

One hundred-twenty (120) adult teeth collected as dental waste from private dental offices were examined and categorized by a dentist according to tooth type (molars, incisors, premolars, and canines) and any abnormalities such as dental caries (cavities), open apices, calcification, and discoloration were noted. The teeth were incubated in soil, cement, and water from the East River in New York City, or physiological saline from 1 to 48 weeks. Two specimens from each environmental condition had the pulp tissue extracted at weeks one, two, three and four and every four weeks thereafter using a dental pulpectomy procedure. The pulpectomy involved removing tissue from the pulp chambers using nerve broaches utilized by dentists when performing a root canal. A phenol:chloroform:isoamyl alcohol (PCIA) extraction was performed on the pulp tissue. Each DNA sample was quantified, amplified with the Applied Biosystems (ABI) Identifier<sup>®</sup> kit (28 cycles), and alleles detected using the ABI PRISM 3130xl Genetic Analyzer.

The current method used to extract DNA from teeth involved grinding the entire tooth using a freezer mill. This destroyed the morphology of the tooth and successful DNA results fluctuated and were unpredictable. Results from this study greatly improved examination procedures; the morphology of the tooth structure was maintained while examination efficiency increased by accessing the DNA in the tooth through pulpectomies.

By week 40, 30.8% generated full profiles and 16.7% generated high partial profiles all eligible for entry into CODIS. Overall by week 40, the number of identified loci decreased over time in most conditions but DNA extracted from dental specimens buried in soil showed the strongest reduction in identifiable loci.

Prior caries status of a tooth was not a factor in DNA extraction as those teeth yielded sufficient amounts of DNA. Conversely, teeth with open apices, that were calcified, or had no visible pulp tissue did not yield DNA or yield sufficient amounts of DNA for concentration or amplification.

Overall, the goals of this study thus far have been met. Improvements to the examination of dental specimens through pulpectomies require less examination and hands-on time compared to the conventional freezer mill method. Pulpectomies preserve the morphology of the tooth and yield similar DNA results. The dental specimens exposed to cement, soil, water, and even physiological saline show degradation over time as evidenced by the decrease in DNA concentration and quality of DNA profiles obtained.

#### **DNA, Teeth, Extraction**

### **A53 Low-Level Variant Detection in Mitochondrial DNA Using the Illumina® GA IIx Next-Generation Sequencing (NGS) Platform**

*Brandon Smith, BS\*, and Brittanica J. Bintz, MS, Western Carolina University, 111 Memorial Drive, NSB #231, Cullowhee, NC 28723; and Mark R. Wilson, PhD, Western Carolina University, Forensic Science, Department of Chemistry/Physics, Cullowhee, NC 28723*

After attending this presentation, attendees will be more familiar with the concept of heteroplasmy and interpretation bias drawn from mitochondrial DNA sequence data using current sequencing methodologies. An overview of the Illumina® Next-Generation Sequencing (NGS) chemistry and workflow will be presented. Data elucidating depth of coverage required for variant detection at or below a 1% threshold will be shown. Per sample cost, training issues and implementation strategies will also be mentioned.

This presentation will affect the forensic science community by suggesting an alternative method for mtDNA sequence analysis, one that is able to reliably detect low-level variants in mtDNA down to the single molecule level.

Heteroplasmy is defined as the presence of more than one mitochondrial DNA (mtDNA) type within an individual (Melton 2004).<sup>1</sup> These differing mtDNA types may occur between populations within an individual, within a single cell, or within a single mitochondrion. Furthermore, heteroplasmy has been observed in two distinct forms including sequence and length heteroplasmy. Sequence heteroplasmy occurs when two nucleotides are present at a single nucleotide position, while length heteroplasmy occurs when two different lengths of a homopolymeric C-stretch are present within the mtDNA sequence. Higher mutation rates (Bogenhagen 1999)<sup>2</sup> and fewer DNA repair mechanisms than nuclear DNA (Kunkel 1981)<sup>3</sup> lend credence to the idea that millions of mtDNA molecules within an individual are not likely to have a single, uniform mtDNA type. As a result, it is presumed that all individuals are heteroplasmic at some level and therefore, mtDNA sequences from single source samples are a mixture of mtDNA types. Heteroplasmy may go unnoticed given the limits of detection of traditional Sanger sequencing methods, whereby a single sequence is generated from amplified mtDNA products. Failing to detect heteroplasmy gives bias to interpretation of mtDNA sequence data. With the advent of sequencing-by-synthesis technologies, it is possible to resolve and quantify mixtures of mtDNA at much lower levels than current technologies (Andréasson *et al.* 2006).<sup>4</sup> These findings suggest that sequencing-by-synthesis methods may be an attractive alternative to current methods used in Forensic laboratories by allowing for the detection heteroplasmy below a threshold of 1%. The Illumina® GAII<sub>X</sub> is a massively-parallel sequencing platform which utilizes a unique chemistry that is unlike most pyrosequencing technologies currently on the market. The Illumina® GAII<sub>X</sub> utilizes a proprietary flow cell that is covered with a dense lawn of primers which will bind to flanking adapters incorporated into amplified DNA products (Illumina® 2010).<sup>5</sup> Clonal amplification of captured products results in tens of millions of clusters per square centimeter of the flow cell. Each cluster is then sequenced and analyzed independently of one another. This allows for the generation of sequence data on a single molecule scale.

In this effort, the Illumina® GAII<sub>X</sub> was evaluated for mixture analysis of mtDNA. Initially, DNA was extracted from forensically relevant sample types including donated hair, buccal, and blood samples. The DNA was amplified using a novel amplification strategy described by Bintz *et al.*, and reference sequences were obtained from the mtDNA HV region of all 20 donors using Sanger sequencing methods. Artificial mixtures were then prepared using distinct templates from the samples described above in ratios of 99%/1%, 98%/2%, and 95%/5%. Multiplex assays were designed using the resulting mixtures. Resulting data shows the reliable detection of variants at a target level of 1%, which is significantly lower than the 10% threshold of current methods. Additionally, we were able to quantify the depth of coverage needed to detect variants at a level of 1% at an average of 250X coverage from approximately 50 individuals per Roche GS-Junior run.

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#### **Next Generation Sequencing, Mitochondrial DNA, Heteroplasmy**

### **A54 Development of Streptavidin-Biotin Binding of DNA Amplicons Methods for the Typing and Re-Typing of Forensically Relevant Short Tandem Repeats**

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After attending this presentation, attendees will be informed of a method which would allow the re-amplification of a DNA target that has already undergone the Polymerase Chain Reaction (PCR) and is a now a constituent of the amplified work product. This will be accomplished by removing the amplicons and other PCR components such primers, dNTP's, etc, to sequester the original target DNA such that it is available for re-amplification with additional human identification chemistry. This would allow DNA crime laboratories and their analysts to genotype limited or exhaustive samples using a variety of kits/chemistries without the need for additional extraction or evidence processing.

This presentation will impact the forensic science community by demonstrating an approach that would allow for: (1) testing of both autosomal- and Y- STRs for limited sexual assault samples; (2) the ability of the analyst to re-amplify with mini-STR's or an enhanced amplification scheme (i.e., more Taq Polymerase, repair enzymes, more BSA) if it is realized that the DNA was degraded, damaged, and/or PCR inhibited; and, (3) the re-amplification of an exhaustive sample due to an amplification failure.

The first stage of development concentrates on maximizing PCR efficiency with primers that have been functionalized with biotin. In this study, the TPOX locus was used to test this optimization. Amplification of the TPOX locus was optimized by varying the concentrations of magnesium chloride (MgCl<sub>2</sub>) and primers. The amplified products were then electrophoresed and stained with GelStar® Nucleic Acid Gel Stain

(Lonza Inc, Walkersville, MD). The resulting bands were analyzed using ImageJ - a public domain open source image processing software.<sup>1</sup> The optimal concentrations were determined to be those used with Sample 3-5 (i.e. 2.25 mM MgCl<sub>2</sub> and 0.06 μM primers) (Figure 1).

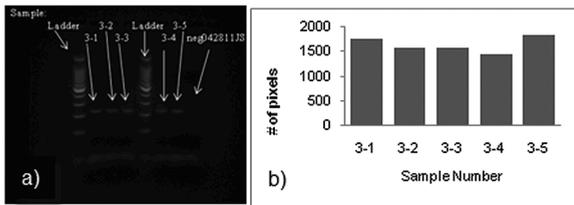


Figure 1: a) Agarose gel electrophoresis of the amplified product with non-biotinylated primer and varying concentrations of MgCl<sub>2</sub> and primer; b) Number of pixels (signifying signal intensity) of the amplified product analyzed with ImageJ.

Once optimized conditions for the amplification of TPOX were determined, it was of interest to confirm whether modification of the forward primer with biotin had a significant impact on amplification. As a result, new forward primers of the same sequence which were functionalized with biotin were used during amplification and the reagent concentrations once again varied. Agarose gel electrophoresis was performed as with the amplicons originating from the PCR using the non-biotinylated primer and the gel analyzed using ImageJ as previously described. In contrast to the aforementioned results, the optimal reagent concentrations were determined to be those used with Sample 4-4 (2 mM MgCl<sub>2</sub> and 0.04 μM primers) (Figure 2). This indicates that functionalization of the forward primer had an impact on the PCR, suggesting that optimization of the amplification for methods which use modified primers must be performed with the specific primer of interest.

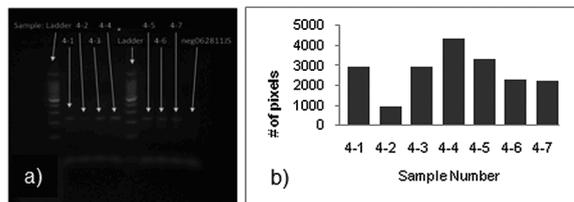


Figure 2: a) Agarose gel electrophoresis of the amplified product with biotinylated primer and varying concentrations of MgCl<sub>2</sub> and primer; b) Number of pixels (signifying signal intensity) of the amplified product analyzed with ImageJ.

Once the reaction is optimized, the clean-up procedure can be used on 1ng of DNA. First, the amplified DNA product is run on the 3130 Genetic Analyzer. Then the remaining sample is cleaned using post PCR purification to remove salts and any unincorporated primers. The amplicons can then be removed from the sample using Streptavidin coated magnetic beads. More specifically, the beads bind to the biotin on the primer (now hybridized to the amplicon) leaving the original DNA in the supernatant for subsequent re-amplification.

**Reference:**

<sup>1</sup> <http://rsbweb.nih.gov/ij/>. Accessed August 1, 2011.

**Streptavidin, Biotin, PCR Optimization**

## A55 Rapid and Direct CODIS STR Screening Using Short Microchip Capillary Electrophoresis

Maurice J. Aboud, MS\*, FL International University, 11200 Southwest 8th Street, Chemistry & Biochemistry CP304, Miami, FL 33199

After attending this presentation, attendees will understand the development of a fast and portable DNA screening method that uses microchip electrophoresis for the rapid detection of a set of seven CODIS STR markers. They will gain an understanding of how this system works, the limitations of the system, and how these limitations were surmounted to achieve the desired resolution and capability to genotype on the microfluidic chip.

This presentation will impact the forensic science community by addressing the problems and limitations encountered with the current commercial microfluidic systems for DNA separation, such as poor resolution, and provide a useful tool for quick CODIS screening of DNA samples.

There are situations in which it is very important to quickly and positively identify an individual. Examples include suspects detained in the neighborhood of a bombing or terrorist incident, individuals detained attempting to enter or leave the country, and victims of mass disasters. Systems utilized for these purposes must be fast, portable, and easy to maintain. Current DNA typing methods provide the best biometric information yielding identity, kinship and geographical origin, but they are not portable. Currently DNA typing is performed by large-scale sequencers using multichannel fluorescent capillary array electrophoresis. Complex robotic extraction and PCR processing creates economies of scale and time, permitting large numbers of samples to be efficiently processed. Unfortunately this process is not flexible enough for many applications in the field and is not quick on a per sample basis.

The proposed alternative, microfluidic DNA typing, holds great promise but constraints on resolution and problems with coupling inline extraction, inline PCR, and multicapillary analysis make these systems highly complex. These systems require complicated integration of engineered components making them highly vulnerable to clogging, misalignment, and voltage leakage. The issues with large-scale integration of extraction, amplification, and DNA electrophoresis also make these systems less than portable. While there is no doubt that the technological issues may someday be solved, there are alternative modular approaches to perform this task that do not require extensive engineering and do not require complete system integration. Such modular systems are easily repaired in the field and can be quickly switched if problems occur. Furthermore, new advances in the field of DNA typing involving direct PCR make it possible to eliminate complex extraction steps and simply amplify DNA directly from a paper punch. This has the potential to greatly simplify analysis. Other advances such as commercially available high speed PCR and disposable short channel microchips provide off the shelf solutions to the problem of portable high-speed DNA detection. This approach is simple and does not require major engineering or retooling of equipment.

This project was designed to optimize and combine off the shelf components (direct PCR, high speed thermal cycling, and short channel microfluidics) to produce a high-speed genotyping system. In this project, we are utilizing known biological principles to accelerate the amplification rate of direct PCR and couple this fast amplification to a beta version of an Agilent 2100 Bioanalyzer that has been upgraded to permit ssDNA typing. The microfluidic chip separation channel is 1.5cm, placing unique restraints on resolution and sizing that have been solved using a specialized polymer matrix and multichannel fluorescence detection. Per sample run times are 80 seconds and fast and direct PCR was utilized to minimize sample-processing times. The efforts of this study demonstrate a simple, highly portable DNA typing procedure that should prove valuable for applications involving a need for a robust and rapid analysis.

**Microchip, DNA Screening, CODIS Markers**

## A56 Separation of Semen From Superabsorbent Polymers for Forensic Analysis

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After attending this presentation, attendees will gain an understanding of the challenges in separating cellular material from the superabsorbent polymer (SAP) materials and the fibrous matrices found in evidence such as diapers, sanitary napkins, absorbent medical pads, and other related forensic evidence, and will learn a protocol that can successfully separate the cellular material from the substrate will be presented.

This presentation will impact the forensic community by describing best practices for isolating semen from evidence containing absorbent and superabsorbent materials, and the impact that SAPS have on downstream DNA analysis.

The presence of super absorbent polymers in forensic evidence has been found to complicate spermatozoa isolation for body fluid identification and inhibit organic DNA extractions. In certain cases, primarily involving women, small children, and infants, the successful isolation of cellular materials, and subsequently DNA, from SAP-containing materials such as feminine pads, diapers, and absorbent medical pads, is a difficult task, and failures of body fluid identification tests and DNA isolation have been observed. These super absorbent polymers are designed to absorb and retain large volumes of water or organic liquids, but when they are submitted as forensic evidence, they impede testing by sequestering the cellular material in the process. The purpose of this research was to develop a method to separate semen from SAPs found in materials such as diapers and feminine pads, with the ultimate goal of maximum yield from the evidence, and minimal inhibition of downstream forensic molecular testing.

Multiple centrifugal methods using size filtration were investigated to separate the SAP gel and fibrous material from the rest of the liquid sample containing the desired spermatozoa and other cellular material. Factors for a successful separation required determination of an optimal filter pore size, centrifugal speed and time that would allow cellular materials and liquid to flow through the filter, while preventing the polymers and cellulose fiber from flowing through. After a successful separation protocol was developed, sperm counts were used to compare percent yield and success of the protocol. Percent yield between isolation from just the top fabric layer of the item and a "core" of all layers of the item were also compared. DNA isolations and STR analysis were conducted on filtered SAP samples to determine levels of inhibition.

Finally, direct DNA isolation of the biological fluids on evidence with SAPs was evaluated using two silica-based DNA isolation methods. Since the silica methods have a direct affinity for DNA, samples were isolated using silica-based differential extraction methods to determine if direct DNA isolation is possible in the presence of SAPs, and if so, how those isolations are impacted. If the isolation methods are not affected by SAPs, laboratories may have the option to directly isolate DNA and determine presence of seminal fluid using molecular biology methods.

In conclusion, difficult samples such as evidence containing superabsorbent polymers can impact both body fluid identification and DNA analysis, and a modified approach was developed that can directly impact casework. This method uses standard materials and reagents found in most laboratories, and thus could be implemented quickly for significant impact.

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### Seminal Fluid, Semen, Diapers

## A57 High-Throughput Human Mitochondrial DNA Database Development Using Automated Electrospray-Ionization Mass Spectrometry

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The goal of this presentation is to demonstrate the use of a fully-automated electrospray-ionization mass spectrometry (ESI-MS) system in the high-throughput development of a human mitochondrial DNA profile database.

This presentation will impact the forensic science community by revealing how, over the course of five years, the Ibis platform was used to develop a database of one hundred thousand human mitochondrial DNA profiles at a single site, allowing an unprecedented ability to interrogate the prevalence and extent of genetic variation in the non-coding region, including length and point heteroplasmy.

Forensic mitochondrial DNA (mtDNA) analysis is performed when DNA quantity/quality is insufficient for nuclear DNA analysis, or maternal-lineage DNA is required. Analysis involves sequencing mtDNA segments, a lengthy and labor intensive technique. Ibis Biosciences has developed a high-throughput mass spectrometry-based mitochondrial DNA profiling assay suitable for automated analysis of mtDNA control region segments that retains approximately 94% of the individually-discriminating information of sequencing the same regions. The assay amplifies HVI 15924-16428 and HVII 31-576 and provides more discriminating information than sequencing the minimum HVI and HVII regions of 16024-16356 and 73-34, respectively. Twenty four (24) overlapping PCR primer pairs are used to amplify 1051 bases of mtDNA hypervariable regions HVI and HVII in eight triplexed PCR reactions. Pre-fabricated 96-well kit plates are configured such that one sample occupies one column of an assay plate and 12 samples may be run on a single plate. All reaction components are supplied in frozen kit plates such that only DNA extract (5ul per well) needs to be added prior to thermal cycling. After thermal cycling, assay plates are placed directly onto an Ibis T5000™ or PLEX-ID™ instrument and all analytical steps through mass spectrometry data processing are automated. Amplified PCR products are desalted using an anion-exchange matrix coupled to paramagnetic beads and analyzed by ESI-MS in a time-of-flight (TOF) mass spectrometer. Forward and reverse strands of each PCR product are weighed with sufficient accuracy to calculate the base composition (A, G, C, and T count) of each amplicon in multiplexed PCR reactions. Base composition indexed by rCRS coordinates of amplified regions define 24-module mtDNA profiles that can be compared directly to each other or used to search a lookup or population database derived from mass spectrometry, sequence data, or both. Likewise, sequence data can be used to search a base composition-based database by converting the sequence(s) to base compositions corresponding to the primer pairs employed in the assay. Base composition profile comparisons to a population database are amenable to the same counting and confidence interval (upper bound frequency) estimates used for sequence comparisons.

The Ibis platform was used over the course of five years at a single laboratory to generate a searchable database of 117,278 human mitochondrial profiles. Profiles were developed as a list of PCR product base compositions referenced by revised Cambridge Reference (rCRS) coordinates. Profiles may be directly compared to each other, compared to a database of base compositions, or compared to a population database of sequence profile by converting sequence profiles to base compositions prior to comparisons. Frequency statistics for profile matches within a population database may be calculated in a manner identical to sequence database comparisons. The assay is not hindered by the presence of sequence length heteroplasmy, is capable of resolving mixtures, and can be

used to quantify the relative contributions of both length and point heteroplasmic variants. Statistics will be presented detailing the development of an mtDNA profiling system, sample processing rates over time using two Ibis instruments in parallel, rates of profile completion, heteroplasmic variants, and precision and reliability of assay controls.

**mtDNA, Mass Spectrometry, Base Composition Analysis**

## **A58 Concurrent Internal Validation of Four Forensic STR DNA Profiling Kits Using a Single Genetic Analyzer**

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After attending this presentation, attendees will be familiar with the validation study performed and how the results were used to set mixture interpretation guidelines and policy for the Department of Biology of the National Institute for Criminalistics and Criminology of the Algerian Gendarmerie Nationale (INCC).

This presentation will impact the forensic science community by presenting the results of a single combined internal validation of four STR kits: AmpFISTR® Identifier™, PowerPlex 16™, AmpFISTR® SGM Plus™, and PowerPlex® ESI 16™ using the same analysis conditions and a single instrument throughout the entire study. With these concurrent validations, relative kit sensitivities, precision, analytical, and stochastic thresholds were determined. This study has allowed the authors to determine a hierarchy of kit use during analysis and has allowed us to create fixed interpretation guidelines for interpretation of DNA profiles in forensic casework at the biology department INCC-Algeria.

Forensic casework very often involves samples that may not produce DNA results. When results are obtained, the DNA profile information may not be interpretable. This may be due to the variable quality of biological material that can be obtained from forensic evidence exhibits, but this also may be due to the inherent variability of current STR DNA profiling technology. Every technology has a limit at which usable or reliable data can be obtained or cannot be interpreted for conclusive analysis. Forensic DNA profiling technology has two such limits.

Currently, forensic DNA profiling technology is centered upon the use of a very finite number of commercially available forensic human identity (HID) kits which produce amplified products via the polymerase chain reaction (PCR). PCR can fall prey to stochastic effects. An even more finite set of commercially available genetic analyzers are currently used in forensic DNA laboratories. The genetic analyzer is merely an analytical instrument used to observe the amplification products. These analytical instruments individually have their own limits of linearity and detection. A thorough comparative assessment of HID kit performance has not been truly executed, in the author's opinion, because the performance of these kits has been assessed under greatly varying analytical conditions, differing analysis equipment, and analysis conditions. Widely differing DNA interpretation methods are also used to evaluate the data generated, greatly exacerbating the already highly variable results that may have been obtained during the kit performance assessments.

Laboratory interpretation variability has been highlighted in recent years by inter-laboratory studies and surveys that have been performed. This interpretation variability appears to be the result of an oversimplification of observations on an analytical device without taking into account its limits and the disregard for an HID kit to produce non-stochastic DNA amplification during PCR depending upon input template concentration in PCR. In an effort to minimize the use of non-reliable PCR

amplification results, several oversight committees have suggested new DNA interpretation guidelines which incorporate methods for determining and handling analytical and stochastic limits within DNA profiled data to ensure it is accurate analytically and reproducibly amplified.

These newly revised interpretation guidelines have recently been adopted by many accrediting bodies internationally in the last few years. In preparation for accreditation, the INCC was in the unique position to carry out validation studies on the four commercial STR HID kits currently in use using the new interpretation guidelines as part of the assessment of performance of each kit. To this end, a single combined internal validation of four HID STR kits (AmpFISTR® Identifier and AmpFISTR® SGM Plus from Applied Biosystems and PowerPlex 16 and PowerPlex® ESI 16 from Promega) was designed specifically to observe and compare the performance abilities of these HID kits using a single dilution series of template DNA for amplification, the same post-amplification analysis conditions, and using the same multicapillary electrophoresis instrument throughout the entire study.

With the concurrent data generated by this validation study, it was determined that the relative sensitivities, precisions, and analytical/stochastic thresholds did not vary significantly among these four kits. However, due to the varying size of reporting amplicons within each kit, it was found that certain kits are able to complement each other to allow more complete results. The author's believe the use of new fixed interpretation guidelines for the interpretation of the DNA profiles in their validation study was key in determining that no single kit was able to outperform the others. This allowed consolidation of methods for profiling forensic casework at the biology department of the INCC-Algeria.

**DNA STR Kit Validation, DNA STR Kit Sensitivity, Analytical and Stochastic Thresholds of STR Kits**

## **A59 Comparing Wearer DNA Sample Collection Methods for Determining the Best Method for the Recovery of Single Source Profiles**

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After attending this presentation, attendees will have a better understanding on how to most effectively collect wearer DNA. Attendees will also see how the currently used collection methods compare to a new gel film method.

This presentation will impact the forensic science community by demonstrating which collection method best collects DNA from the last wearer of an item. This may allow for a reduction of mixtures that are not interpretable, often associated with wearer DNA.

Wearer DNA is the deposit of epithelial cells on clothing worn by an individual. Detection of the last individual to handle or wear an item is often an important determination in forensic science.

The most commonly used collection methods for wearer DNA include swabbing and scraping. These often result in mixture profiles. The detection of a single individual who last wore or came in contact with an item is desirable. Recently, adhesives have been introduced as a possible reliable method for the collection of biological evidence. Adhesives have a tendency to recover less, but more recently deposited particulate than the current methods because they are less invasive. The ability to observe the collected cells with the aid of a microscope is another advantage of using adhesives.

The goal of the research was to compare the current collection methods of swabbing and scraping with a gel film called Gel-Pak '0' which shares similar properties with adhesives. Gel-Pak '0' has been previously studied in comparison to other adhesives for the collection of epithelial cells, and was shown to recover the top layer of loose particulate. The particulate was deposited by the individual who last came in contact with an item. Therefore, in comparison to the other two collection methods, Gel-Pak '0' was hypothesized to recover single source profiles on clothing items from the most recent wearer.

DNA analysis was performed on samples collected by the three methods from various clothing items including baseball hats, t-shirts, sweatpants, socks, and other items commonly submitted to crime labs for DNA analysis. The habitual wearer and second/last wearer wore each item for a predetermined amount of time.

Research findings revealed that Gel-Pak '0' collected less DNA compared to the other two methods for the majority of items sampled but did not recover single source profiles from the last wearer. Instead, all three methods resulted in DNA mixtures. Low levels of DNA associated with wearer DNA often resulted in peak height imbalance and stochastic effects. This prevented the determination of major and minor contributors for the majority of items sampled. Even with mixed profiles, the last wearers' profiles were more discernable with Gel-Pak '0' and swabbing, while scraping had a tendency to recover more DNA from the habitual wearers. There was no significant difference in which swabbing or Gel-Pak '0' most frequently collected more of the last wearer's DNA. However, swabbing resulted in slightly more interpretable profiles from the last wearer and an increase in overall CODIS eligible profiles compared to Gel-Pak '0'.

This research may reveal how best to collect wearer DNA. Swabbing and the use of a gel film or adhesive preliminarily shows to be more effective in detecting who last wore a piece of clothing while scraping best determines the habitual wearer. Revealing individuals who last wore an item can be of great importance in forensic science, and therefore, further research with various adhesives and gel films could be vital for solving forensic investigations.

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**DNA Collection, Wearer DNA, Gel-Pak '0'**

## **A60 Combined Chemical and Biometric Field Analysis of Human Fingerprints**

*Jessica L. Staymates, MFS\*, and Julie Flanagan, MFS, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8731, Gaithersburg, MD 20899; Shahram Orandi, MS, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8940, Gaithersburg, MD 20899; and Greg Gillen, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8371, Gaithersburg, MD 20899*

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After attending this presentation, the attendees will learn of a novel approach to combine quick field-deployable chemical trace detection analysis and a unique latent fingerprint collection method in a single sample.

This presentation will impact the forensic science community by offering a unique latent fingerprint collection method that is compatible with field-deployable chemical analysis techniques that are currently in use around the globe.

There are currently several existing methods to collect and analyze latent fingerprints for human identification with forensic applications. There are also numerous techniques to collect and detect trace levels of contraband materials from various surfaces within a few seconds. However, there is not yet a field deployable technique to collect a latent fingerprint and investigate that print for trace explosives or narcotics contamination in the field within a single sample. This presentation will describe a method to collect latent fingerprints and analyze them with a common trace contraband detection technique, ion mobility spectrometry (IMS).

IMS is a rapid screening technique commonly used in airports, prisons, and border control checkpoints to examine various surfaces for trace levels of explosives or narcotics. Surfaces are typically swiped with a collection material to collect microscopic particles, and the swab is then heated to temperatures exceeding 200°C for analysis. Adding a heat-sensitive and low out-gassing silicone adhesive to the collection swabs has been shown to improve the particle collection efficiency by a factor of 12.<sup>1</sup> This adhesive can also be applied to a surface for collecting a latent print, and, due to its heat resistance, is compatible with trace detection techniques such as IMS.

Collection swabs for lifting fingerprints were made by applying a heat-resistant silicone adhesive to opaque smooth Teflon swabs. Latent fingerprints containing trace levels of C-4 explosive were created by a volunteer on glass slides, and forensic magnetic fingerprint dust was brushed over the fingerprint for development. The adhesive swab was used to pull the latent fingerprint off the glass surface, similar to collection of a forensic tape pull. In addition to the latent fingerprint, a full set of ink-rolled exemplar fingerprints were also captured from the same volunteer. The latent print on the adhesive swab was analyzed directly with IMS. All fingerprints including both the exemplar and the latent were then scanned to digital form using an FBI Appendix-F certified scanning station. All images were cropped of most white-space, and the latent fingerprints were inverted across the vertical axis to correct for inversion resulting from the lift-capture. The digital fingerprints were measured for relative quality using the NFIQ algorithm<sup>2</sup>, processed through the MINDTCT minutiae detector<sup>3</sup> and the resulting minutiae templates matched using the BOZORTH matcher<sup>3</sup> to verify that a match can be made between the latent and the matching exemplar finger. Preliminary results show that the fingerprint swabs were capable of producing an IMS response for the explosive, and the lifted prints had enough fingerprint detail to make a positive match using the algorithm, even after thermal desorption.

This presentation will introduce this novel approach of combining trace contraband detection with biometrics for field applications. Having the ability to collect a latent print and heat it for chemical analysis while keeping the print intact could be extremely useful for law enforcement and military operations. It could potentially reduce the cost and delay of sending fingerprint samples to a lab for chemical analysis. Chemical analysis in the laboratory may also affect the ability to analyze the fingerprint for biometric purposes. This work is beneficial to the forensic community by offering a unique latent fingerprint collection method that is compatible with quick field-deployable chemical analysis techniques that are currently in use around the globe.

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### **Latent Fingerprint, IMS, Trace Detection**

## **A61 Shape Measurement Tools in Fingerprint Analysis: A Statistical Investigation of Distortion**

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The goal of this presentation is to examine one of the concerns stated in the 2009 National Academy of Sciences Report (NAS), that of shape change (distortion) of a print as it is impressed on various substances with different mechanics of touch (i.e., pressure).

This presentation will impact the forensic science community by describing statistically the changes that occur when a print is impressed on different substances under varying pressures. This will allow for a quantifiable means to describe shape change of a fingerprint relative to these variables.

In the 2009 NAS report summary assessment on friction ridge analysis, it was stated that the impression left by a finger would invariably change each time due to degree of pressure used and impression medium touched. As per the NAS report, "None of these variabilities – of features across a population of fingers or of repeated impressions left by the same finger – has been characterized, quantified, or compared." It was the goal of this project to investigate the latter portion of this statement.

A well-developed method to study shape change is that of geometric morphometric analysis (GM). This involves the placement of landmark points on digital images. The data from the images is extracted and compared statistically, allowing for multivariate quantitative comparison.

Shape information can be visualized by plotting landmark positions in Procrustes superimposition. Procrustes distances can be used to summarize variations in populations, to express the degree of similarity of individual specimens, means of populations, or to search for matches between specimens.

The tools available for statistical analysis include Principal Component Analysis (PCA) with which the principal variations of shape can be plotted and visualized. This allows for determination of which shape aspect is responsible for the most variation and reveals patterns of covariance or related structure in data. Canonical Variates Analysis (CVA) can also be used to determine if shape information is distinguishable between different categories of data. CVA is a multi-axis discriminant function and it attempts to sort individuals into groups based on multivariate measurements.

It was the goal of this project to determine if these methods would translate to fingerprints describing changes in a print relative to pressure and substrate. A series of fingerprints were acquired from a volunteer who is also a fingerprint examiner. These prints were impressed on 10 print cards, computer paper, soft gloss photographic paper, and retabs. Each series was impressed with heavy pressure, normal pressure and light pressure. Ten prints were obtained in each series for a total of 114 prints of the same finger under the varying conditions of substrate and pressure.

The prints were then scanned on a flatbed scanner with a calibrated ruler in place and digital images created (1000dpi). Eighteen (18) landmarks were placed on the print and two on the scale for reference with tpsDig freeware. The landmarks were chosen at points of minutia that maximized the area of the entire print. The data was then extracted and the prints compared statistically with IMP freeware. Repeated measures were used to determine the variance, which was described by Root Mean Square (RMS) as the data was multivariate and not normally distributed.

Results indicated that there was a high degree of reproducibility of the prints at the varying substrates and pressures as the variance was very low at 0.000886 with an RMS of 0.029. PCA analysis determined that 56% of

the variance in the prints can be seen in movement of the ridge structure in the fingertip portion of the print while the bottom portion remains almost stationary.

CVA plots of scores can separate specimens into groups (i.e. by pressure+ substrate). Cross-validation estimates of assignment tell how effectively the measurements are in performing this task of assigning specimens to groups. There was a high correct assignment to pressure based on substrate in all groups except the 10 print cards where the results were not pronounced, indicating that 10 print cards are relatively immune to pressure affects. Substrate differences were always detectable, an effect which increased with increasing pressure.

As per the NAS report, "Formal research could provide examiners with additional tools to support or refute distortion explanations." This study is a beginning attempt to understand affects of substrate and pressure on distortion of a fingerprint.

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**Forensic Science, Fingerprints, Geometric Morphometric Analysis**

## **A62 Geographic Information Systems and Spatial Analysis – Part 1: Quantifying Fingerprint Patterns and Minutiae Distributions**

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After attending this presentation, attendees will understand how Geographic Information Systems can be used to quantify spatial relationships in fingerprints. Furthermore, attendees will learn how custom GIS tools can be used to run fingerprint matching simulations and calculate false match probabilities.

This presentation will impact the forensic science community by making a significant contribution toward forensic practice in the laboratory by establishing a degree of certainty for fingerprint uniqueness and by defining the limits for latent print identifications.

A Geographic Information System (GIS) is a collection of hardware and software components that integrate digital map elements with relational database functionality. GIS data is typically captured in the form of either raster grids (i.e., pixels) or vector features (i.e., points, lines, and polygons) with points in space using x, y and sometimes z coordinate values. While GIS is widely used for crime pattern analysis and emergency management applications, its spatial analysis capabilities have not been applied to fingerprint characterization and pattern recognition. The methodologies presented herein provide a framework for cataloging, characterizing, and quantifying fingerprint patterns and minutiae using custom and standard GIS tools. The power of a GIS is in its ability to allow users to integrate, store, edit, and analyze spatial features and relationships, as well as query and display spatial information. Such systems include traditional mapping capabilities (e.g., land surveying and aerial photography) and provide users with tools to interactively search and analyze spatial information. GIS is increasingly being applied to a field of study referred to as spatial analysis, which involves analyzing the positions, patterns, and relationships between objects located in a defined space. Collections of objects in a defined space may be linked or associated with one another geometrically or by functional associations. Given that fingerprint analysis and latent print identification is based on spatial associations between minutiae and ridge lines (e.g., ridge counts and minutiae locations, directions, and distributions), GIS-based tools for spatial analysis are a natural extension. Utilization of GIS in conducting dactylographic research is particularly appealing given that

fingerprint minutiae and ridge patterns are very analogous to geometries reflected in Earth surface topography, a traditional focus of GIS.

The Python programming language was used in conjunction with GIS software to create custom tools and automate complex project workflows. Python tools designed for fingerprint data collection, pattern characterization, and statistical analysis were created and deployed within a GIS toolbox. Initial minutiae detection was conducted using latent fingerprint processing software, with results exported as ANSI/NIST Type-9 records. A tool was created to parse finger and minutiae information from a Type-9 text file and transpose the data into Cartesian coordinate graph space. Coordinate referencing places the image and minutiae in quadrant I (+,+) of a Cartesian coordinate system. The Type-9 ULW summary output file was georeferenced to adjust the X-axis and Y-axis origins -100mm from the fingerprint core. Thus, all cores of each fingerprint image were positioned at (100,100mm) within Cartesian coordinate space. Once imported and registered in GIS, minutiae were subjected to an additional level of quality control and secondary processing. Minutiae were re-positioned to more accurately mark their placement and falsely marked minutiae were corrected. Minutiae files were aggregated within a GIS geodatabase for querying and spatial analysis. Custom GIS tools were built and implemented for extracting specific fingerprint metrics. A ridge counting tool provides the capacity to count fingerprint ridges between all minutia, and a ridge skeletonizing tool converts ridges into vector polylines, which allows for additional levels of analysis compared to raster grids. Pattern characterization tools were created to analyze point density and percent minutiae frequency by spatially intersecting minutiae with pre-established templates, such as a 2mm grid overlay and a core-centered “dart board” diagram. Distance and azimuth (0-360 degrees) from the finger core were calculated for all minutiae within the database. Using these values, minutiae were combined by print type and summarized in rose diagrams, which provide succinct figures for displaying minutiae distributions relative to the core. In addition, a Monte Carlo simulator was built to randomly sample  $n$  minutiae from a given fingerprint and iteratively match said minutiae against a database of fingerprints. This statistical analysis tool creates data suitable for building false match probabilities. Impacts of this work include making a significant contribution toward forensic practice in the laboratory by establishing a degree of certainty for fingerprint uniqueness and by defining the limits for latent print identifications.

This project was supported by Award No. 2009-DN-BX-K228 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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#### GIS, Fingerprint, Analysis

### A63 Geographic Information Systems and Spatial Analysis – Part 2: A Monte Carlo Approach to Estimating Probabilities for Latent Print Identification

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After attending this presentation, attendees will understand the use of Geographic Information Systems as a means to quantify fingerprints and evaluate fingerprints.

This presentation will impact the forensic science community by addressing issues raised by the NAS report to apply a scientific approach to fingerprint analysis. The probabilities being produced are addressing the uniqueness of fingerprints.

A Geographic Information System (GIS) is a collection of hardware and software components that integrate digital map elements with relational database functionality. GIS data are typically captured in the form of either raster grids (e.g., pixels) or vector features (e.g., points, lines, and polygons) with points in space using  $x$ ,  $y$  and sometimes  $z$  coordinate values. GIS allows for the placement of spatially rich objects, such as fingerprints, in a shared spatial environment, and allows for comparisons among the objects. For this study, approximately 950 fingerprint images from digits one, two, six, and seven were geo-referenced by placing the core at 100mm in Cartesian coordinate graph space consequently positioning every print in positive graph space. Fingerprint parameters including minutiae type placement, direction, and the spatial relationship of minutiae were quantified and were used to evaluate the uniqueness of fingerprint regions. The distances and directions between minutiae and between core and minutiae were calculated. In addition, a Monte Carlo simulation, more specifically a bootstrapping simulation, was used to estimate the probabilities of occurrence of different spatial configurations of minutiae within print types. These probabilities were created first for a reduced model of position only ( $x,y$ ) with a margin of error ( $\pm 0.32$  mm) placed around each minutiae position, and then for models of increased information and complexity. The simulations consisted of  $n$  minutiae randomly selected from a specific fingerprint region. The selected minutiae were then used to query our database for fingerprints with the same minutiae configuration. This procedure was iterated 1,000 times to obtain a distribution of false matches. This procedure was performed on nine predefined, overlapping regions of a fingerprint that represent the entirety of a fingerprint. These regions were defined in such a manner as to reduce any artificial boundary effects created by the boxes. For every fingerprint used in this procedure, nine separate probabilities were obtained. The simulations were run on 50 randomly chosen fingerprint images per pattern type (right slant loops, left slant loops, whorls, arches) for a total of 300 fingerprint images sampled and 50,000 iterations performed per region within a pattern type and for the number of minutiae chosen. These results were then used to analyze whether different regions have a lower chance of false matching. Simulations were performed with three, five, seven, and nine minutiae chosen. As anticipated, as the number of minutiae selected increases, the chance of obtaining a false positive decreases. The overall means for all regions and print types were  $\bar{x}_3=0.16$ ,  $\bar{x}_5=0.000714$ ,  $\bar{x}_7=0.000005$ ,  $\bar{x}_9=0.00$ . In addition, our simulations showed that the area above the core has a lower probability of matching anything not itself. The probability of false positives drastically decreased as more information, such as minutiae type and direction, was added to the model. Model simulations were performed both within print type and among all print types, and the findings for both approaches are similar. The research described addresses issues raised by the NAS report by applying a scientific approach to fingerprint analysis. The probabilities being produced are addressing the uniqueness of fingerprints.

This project was supported by Award No. 2009-DN-BX-K228 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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#### Fingerprints, Statistics, GIS

## A64 Novel Nanoparticle Polymer Applications in Lifting or Preserving Imaged Latent Fingerprints

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After attending this presentation, attendees will be introduced to a novel application of a polymer embedded with nanoparticles in the lifting and preservation of imaged latent fingerprints.

This presentation will impact the forensic science community by being aware of the research that has developed a clear polymer capable of lifting positive imaged latent prints from a wide variety of irregular surfaces as well as a nanoparticle-doped polymer capable of preserving imaged latents on a wide scope of paper documents. These constitute significant advances in the latent print discipline.

The 2005 on-going application investigation for a novel polymer began to focus on the lifting of imaged latent fingerprints from irregular and highly irregular surfaces such as plastic beverage containers, automobile dash boards, bank countertops, and mottled appliance surfaces including handles. Additional research demonstrated that the polymer provided a mechanism for preserving imaged latent prints on virtually any paper surface. Furthermore, accelerated aging tests involving heat and humidity sufficient to simulate ten years of aging were conducted. Experiments then focused on refinement of aerosol delivery of the polymer and solvent system.

Fingerprint samples were collected from these surfaces and the ridge detail assessed by five students who completed a three-credit course in fingerprint identification. Results demonstrated that the polymer spray provided an outstanding means of preserving imaged latent prints as well as lifting positive images of dusted latent prints from the aforementioned irregular surfaces with minimal stretching, distortion, or loss of ridge detail. Additional experimentation utilized latent prints collected from six volunteers on index cards at the same time of day, each day, for one week. The collected latents were dusted using magnetic black powder. One-half of the prints were sprayed with the polymer while the remaining prints are treated with a silicone-based product producing a negative image. Statistical results comparing the two lifts are presented. A semi-quantitative rubric was developed to compare ridge detail of the positive print images generated by this clear polymer and the negative print images generated by commercial available, silicone-based products for the index card experiment. Together, these studies demonstrate the viability of this polymer in the lifting of positive images of latent prints from nearly all surfaces.

In the preservation studies, simulated aging tests with heat and humidity demonstrated that the paper substrate yellowed prior to polymer degradation during tests designed to mimic ten years of aging. This led to the incorporation of zinc oxide (ZnO) nanoparticles into the polymer solution. The addition of the ZnO nanoparticles resulted in a clear solution given the nanochemistry of ZnO and filtration. The presence of the ZnO nanoparticles was confirmed by UV spectrometry scans ranging from 200nm to 400nm. Experiments demonstrated an increased resistance in simulated aging including UV light tests. In these experiments, newsprint, various cotton bonds, and copier paper were printed, dusted with magnetic black, and treated with the ZnO embedded polymer. The latent print was over-sprayed by a radius of approximately three centimeters. This enabled a comparison of the paper and the polymer coated paper outside of the latent print to assess the general effects of the polymer system on preventing paper oxidation. With regards to the effects of the embedding of the ZnO nanoparticles on the small potential of stretching distortion of ridge detail with the polymer-only application, the results indicate further reductions of distortion may be possible by this incorporation of the ZnO nanoparticles.

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**Nanoparticle, Latent, Fingerprint**

## A65 Assessing the Quantity of Friction Ridge Characteristics as a Function of Human Perception

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After attending this presentation, attendees will understand the degree of variation in the perception and interpretation of friction ridge skin characteristics by Ten-Print and Latent Print Examiners.

This presentation will impact the forensic science community by discussing the variation in the quantity of friction ridge skin characteristics as perceived and interpreted by practicing Ten-Print and Latent Print Examiners.

Friction ridge skin characteristics (bifurcations, ridge endings, dots) and their unique arrangements are the primary information content evaluated by Ten-Print and Latent Print Examiners when comparing unknown friction ridge skin impressions to known (record) impressions. During the comparison process, the information content (characteristics) of these friction ridge skin impressions are perceived and interpreted by the human examiner. This study seeks to understand the variability associated with the human perception of friction ridge skin characteristics.

Fifty (50) high quality friction ridge skin impressions were evaluated and the quantity of friction ridge skin characteristics (bifurcations, ridge endings, dots) were reported by eight practicing Ten-Print Examiners and fifteen practicing Latent Print Examiners (total n=23). Each impression was prepared by the same individual recording the impressions under controlled conditions from nine different donors using standard black printers ink and a standard fingerprint card. Each impression was scanned into a digital format at 1,200 dpi and image samples used in the evaluation were cropped from various areas in the fingerprints at a set size of 6x6mm<sup>2</sup>. Of the fifty impressions, two pairs of impressions were duplicate images to assess any variation in perception from the beginning of the study to the end of the study. Each of the fifty impressions was presented to the study participants in a digital format for evaluation. Examiners were asked to count and record how many bifurcations, ridge endings, and dots are perceived and interpreted by them for each impression.

Preliminary results from these twenty-three participants reveal more variation than expected in the perception and interpretation of friction ridge skin characteristics. Statistical analyses found no significant difference in the perception of friction ridge skin characteristics due to gender, age (<40yrs vs >40yrs), experience (<10yrs vs >10yrs), and specialty (Ten-Print Examiner vs. Latent Print Examiner). A slight variation was observed in the quantity of friction ridge characteristics perceived in the two pairs of duplicate images; however these variations were not statistically significant.

Preliminary results suggest the information content relied upon by Ten-Print and Latent Print Examiners may vary due to perception and interpretation differences by the human examiner. These preliminary results warrant further research to compare with computer based interpretations using the automated fingerprint identification system, to better understand the inter-examiner variability, and to determine whether these results have any impact on the conclusions generated during the comparison process.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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**Forensic Science, Fingerprint Characteristics, Fingerprint Perception**

## A66 Importance of Third Level Details in the Analysis of Fingerprints

Mukesh K. Thakar, PhD\*, and Haroon N. Khan, MS, Punjabi University, Department of Forensic Science, Patiala, Punjab, 147002, INDIA

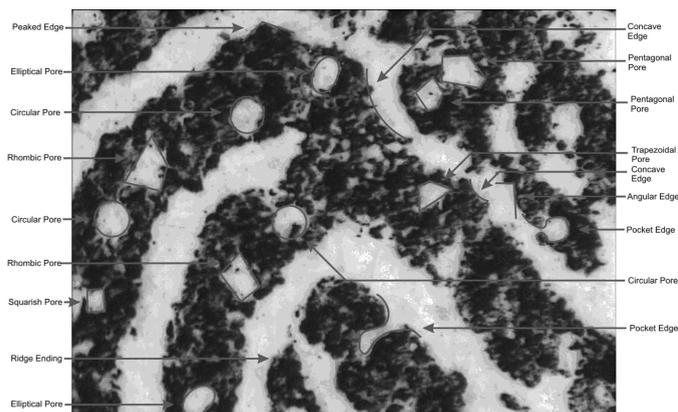
After attending this presentation, attendees will have exposure to the international community of forensic science which will help in exploring other areas of research.

This presentation will impact the forensic science community by being aware of lesser known characteristics that can produce very good results through innovation (i.e., measuring third level details which will provide an alternate method for identification of partial, smudged, or fragmented fingerprints).

The influence of cinema, television, and detective fictions has projected fingerprints as one of the most important types of evidence. The public has become most familiar with fingerprints as the best means to prove the identity of a criminal. These familiarities with fingerprints tend to provide them a greater importance in the field of criminal investigation. Fingerprints are most commonly available at the crime scene; their permanence and uniqueness leads to absolute identification of the person.

Prints recovered from the scene of crime are identified for their pattern types and compared with specimens obtained from suspects. During the process of comparison, 8-21 ridge characteristics are required to give a positive or negative opinion in the court of law (the number of characteristics varies from one country to another). If a sufficient number of characteristics is not available, the fingerprints cannot be used for identification. In many cases, the recovered fingerprints are partial, smudged, or fragmentary where the required numbers of ridge characteristics are not available. In such cases it becomes a great handicap for experts to give an opinion. In this situations, there is the need to include third level details (besides first and second level) such as the number, shape, and measurements of relative position of sweat pores and shapes of the edges of ridges which can be used to supplement the shortfall in the number of ridge characteristics to establish identity.

In the present study, an attempt has been made to collect 100 samples of partial, smudged, or fragmentary fingerprints along with complete prints from 53 males and 47 females on different types of papers. Second and third level characteristics (edgeoscopy and poroscopy) for each compromised print were marked at their correlative position to prove identity. After this, a further attempt was made to measure the distance between them. The results obtained were analyzed statistically and found significant. The range of ridge and edge characteristics (including pores) varies from 2-8/ridge; an example is given below in the photomicrograph (40X magnification).



Photomicrograph at 40x Magnification showing Second and Third Level details (ridge characteristics, shape of pores and edges)

Although the edge characteristics on fingerprints can be affected by a number of factors such as pressure applied, type of ink used, surface on

which the prints are taken, and donor, the results obtained from this study are very encouraging and will be of great use to fingerprint examiners, particularly those who identify individuals from smudged or partial/fragmentary fingerprints, or any fingerprints in which only few ridges are available for comparison.

## Fingerprints, Poroscopy, Edgeoscopy

## A67 LIMS: Getting What You Want

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After attending this presentation, attendees will know a minimum of ten concepts necessary for success when planning an information management project. The focus of the presentation is on project management ideas.

This presentation will impact the forensic science community by providing clear project management guidance to laboratories planning to implement a LIMS in the future or working to improve an existing LIMS. It will be beneficial to all laboratories with LIMS design or implementation aspirations.

Transitioning a laboratory from a traditional paper-based record keeping system to an automated information management system is a process that presents multiple challenges. Many crime laboratories either have faced this challenge already or will in the near future. Some are facing it for a second or third time due to dissatisfaction with initial attempts. The most common experience of this type is with the implementation of a laboratory information management system (LIMS).

During the three year period 2009-2011, the DNA database unit at the United States Army Criminal Investigation Laboratory contracted for, customized, and implemented an information tracking system. In the process, a number of key project management elements that proved important to the success of the system were discovered. Some elements were discovered because they were done well. Others were found because they could have been done better. The lessons learned are presented so that other laboratories may take advantage of them and avoid common pitfalls.

During the contracting phase, the goal must be to articulate and document detailed requirements for the system. No assumptions whatsoever can be made. The requirements document (or statement of work) will become the foundation of the development process. Items that are vague or left open to interpretation may become points of contention in the future and the system may not perform as expected. It was found to be highly beneficial to obtain statements of work from other laboratories as well as to visit other laboratories and observe similar systems in action. A contract officer representative who is not a member of the group writing the requirements should become involved in this phase. Where possible, it is advisable to engage an information technology specialist in order to ensure the system will work as planned on the laboratory's information services platform.

In the second phase of the project, design, or customization, laboratory management make a number of strategic decisions that determine the level of success. The composition of the design team is crucial. The most productive team was determined to be one which included someone who had performed every task the system would be expected to perform.

Perspectives of different users ensure that no critical functions were overlooked. It was found to be imperative to establish a documentation system for recording all interactions with the vendor early in the design/customization process. Phone conversations and emails on a multitude of minute details can quickly become difficult to manage. Reliable documentation can prevent misunderstandings and help to ensure the final product is satisfactory.

Once the system is designed/customized, the work is by no means over. Implementation is a very involved process. Many issues must be considered including entry of static data, conversion of legacy data, and interactions with other databases. Finally, the implementation phase must include a formal user training program with carefully constructed training exercises designed to cover every routine function.

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#### **LIMS, Information, Management**

### **A68 Turn a Box of Case Files Into One Click on Your Screen Using a Specialized Laboratory Information Management System (LIMS) to Improve the Efficiency and Quality of Serology in a Forensic DNA Laboratory**

*Tian Liang, BS\*, Stephanie Masters, BS, Kari Killian, BA, Daniel Hellwig, MSFS, and Martyna Shallenberg, BS, Sorenson Genomics, 2495 Southwest Temple Street, Salt Lake City, UT 84115*

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After attending this presentation, attendees will be shown how a specialized form of LIMS system can significantly reduce the process time and human errors of serology in a DNA laboratory. They also will learn the principles of how to design a more efficient LIMS system for different types of crime laboratories.

This presentation will give the forensic science community a different aspect of how to manage and track laboratory information in a superior way. It will compare the specialized form of LIMS to the traditional paper trials.

DNA analysis has become one of the most important criminal investigation tools. Serology, the first step of the DNA process, cannot be automated as other parts of this procedure due to the variation and complexity of the work and consequently more human errors could occur. *Sorenson Forensics, LLC* has improved both efficiency and quality of serology by utilizing a LIMS system with a software program, UNIFlow, developed by *UNICconnect*. UNIFlow is a software program that consists of different modules which are easily programmed by end users. After modification, it electronically tracks and manages every step of evidence and DNA sample handling, including case log-in, serology, DNA extraction, DNA analysis, and report generation.

This specialized LIMS system has significantly reduced the time of serology, averaging 20 minutes per case, by eliminating unnecessary work including filling out six handwritten forms (e.g., case evidence list, stain cutting list, reagent log, serology notes, case notes, and case management database). Uniflow also accesses four software programs (word processing, spreadsheet, database, and portable document software) to automatically input the same information repeatedly. Every detail of a case such as chain of custody (COC), case notes, serology notes and results, are entered real-time in LIMS. Hence, the COC can be recorded more precisely which protects the integrity of the evidence. In addition, backdating case notes is impossible which provides a truer representation of the evidence and DNA samples. All information can be corrected or changed if a mistake ever occurs; the changes are also tracked by LIMS and displayed on the final report. Moreover, by tracking information electronically, it largely prevents human errors such as misspellings, errors due to poor handwriting, and mislabeling tubes. By eliminating such human errors, LIMS largely reduces the possibility of sample handling errors. After serology is finished on each piece of evidence, the portion sent for DNA analysis is stored in an extraction tube with a unique barcode. The barcode is linked with all the

information of the evidence and tracked in LIMS through every step of the DNA process. Therefore, sample swapping is almost impossible.

One of the most significant advantages of UNIFlow LIMS system is that it is highly flexible and can be programmed to meet the specific needs and preferences of various types of forensic and non-forensic laboratories. *Sorenson Forensics, LLC* had researched many different LIMS systems available on the market (e.g., *Beast and Justice Trax*) before establishing UNIFlow. There was no other forensic LIMS software that could be tailored to coordinate the laboratory work flow in extensive detail. Therefore, the company utilized UNIFlow software and literally built a specialized LIMS system from the ground up. However, certain laboratory personnel needed to be trained to program UNIFlow software, which may increase the initial input time and cost of this LIMS system.

Overall, by implementing the specialized LIMS system in a forensic DNA laboratory both the efficiency and quality of serology and the DNA process have significantly improved and human errors have been reduced practically or totally eliminated. This system is easily adjusted to various types of forensic laboratories which can enjoy the same benefits.

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#### **LIMS, Efficiency, Serology**

### **A69 A Sample Tracking System to Automate and Increase Workflow in a Forensic DNA Casework Lab**

*Cindi L. Hricko, MS\*, 1020 Brand Lane, Apartment 1538, Stafford, TX 77477; and Michael A. Donley, MS, and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX*

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After attending this presentation, attendees will understand the steps needed to create and use barcodes with a sample tracking system in a high-throughput, automated forensic DNA lab. The audience will be led through casework examples using the HCIFS Forensic Biology Laboratory system as a model and be shown that this type of system used with barcode-labeled samples will increase efficiency, limit human error, and help ensure the integrity of each sample.

This presentation will impact the forensic science community by demonstrating how barcode labeled samples and a sample tracking system can increase laboratory efficiency and decrease human error during DNA processing. This type of system will benefit the criminal justice community by demonstrating a way to ensure the integrity of casework samples while providing a step by step snapshot of the automated verifications performed throughout our DNA testing process.

Tracking samples correctly and efficiently is a critical priority that becomes increasingly difficult with increases in caseload. In a batch-testing system, quality concerns increase as batch sizes increase. Verifications are required for each tube change and each time a plate is moved to a new station. Manual verification (i.e., by a second analyst) of casework samples tends to be time consuming and requires second analysts to be available to verify that paperwork and labels match. This is inefficient as well as a source of potential error. Barcode labeled samples, in conjunction with a sample tracking system, help reduce labeling errors and provide automated verification.

The HCIFS Forensic Genetics Laboratory currently utilizes an electronic LIMS to track the chain of custody of evidence *items*. However, the LIMS does not track evidence *samples* throughout the DNA process. To overcome this limitation, the HCIFS Forensic Genetics Laboratory designed and implemented a sample tracking system that generates a barcode for each sample and utilizes a SQL database to maintain the sample information. The system reduced the number of second analyst verifications from approximately twenty to one.

Within the laboratory, twelve barcode stations are set up to quickly and efficiently label samples. Each station consists of a barcode printer, a scanner with 1D and 2D capabilities, and either a desktop computer or a mobile tablet with sample tracking software installed. The software was

written to allow us to alter and customize it to accommodate evolving manual or robotic procedures. The sample tracking system generates a 1D and a 2D barcode label for each sample that enters DNA processing. The barcode label is scanned and tracked electronically from that point forward. The system allows samples to be prioritized (e.g., stat. rush, normal) and then orders them by the date they were entered into the database. Batches are compiled by the analyst based on these criteria. Manual, second-analyst verifications have been replaced by automatic verification as the analyst scans the source tube and then the destination tube to ensure that labels match before transferring a sample. The system has numerous safeguards to prevent bypassing mandatory verifications or incorrectly scanning a sample. For example, if an analyst tries to scan the same tube for both the source and the destination tubes on a tube transfer verification, or the analyst scans the top label twice on a label verification, an error message will appear alerting the analyst that the verification failed and won't allow the process to continue until the verification is successful. Database management procedures are also included with the sample tracking system so that cases can be closed and stored in a separate database to prevent the program from running slowly.

To further automate the barcoding and sample tracking, the software is used with liquid handling robot with an integrated barcode scanner. This allows the robot to identify the position of each sample on the deck according to its barcode; the user does not prepare a fixed-order list of samples to begin the process. A full description of the sample tracking system and how it integrates into an automated lab will be presented.

#### **Sample Tracking, Barcodes, Automation**

## **A70 Investigative DNA Databases That Preserve Identification Information**

*Mark W. Perlin, PhD, MD\*, Cybergenetics, 160 North Craig Street, Suite 210, Pittsburgh, PA 15213*

After attending this presentation, attendees will understand how investigative DNA databases can be improved to better preserve DNA identification information. When databases store and match probabilistic genotypes, instead of allele lists, all the biological evidence information can be preserved.

The presentation will impact the forensic science community by enabling DNA databases to make better use of biological evidence for investigations. By moving to a more informative probabilistic genotype representation, databases like CODIS can become far more sensitive and specific. This sharpened information capability makes DNA databases more successful in connecting the right criminal to DNA mixture evidence.

A DNA database can link crime scenes to suspects, providing investigative leads. These DNA associations can solve cold cases, track terrorists, and stop criminals before they inflict further harm. However, current government databases do not fully preserve DNA identification information and cannot maximize public safety.

DNA data is summarized in a genotype. The genotype can be stored on a database, and compared with other genotypes to form a likelihood ratio (LR) match statistic. Data uncertainty, present in most evidence, particularly DNA mixtures, translates into genotype probability.

Highly informative DNA mixture interpretation uses all the quantitative data, placing higher probability on more likely genotype values. Most evidence, though, is currently interpreted by a qualitative human review that diffuses probability across infeasible solutions. Since the LR is proportional to the true genotype probability, weaker interpretation methods lead to weaker (or nonexistent) DNA matches.

The weakest DNA mixture interpretation method is the Combined Probability of Inclusion (CPI), also known as Random Man Not Excluded (RMNE). CPI uses thresholds to truncate quantitative data into all-or-none qualitative "allele" events. The current DNA databases (including CODIS)

use a CPI allele representation that discards considerable genotype information, losing sensitivity and specificity.

The "probabilistic genotype" representation described by SWGDAM<sup>1</sup> is part of the new ANSI/NIST-ITL data exchange standard.<sup>2</sup> Unlike allele lists, a probability representation can preserve all DNA evidence identification information on a forensic database and calculates accurate LR statistics as it matches across the database.

ISFG's 2006 mixture guidelines<sup>3</sup> recommend the more informative LR over CPI. Unfortunately, current databases transform hard won LR genotypes into less informative CPI alleles. This presentation will explain how genotype probability can preserve identification information for DNA investigation.

Forensic DNA is an information science with DNA databases having the potential for considerable identification power. However, current database implementations discard most of the information in DNA mixture evidence. This presentation will help practitioners understand how to build and use investigative DNA databases that preserve all of the identification information present in their biological evidence.

#### **References:**

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2. SWGDAM. Interpretation guidelines for autosomal STR typing by forensic DNA testing laboratories. 2010; Paragraph 3.2.2 (probabilistic genotypes).
3. Carey S. Data format for the interchange of fingerprint, facial & other biometric information, ANSI/NIST-ITL 1-2011. Gaithersburg, MD: American National Standards Institute (ANSI) and National Institute for Standards and Technology (NIST) 2011; Sections 18.020-18.021 (probabilistic genotypes).

#### **DNA Database, DNA Mixture, Likelihood Ratio**

## **A71 The Imperial Avenue Strangler: Issues in Crime Scene Documentation and Evidence Collection**

*Curtiss L. Jones, MS\*, and James T. Wentzel\*, Cuyahoga County Medical Examiner's Office, 11001 Cedar Avenue, Cleveland, OH 44106*

After attending this presentation, attendees will understand challenges to documenting a complex homicide scene involving multiple victims killed at various times and will become acquainted with existing and emerging technology for crime scene processing and documentation.

This presentation will impact the forensic science community by serving as a key aspect of crime and/or death scene documentation by augmenting traditional still photography with full spherical images, scene diagrams, and other pertinent information relevant to the ongoing investigation, presenting viewers with an ability to have unrestricted views of crime and death settings as if they were on-site.

The Imperial Avenue Strangler case presented the challenges of documenting the locations of 11 fatalities within the same crime scene area, the collection of evidence pertaining to the 11 fatalities from both inside and outside of a three story residence, and the recognition and collection of evidence from inside of the residence from an unknown number of non-fatal sexual assaults. Of the 11 fatalities, six were located within the residence and five were buried in shallow graves in the rear yard. Recognition and collection of probative evidence linking the suspect to the 11 fatalities, regardless of the location from which each body was recovered, was also a major consideration. Recovery and documentation of the buried remains located in the rear yard proceeded in typical fashion. The overburden covering the shallow graves was removed mechanically. Recovery of bodies and foreign material was performed by shovel, trowel,

and hand. Each of the victims recovered from the rear yard was wrapped in plastic, cloth, or a combination of both. The wrappings were robust enough to keep each victim's remains encapsulated. Had the victims not been wrapped, it would have necessitated an "archeological" style dig to recover all of the victim's clothing, property, and remains.

Inside the residence, an in-depth multi-format photographic documentation scheme was employed. Still photography combined with 360 degree panoramic high dynamic range image capture ensured a complete digital image recording of each room within the scene. Using software, the photographic narrative was then overlaid onto detailed drawings allowing for "virtual" navigation within the resultant digital scene. Any documentation and evidence collection had to be exhaustive because there was a real possibility that the residence would be completely destroyed once the scene was released. Once the scene documentation was complete, each room was subjected to evidence collection focused on obtaining probative items pertaining to the known fatalities and/or suspected non-fatal sexual assaults. It was decided that any items with potential probative value would be collected from the residence. The broad spectrum collection scheme would ensure that a high percentage of useful evidence items would be collected, even if the probative value of a specific item wouldn't be realized until a later date or at all. Specific attention was applied to the collection of known samples from the residence which could at a later date be compared to evidence items collected from the wrappings received with the victims' bodies. Examples of reference samples collected from the residence included tape, plastic bags, carpet fibers, and carpet pad. The experience of the Imperial Avenue Strangler case has shown that to be effective in scene documentation and evidence collection for a complex multi-victim crime scene, a strategic plan incorporating input from investigative agencies is necessary. Also, a comprehensive image capture scheme beyond typical still images is desirable to ensure that the condition of the crime scene is recorded and can be accessed at will even if the physical scene is ultimately destroyed.

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**Crime Scene Documentation, Evidence Collection, Serial Homicide**

## **A72 Cold Case Criminalistics: An Anthropological Approach to Cold Homicide Casework at the Boston Police Department Crime Laboratory**

*Sarah A. Kiley, MS\*, University of Florida, C.A. Pound Human Identification Laboratory, 2033 Mowry Road, Room G-17, Gainesville, FL; and Donald R. Hayes, MS, Boston Police Crime Laboratory, 1 Schroeder Plaza, Boston, MA 02120*

After attending this presentation, attendees will be briefed on the complexities of cold case criminalistics and how collaboration between investigators, anthropologists, and criminalists has contributed to the successful evaluation and examination of cold case evidence at the Boston Police Department Crime Laboratory.

This presentation will impact the forensic science community by discussing the value of an interdisciplinary approach to cold case analysis to overcome the inherent challenges of missing documentation and degraded biological samples in an effort to expeditiously identify an aging suspect population in a modern crime laboratory; the implications of which contributes significantly to resolving investigations and successful prosecution.

In the United States, the definition of a backlogged case varies by jurisdiction, but has been defined by the National Institute of Justice as any case that has not been tested within 30 days of submission to a crime laboratory. Currently there is a considerable backlog of cold case homicides in the United States from the pre-DNA era. Federal grants, such as those offered by the National Institute of Justice DNA Backlog Reduction Program - Cold Case Initiative, provide eligible state and local authorities with additional funds to re-examine cold cases. Selection criteria for re-examination of cold cases include violent crimes where there

is close contact and a significant potential for Locard's exchange of trace and biological material, e.g., homicide, rape/homicide, stabbing, strangulation and blunt force trauma.

Forensic anthropologists trained in taphonomy, trauma analysis, and human anatomy are particularly well-equipped to sequence events, distinguish between extraneous environmental modifications of human remains, and interpret patterns of evidence and biological materials associated with cold cases. In most areas of the country, forensic anthropologists work as consultants to local law enforcement as well as medical examiners' and coroners' offices to assist with the identification and/or recovery of skeletonized human remains. Forensic anthropologists are often asked to examine evidence without being present at the time of recovery or during transportation, autopsy, or testing. Careful examination of the condition and pattern of evidence is synthesized by the anthropologist along with photographs, case files, police reports, newspapers, and climate data to recreate the circumstances surrounding the time of death. Additionally, training in human gross anatomy and an understanding of biomechanics and trauma analysis are valuable in identifying and collecting aberrant biological materials from cold case evidence such as blood, semen, and other bodily fluids from a victim's clothing, especially when coupled with an autopsy report's description of the type, nature, location, and size of injuries sustained by the victim.

Criminalists investigating cold cases are similarly responsible for recreating the circumstances surrounding the time of death as well as understanding the taphonomic history of the forensic evidence since collection. This often involves researching internal institutional forensic documentation standards for collection, modalities for the retention and storage of evidence, documentation, testing practices, and understanding how these have changed over time. Additional information gathered from supporting agencies such as medical examiners' offices, hospitals and outside investigative service organizations can help locate evidence and reference samples and guide investigations. Another agency's commonplace practices - particularly practices in place prior to the field's understanding of and ability to use DNA evidence - may provide biological comparative or reference samples (e.g., slides, swabs, evidence, or blood) that were retained without extensive documentation. Finally, consideration of the type of biological material, as well as the medium, size, and estimated robusticity (given the taphonomic history) of the sample, should be considered prior to collection. A review of the literature and consultation with a DNA analyst should be part of the evaluation of the collection and retention of small samples from evidentiary items. Traditional criminalist screening methods for evaluating evidence should be amended to reflect the quality and quantity of a cold case sample.

The successful prosecution of cold cases is dependent on the ability to evaluate evidence and select the case specific DNA test that is most appropriate to conduct in light of the documentation available. A case series history from the Boston Police Department Crime Laboratory will be provided to illustrate some of these challenges.

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**Criminalistics, Cold Cases, Forensic Anthropology**

## **A73 Quality Control on Crime Scene for First Response and "On the Spot Activities"**

*Donatella Curtotti, PhD\*, University of Foggia, Via Smaldone 21, Foggia, 71100, ITALY; and Luigi Saravo, PhD\*, RACIS, Viale Tor di Quinto, Rome, 00123, ITALY*

After attending this presentation, attendees will understand that the lack of Standardized Operation Procedures (SOPs) for the management of crime scene evidence in the judiciary system commonly leads to acquittal decisions due to the unreliable nature of the scientific investigations of the police.

This presentation will impact the forensic science community by highlighting the importance of Standardized Operation Procedures (SOP)

in the first response and “on the spot activities” at the crime scene. It is intended to be a call to action to the scientific community to become involved and proactive in the creation of such procedures in the countries where a void still exists in this area.

**Introduction:** Crime scenes that involve offenses of a violent nature are worthy of special note for two reasons. Such crime scenes very often involve the distribution of blood, body tissue, and several types of traces from the offender. For this reason, they can provide excellent contact trace material and potential evidence. But the investigation of these violent crime scenes can be quite complex both in the first response and in the crime scene investigation. There are a variety of techniques and tools that can be used to visualize and collect all types of evidence. Mistakes or oversights made during the management of crime scene can have a great impact on the final outcome of the case. For a crime involving scientific evidence, it needs to be processed with Standardized Operation Procedures (SOP) which detail the sequential steps of the investigation.

**Objective:** Standardized Operation Procedures (SOP) for the management of forensic evidence at the crime scene are missing in many countries, including a large part of the European nations. The study presents a statistical analysis of several Italian and other European countries’ decisions in which, despite the large amount of scientific evidence collected at the crime scene, the supreme criminal courts were unable to condemn the accused. The forensic investigations were not considered reliable enough to determine the standard of proof required by the law because the management of the process of the evidence in the first response and “on the spot activities” (such as initial assessment, identification of key evidence areas, preservation of the scene, crime scene documentation and recording, packaging and removal of traces) were conducted by the police without complying with Standardized Operation Procedures (SOP). Commonly, the practices introduced by the police for the processes of recovery, packaging, and storage of scientific material have been considered inadequate by the court to avoid contamination.

**Goal:** The goal of the study is to underline the importance of incorporating an accreditation process into crime scene investigations and to highlight the role played by SOP in ensuring both the quality of scientific investigations at the crime scene and the fairness of the judicial outcome.

**Result:** As a result of the efforts described in this study, a committee was recently formed in Italy to create the first Italian SOP for the activities of first response and crime scene investigations. This multidisciplinary working group is composed of members of police forces, magistrates, lawyers, forensic sciences experts, and academics. At the moment, it has drafted standardized guidelines on each step of crime scene investigation (such as observation, pre-examination, multi-communication, preservation, initial report, initial strategy, choice of methods and techniques, collection, packaging, chain of custody from crime scene to office/lab, storage items, and final report).

The procedures have been submitted to a large group of crime scene investigators attending training courses at the high schools of the police departments. At the end of the training courses, investigators have been asked to highlight the advantages of the introduced procedures, to focus on possible points of weakness of the procedures, and to strictly apply them when investigations are in progress.

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**Crime Scene, Forensic Investigation, SOP**

## **A74 Obtaining DNA From Spent Bullet Casings: A Review**

*Lisa R. Ludvico, PhD\*, and Ronald Freeman, BA, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282*

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After attending this presentation, attendees will understand the many research approaches and techniques employed in trying to recover DNA from fired bullet casings.

The presentation will impact the forensic science community in that often the only physical evidence linking a murderer to the crime scene are spent bullet casings. Maximizing DNA recovery from such a comprised source (spent bullet casings) will aid law enforcement in identification of suspects.

Law enforcement officers have found in many cases that the sole evidence recovered at a firearm-related crime scene is spent bullet casings. Obtaining even a partial DNA profile from spent bullet casings would be of utility in eliminating possible suspects. It was previously believed that the high firing temperatures would destroy any touch DNA (tDNA) that transferred to the bullet. As reported at the 2009 American Academy of Forensic Sciences meeting, Nase *et al.* found that the time the bullet is in the firing chamber is not sufficient to destroy tDNA. The second great hypothesized obstacle was the quantity and quality of the DNA left on the casing. The Dawson-Cruz laboratory (2008) demonstrated that DNA concentration can be enhanced using both pre- and post- amplification techniques. Partial profiles can be generated using both pre and post amplification modifications.

The research scope of this talk includes demonstrating that tDNA can withstand the high firing temperatures with single shots as well as firings from an entire handgun magazine. Swabbing techniques using different swabbing matrices and a variety of surfactants (1, 2%, and 20% SDS) and alcohols, ethanol and 2-propanol, have been examined. Moreover, “target swabbing” was conducted on both spent and unfired (though handled) casings with hyper-imaging technology and locate areas believed DNA to be present. However, target swabbing via imaging instrumentation had little effect on total DNA recovered in this study. Partial profiles were generated using both pre and post amplification modifications.

Another study followed the Dawson-Cruz pre-amplification and post-amplification procedures, but used shotgun shells. The shells were used in place of hand gun casings to determine if a profile could be generated for a Pennsylvania Game and Wildlife open case involving a shotgun.

The effect of “pooling” DNA from all casings found at a crime scene is also being studied. The pooled findings from the various studies show that loading order has an effect on DNA deposition, with the first and last loaded bullets having the highest DNA concentrations. No significant difference in DNA recovery exists from firing one shot vs. an entire clip.

Currently, the determination of which of the major components of primers (antimony, barium, and lead) may be contributing to downstream inhibition in DNA processing and how much DNA is lost in the packaging of spent bullet casings.

A review of all in-house DNA recovery research spanning three years, seven interconnected studies and the published literature, will demonstrate the status of the research on obtaining DNA from used casings is currently and what techniques have been most promising.

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**DNA Recovery, Spent Bullet Casings, Low Template DNA**

## **A75 Trace DNA From Fingernails: Increasing the Success Rate of Widely Collected Forensic Evidence**

*Lisa M. Hebda, BS\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824*

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After attending this presentation, attendees will appreciate the need for standardization in collecting and processing fingernail evidence and will be informed about best practices for recovering foreign DNA from fingernails and which DNA analysis methods are most successful for producing a DNA profile.

This presentation will impact the forensic science community by disseminating an optimized method of collecting and processing fingernail evidence which currently does not exist. The research has the potential to have a substantial impact on the way forensic pathologists, forensic nurses,

and forensic biologists conduct their work. The experiments were specifically designed to quickly and directly benefit practitioners so they will know how to best collect and process nail evidence.

Direct contact between an assailant and victim occurs during sexual assault and many other violent acts. As the victim attempts self defense, biological material from the assailant may be left, particularly under fingernails. Forensic nurses, emergency personnel, and pathologists often collect fingernails or material beneath fingernails from surviving or deceased assault victims for DNA testing. Unfortunately, very little is known about the utility of such collections, including if the existing methods for obtaining and testing fingernail/DNA evidence are optimal for producing probative evidence. Procedures for fingernail evidence collection and examination have never been standardized or optimized, nor has their subsequent genetic testing. In different jurisdictions nails can be clipped, scraped, swabbed, or simply not collected at all. A new set of clippers might be used for each hand, for each case, or the same set used for all cases. Rarely are nails treated individually, but instead are collected and processed for the right and left hand, raising the possibility of cross contamination or dilution of an assailant's DNA if it resides under only one nail.

Given the thousands of nail samples collected following sexual assault or upon autopsy each day in the United States, a surprisingly small amount of actual research has been conducted on nail evidence. In consultation with several forensic practitioners, the research to be presented was designed to address these questions in an objective and statistically reliable manner. First, the general level of foreign DNA found under nails was examined. Next, volunteers scratched one-another's forearm under a constant level of pressure, using the middle three fingers of each hand. A buccal swab was also provided. Multiple methods for collecting nail evidence were tested, including swabbing the underside of a nail with a wet swab moistened with an SDS solution, a wet swab followed by dry swab, scraping beneath the nail, and clipping and analyzing an entire nail. Likewise, the utility of processing nails individually or combining all nails from a hand together was examined. DNAs were quantified from each collection method and various DNA analysis procedures widely used in crime laboratories (STRs, miniSTRs, YSTRs) were evaluated using commercially available kits. Finally, experiments were conducted to examine if the results could be enhanced by increasing the DNA injection time (30 sec), injection voltage (3 kV), post-PCR purification, and increasing the volume of amplified DNA loaded for electrophoresis.

The recovery of exogenous DNA from under the fingernails of average individuals was uncommon using standard collection techniques. In general, a major profile matching the nail donor was seen, and any alleles foreign to the nail were weak and small in number (one or two loci). Likewise, standard STR analysis of post-scratching nail debris produced few alleles from the person being scratched, and occasionally produced a complete profile of the scratcher. In contrast, YSTR analysis tended to lead to more callable alleles from the person being scratched (when females scratched males, as would be most common in a forensic situation). Increased DNA injection time (30 sec), injection voltage (3 kV), or post-PCR purification resulted in an approximate doubling of peak heights, along with some new callable alleles, and in some instances, a complete YSTR profile was obtained. Combining the increased injection time and voltage raised peak heights even more. In contrast, loading a higher volume of amplified DNA did not appear to increase peak heights nor increase the number of callable alleles.

The fact that foreign DNA is relatively uncommon under fingernails accents the significance of those instances wherein a foreign DNA profile is obtained from fingernail evidence. In this regard, utilizing YSTRs when a female is assaulted by a male may be preferable as more data may be obtained than when utilizing standard STRs. Finally, the ability to generate callable alleles can be enhanced by post-PCR purification or by modifying the injection parameters of the genetic analyzer.

#### **DNA, Fingernail, Sexual Assault**

## **A76 Sex Determination Assay for Degraded or Low Quality DNA**

*Amanda Buszek, BS\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will learn about a highly sensitive, pyrosequencing based sex determination assay for degraded or low quality DNA.

This presentation will impact the forensic science community by detailing a more sensitive technique for sexing challenging samples, such as aged skeletal remains or tissue with little or poor quality DNA, than is currently available. The pyrosequencing based method will better allow sexing of samples when the standard amelogenin method is not effective.

Current molecular based human sexing techniques target a region of the single copy amelogenin gene, which exists on both the X and Y chromosomes, with the X chromosome having a six base-pair deletion. When this region is amplified and sized, female samples result in a single allele (peak), while males produce two products. However, this method relies on a sufficient quantity of deoxyribonucleic acid (DNA) being present to produce a strong enough signal for detection, usually 0.5 – 1 ng. Unfortunately, many forensic samples, such as hair shafts, aged bone, or handled objects, have much lower quantities and/or quality of DNA, and traditional molecular sexing methods are unsuccessful.

In order to overcome shortcomings with targeting amelogenin in degraded or low copy DNA, multicopy loci can be assayed which increases the probability of amplification. For instance, X chromosome specific DXZ4 repetitive satellite sequences have successfully been used to help sex skeletal remains. Other sexing techniques utilize an autosomal multicopy Alu sequence and Y-specific multicopy DYZ5 sequence, with sensitivity as low as 4 pg. Different regions of the Y chromosome with even more copies have the potential to be utilized in this manner, including DYZ1, a family of repeats similar to but more prevalent than DYZ5, made up of a 3.4 kb repeat with 2000 to 4000 copies per cell.

In this study, a PCR multiplex for DYZ1 and Alu was created which was assayed using pyrosequencing technology. Pyrosequencing was particularly beneficial in these experiments because it is extremely effective for assaying small DNA fragments, consisting of as little as two primers flanking one or more internal bases. This technique differs from standard Sanger (dideoxy) sequencing as it is based on detection of pyrophosphate release upon nucleotide incorporation, which is converted to adenosine triphosphate (ATP) by ATP sulfurylase. A luciferase, in the presence of ATP, gives off light, while any unincorporated dNTPs are degraded by apyrase. Sequences can be generated beginning at the base directly beyond the primer, which are not possible using standard sequencing methods.

Small DYZ1 and Alu regions (<150 bp) were targeted and PCR primers were optimized and tested for specificity on male and female DNAs. Internal pyrosequencing primers were then developed for both regions which further increased the specificity of the reactions. Once the method was optimized on high molecular weight DNA, the sensitivity and accuracy were compared to traditional amelogenin amplification by analyzing artificially degraded DNA as well as low quality DNA extracted from forensic samples such as hair shafts, fingernails, aged skeletal remains, and touch DNA.

Modifications of standard pyrosequencing methods were also examined with a goal of increasing ease and throughput. Pyrosequencing generally utilizes biotinylated primers for post-PCR clean up, immobilizing single stranded products by strong noncovalent affinity to streptavidin. However, this process is time-consuming and requires a vacuum workstation and multiple costly reagents. Given this, the biotinylated primer technique was compared to an enzymatic purification utilizing the hydrolytic enzymes exonuclease I and shrimp alkaline phosphatase, thereby allowing for direct pyrosequencing of double stranded PCR products.

Ultimately, the DYZ1/Alu assay is far more sensitive and potentially less ambiguous sexing technique than the standard amelogenin method on low quality/quantity DNA, proving successful even with less than 1 pg of DNA. Pyrosequencing of multicopy sex-specific loci, along with autosomal controls, is a straightforward and fast molecular technique that could be highly useful to the forensic community.

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#### **DNA, Sex Determination, Pyrosequencing**

### **A77 Comparison of Methods to Collect Contact DNA From Fabrics**

*Sarah M. Thomasma, BA\*, and David R. Foran, PhD, Michigan State University, Forensic Science Program, 560 Baker Hall, East Lansing, MI 48824*

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After attending this presentation, attendees will learn about the different collection techniques utilized and preferred in acquiring contact DNA evidence from fabric items.

This presentation will impact the forensic science community by increasing knowledge about optimization of different collection methods for use on fabric based evidence. This improvement will assist investigators in choosing a collection technique that best fits the evidence in question and the background of the case. In particular, for clothing or other fabric based items that may have come into contact with multiple individuals, the methods described may improve the chances of identifying the victim or the perpetrator of a crime.

The tremendous sensitivity of current forensic techniques makes it possible, but not certain, to obtain a DNA profile from a small number of cells, including those collected from items that have come into contact with one or more individuals. Given this, it is important to collect as many cells as possible, and hence as much DNA, from the item in order to increase the chance of making an identification. Limited comparative research has been conducted on the common techniques used in forensic laboratories to collect DNA from fabric evidence, some of which may be suboptimal—failing to collect the maximum amount of DNA or cells present. Further, in the case of fabric items that may harbor DNA from more than one source, certain collection methods might be advantageous in helping to avoid retrieving mixed DNA samples while still recovering as much DNA as possible.

Common crime laboratory methods for collecting DNA from fabrics include swabbing, tape-lifts, or cutting out an area likely to have come into contact with an individual. The general effectiveness of swabbing has been studied in some detail, usually examining the type of swab used and how best to apply it to a surface. Direct comparisons of tape-lifts and swabs have been made, but generally only utilized saliva as the DNA source. Finally, objective comparison of either swabbing or tape-lifts to cuttings has not been conducted, even though forensic scientists often use the latter technique.

In this study, the three standard methods for collecting contact DNA from clothing were compared. Volunteers wore items such as t-shirts for a prescribed amount of time, and contact DNA was collected from selected areas of the item (e.g., collar or shoulder seams) using swabbing, tape-lifts, or cuttings. Next, separately swabbing or tape lifting the inside and outside of clothing was compared to see if DNA yields varied substantially. Finally, the t-shirts were purposely contacted by more than one volunteer to simulate evidentiary samples that may contain mixed DNA; for example, the inside and outside of a shirt where the arm/sleeve of the wearer had been grabbed. For comparative DNA isolation effectiveness, equally sized segments of fabric were tested using cotton swabs wetted with digestion buffer (containing 0.1% SDS) and rolled over an area of the fabric, tape-lifts applied until the tape was saturated with fabric and/or no longer adhered, or cuttings placed directly into digestion buffer. A standard organic extraction was used to purify the DNA, and yields were quantified using real time PCR, followed by STR analysis.

In general, significantly more cells/DNA were retrieved via cutting and soaking the fabrics than from swabbing or tape-lifts, although the cutting and soaking method tended to result in additional mixed profiles. More DNA was obtained when swabbing the inside versus the outside of the fabric, while there was no difference in DNA yields when tape-lifts were taken from inside or outside of the item. In spite of this, the frequency of mixtures between swabs and tape lifts was similar. All STR profiles generated from fabric samples that contained 200 pg/ml or more of DNA (optimal DNA input) resulted in callable alleles at least 13 loci. Over 80% of the samples that produced 100 pg/ml or more of DNA showed allelic activity at each locus, although approximately 60% of those contained alleles foreign to the wearer (one or more loci with three alleles). Close to 80% of the samples that contained 30 pg/ml or more of DNA (minimum input of 135 pg of DNA) had allelic activity at each locus, but approximately 50% contained alleles foreign to the wearer. Finally, samples with less than 30 pg/ml provided little information, with few or no loci containing callable alleles. The results indicate that although increased DNA yield enhances allelic activity, it may hinder the ability to identify individuals owing to the presence of additional alleles in the STR profile. Considering the totality of the results, careful consideration needs to be taken when deciding on a method for cell retrieval from fabric based items.

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#### **DNA, Collection Methods, Fabric Items**

### **A78 Optimizing Extraction Techniques for the Retrieval of DNA From Evidence Swabs**

*Michael S. Adamowicz, PhD\*, University of New Haven, Department of Forensic Science, 300 Boston Post Road, West Haven, CT 06516; Dominique M. Stasulli, and Emily M. Sobestanovich, University of New Haven, 300 Boston Post Road, West Haven, CT 06516; and Todd W. Bille, MS, Bureau of Alcohol Tobacco Firearms and Explosives, National Laboratory Center, 6000 Ammendale Road, Ammendale, MD 20705*

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After attending this presentation, attendees will have a better understanding of the possible benefits of potential modifications to the QIAamp® DNA Investigator Kit protocol when used to extract DNA from cells collected on cotton swabs. The methods evaluated include a variety of simple modifications to the manufacturer's "Surface and Buccal Swab" protocol.

This presentation will impact the forensic science community by providing experimental evidence demonstrating that some alterations to the manufacturer's extraction protocol can yield enhanced recovery of DNA from cotton swabs, while others provided no increase in yield or show decreased yield as compared to the published kit protocol. This study may suggest potential changes to extraction protocols that laboratories may implement in order to increase the yield of their DNA samples collected on cotton swabs.

Samples for DNA analysis are often collected from a wide variety of objects using cotton tipped swabs. However, the question remains: are all of the collected cells being released from the cotton fibers of the swab? With the advent of increasingly sensitive STR kits, more and more samples are being submitted for DNA analysis which have small quantities of DNA present, degraded DNA present, or both. Samples such as firearms and other handled objects fall into this category and have become items commonly submitted to forensic DNA laboratories. When processing these types of samples the recovery of the maximum amount of available DNA becomes critical, potentially dictating whether or not a usable profile can be derived for a piece of evidence.

The QIAamp® DNA Investigator Kit is a rapid and effective extraction tool for forensic samples. It can be used to process a wide variety of evidentiary materials and requires relatively little handling of the samples as compared to a phenol/chloroform/isoamyl alcohol DNA extraction. Using the standard protocol as a baseline, the following parameters were altered: incubation time, incubation temperature, stationary incubation,

physical disruption of the swab tip during incubation, and periodic re-suspension of the swab tip during incubation. Each of these conditions was performed as single variable experiments, as well as following up with extractions performed with a combination of conditions. All of the experiments were performed on cotton swabs which had either 10µl of liquid blood or 20µl of a buccal cell suspension dried onto them. All of the experiments were, at a minimum, performed in duplicate with both blood and buccal cells. Blank control swabs were also extracted for all conditions. Equivalent volumes of liquid blood and buccal cell suspension were also extracted in order to assess the retention of DNA on the cotton swabs. The concentration of DNA in each extract was quantified using the Applied Biosystems Quantifiler® Human DNA kit, and approximately one ng of extracted DNA was amplified with the Applied Biosystems AmpFI STR® Identifiler® Kit in order to assess the quality of the extracted DNA. Results indicate that up to 50% of the recoverable DNA may be retained on the cotton swab tip for both blood and buccal cells when using the standard extraction protocol. Stationary incubations performed poorly, with DNA yields falling significantly from those samples that were processed in a thermomixer. Incubation times of 18 or 24 hours showed no gain in the recovered quantities of DNA, and more often yielded less DNA that was of poorer quality. Some modest gains in yield were achieved with a three hour incubation coupled with an increase in temperature to 65°C with buccal cells. Physical disruption of the swabs was also no more effective than the standard protocol done with mixing. However, significant increases in DNA yields were observed using the swab re-suspension method for both blood and buccal cells, bringing the amount of recovered DNA close to the values observed for liquid samples.

In summary, while many alterations to the manufacturer's protocol were examined, only the swab re-suspension technique has shown significant gains in DNA yield to this point. These gains do appear to be substantial though, and further experiments will refine this technique to better enable forensic analysts to maximize the recovery of DNA from evidentiary swabs.

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#### DNA, Cotton Swab, Extraction

### A79 An Investigation of Trace DNA in Binding Materials and Clothing on Decedents in Simulated Crime Scenes

*Michael A. Donley, MS, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Jonathan Lai, BSc\*, 1400 Washington Avenue, Albany, NY 12222-0100; and Roger Kahn, PhD, and Rhonda C. Williams, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

The goal of this presentation is to provide a comparison of different methods used for the collection of trace DNA from a perpetrator left on a decedent at a crime scene.

This presentation will impact the forensic science community by increasing the recovery of trace DNA from a perpetrator from a variety of materials encountered on a decedent at a crime scene.

A low level of skin cells is transferred to an object by contact with skin. This is useful in criminal cases when a perpetrator touches an object. Small numbers of cells containing DNA are transferred to the objects that can be collected as evidence. In death investigations, such items are routinely removed in the morgue and then submitted to the DNA laboratory for analysis.

The transportation of the decedent from the crime scene to the morgue may cause loss of trace DNA. In death investigations when the decedent is bound or the body has been moved, or if there is evidence of blunt force or multiple sharp force traumas, the Harris County Institute of Forensic Sciences will dispatch a Trace Evidence Collection Team to collect DNA or other trace evidence from the decedent at the crime scene. The Team will collect samples directly from the body and from materials binding or

otherwise on the body. In this study, volunteers simulated crime scene scenarios where DNA was transferred onto a variety of materials that might be found on a decedent such as clothing, rope, duct tape and zip ties. Each material has different properties that might require different methods of collection to obtain the highest yield of perpetrator DNA from the item. The goal of this investigation was to determine and establish the best way to collect the perpetrator's trace DNA from various materials on deceased individuals at a crime scene.

The initial study focused on testing several solvents and techniques to collect trace DNA from binding materials such as rope, duct tape, and zip ties, and also to collect exogenous DNA from the victim's sleeve and arm. A single swab method and a double swabbing method were tested. The solvents tested were sterile water and 1% Sodium Dodecyl Sulfate (SDS) detergent.

The average yield of DNA was determined for each collection technique. The double swabbing technique with SDS detergent yielded 1.40ng of total DNA compared to the double swabbing technique with water which yielded 1.21ng of total DNA. The single swab technique with water yielded 0.59ng of total DNA. On average, single swabbing techniques yielded less DNA than double swab techniques.

The second phase in this study was to compare different techniques to the swabbing method for the collection of trace DNA. The techniques consisted of using tape lifts, dissolvable tape lifts, and taking cuttings from the items for recovering trace DNA. The study indicates that for zip-ties and duct tape, taking a cutting from the item obtained the highest yield of DNA. Double swabbing yielded the highest amount of perpetrator's DNA from sleeves. Tape lifts obtain the highest yield of DNA from skin and rope. Further data concerning each method, the quality of the profiles obtained, and recommendations for the collection of trace DNA on decedents will be provided.

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#### Trace DNA, Binding Materials, Crime Scene Collection

### A80 Comparison DNA Preservation Buffers for Low Quantity DNA 4°C Storage

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After attending this presentation, attendees will have a better understanding of the DNA preservation capability of several commonly used buffers for DNA storage at 4°C.

This presentation will impact the forensic science community by assisting other laboratories in selecting the optimal DNA preservation buffer for short term storage at 4°C.

The amount of DNA recovered from some forensic evidence samples such as those from handled items is usually very low. Following extraction, DNA samples are typically stored at 4°C for a few days and sometimes a few weeks until completion of the testing process, and then transferred to a -80°C freezer for long term storage. Protecting DNA from potential loss or degradation during this period is critically important in order to obtain as much information as possible for genetic profiling. For this reason, it is necessary to evaluate the DNA preservation performance of buffers commonly used for 4°C wet storage.

Historically, forensic DNA has been suspended or eluted in water, 0.1xTE or 1xTE with or without additional reagents. For this study, two reagents, trehalose and fish sperm DNA, were considered. Trehalose is considered a very effective DNA protection agent in the dry condition. Fish sperm DNA is sometimes used as carrier DNA to enhance DNA recovery from a Microcon™ purification column. The following ten different DNA

buffers were evaluated: water, 0.1xTE, 1xTE; 15ng/μl fish sperm DNA in water, 0.1xTE or 1xTE; 10% trehalose in water, 0.1xTE, or 1xTE, and 15ng/μl fish sperm DNA in 10% trehalose with 0.1xTE. Human blood was extracted with the Qiagen MagAttract kit and diluted with each of the ten different buffers to a final concentration of 10pg/μl or less. Six replicates for each condition for six different time points were stored for up to 15 weeks at 4°C. Six replicates of DNA stored in 0.1xTE buffer alone were also stored at -80°C for each of six time points as a control. For each time point, DNA was measured with an in-house Alu based qPCR system.

Results from this study show a significant difference in the amount of DNA recovered from the various buffers used. Un-buffered DNA in water degraded quickly; however, the addition of fish sperm DNA increased the yield. Fish sperm also enhanced the performance of 0.1X TE buffer alone. Similarly, 10% trehalose was not effective when dissolved in water, but DNA was preserved better when trehalose was dissolved in 0.1xTE and 1xTE. With both fish sperm DNA and trehalose, 0.1xTE outperformed 1xTE. In order to investigate the difference between 0.1xTE and 1xTE, the affect of extra EDTA on qPCR was explored. Although qPCR was slightly inhibited with 1xTE, this effect cannot account for the dramatic difference between the two stored buffers.

Interestingly, there was no significant difference among the top performing buffers, 0.1xTE with 10% trehalose and/or 15ng/μl fish sperm DNA. More DNA was recovered from all of these buffers than from the controls stored in 0.1xTE alone at -80°C. This study proved that DNA preservation buffers have significant impact on the DNA recovery of low amounts of DNA stored at 4°C for even only a few weeks. Since it is critical to select optimal DNA preservation buffers to ensure the robust downstream genotyping, additional studies are in progress to examine the effect of these buffers on long term storage in both the wet and dry conditions.

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**DNA Preservation, Trehalose, TE**

## **A81 Renewed Efforts to Identify the Victims of the World Trade Center Disaster via DNA Testing**

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After attending this presentation, attendees will summarize the DNA testing efforts used to identify the victims of the World Trade Center disaster with a focus on the work that has been performed since 2006. Anyone attending this presentation will learn about the more recent techniques in DNA analysis used in the identification effort.

This presentation will impact the forensic science community by explaining how DNA testing is used to identify the victims of a large scale mass fatality event.

On September 11, 2001, 2,753 people died in the attack on the World Trade Center (WTC). Approximately 20,000 separate human remains were recovered initially from the site dubbed "Ground Zero," indicating an extreme level of remains fragmentation. New York City's Office of Chief Medical Examiner (OCME) was responsible for not only identifying each victim, but identifying every human remain that was recovered. DNA testing was crucial in the identification effort. Three DNA technologies, short tandem repeat (STR), mitochondrial, and single nucleotide polymorphisms (SNPs), were employed to aid in the identifications. As of October 2005, 1,597 victims and 10,904 remains were positively identified. Ninety percent of the remains were identified with DNA testing alone which suggested any future identifications would likely result from improved DNA technologies. By the end of 2005, such improvements began to take shape. The Bode Technology Group offered its optimized bone extraction method which proved to be successful on the WTC

samples. Also around this time, more human remains were discovered on the roof of the Deutsche Bank building adjacent to Ground Zero. New phases in both the recovery and identification of the victims had begun. The renewed recovery effort included searching of additional roof tops in the Ground Zero vicinity and systematic excavation underneath previously paved over access roads. The excavated material was searched for biological remains by using large sifting platforms. The resulting samples varied in size but were generally small and severely compromised. In order to maximize the success rate for this sample type, the OCME Department of Forensic Biology validated the Applied Biosystems' MiniFiler™ kit and adopted the optimized bone extraction method. Overall, a number of methods have been employed to help identify more of the WTC missing persons. Aside from STR testing with the MiniFiler™ kit, there was also retesting of victims' reference samples, and enhanced data interpretation with Cybergentics' TrueAllele® system. Also, the OCME has incorporated the optimized bone extraction into a high throughput sample flow for retesting of previously collected remains. Most of the new DNA results lead to piece to piece associations rather than finding new identifications. As of July 2011, 1,631 victims and 12,810 remains have been identified; 9,007 remains are still unidentified.

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**World Trade Center, Mass Fatality, Victim Identification**

## **A82 Pyrosequencing Analysis of DNA Labeled Security Ink**

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After attending this presentation, attendees will have learned about the possibilities to analyze DNA from bank notes stained with ink and DNA using a rapid extraction method, a PCR, and then a pyrosequencing assay. The pyrosequencing will reveal the unique DNA tag connected to a specific lot of bank notes or cassette. The analysis procedure, results, deciphering for control samples, and case work samples will be discussed. The use of DNA labeling for other items of value as art, perfume, clothing, and documents will also be discussed.

This presentation will impact the forensic science community by providing information regarding security labeling and the required DNA analysis to identify labeled items. This is of great value for labeling of cash-in-transit by incorporation of a unique DNA tag into the ink. As robberies of cash-in-transit are common in Sweden, this system will impact the handling of these cases and potentially prevent robberies. These issues will be discussed in more detail.

It is important to have systems and methods for security marking and protection of valuable property regardless of the material. Security marking is especially interesting when it comes to cash in transit and ATM machines where vast amounts of money are often handled. One method is to stain the money with either ink dye or dye and smoke. A further step is to add unique synthetic DNA tags that are invisible to the eye. DNA-tagged stolen money can then be traced back to its original location. This system is well developed and is already used in many countries in Europe.

Trace Tag, Inc. has developed the unique tags as well as an assay for analysis of the tags. The DNA tags consist of short synthetic oligonucleotides with a specific section for analysis purpose, and a variable section providing the unique tag sequence. Since there are billions of codes available it enables a unique link between a stained item and a security box, owner, or ATM machine. Moreover, the tags are totally invisible, cannot be replicated or counterfeited and can only be analyzed in authorized laboratories. These features allow analysis of individual bank notes that can be performed rapidly to assist the law enforcement in robbery investigations.

The analysis procedure is robust and user friendly. In short, the DNA tag is extracted from the note, the extract is amplified using PCR, and the

short DNA sequences are determined by pyrosequencing technology. Pyrosequencing is a fast and easy to use sequencing by synthesis method that can run up to 96-samples in less than an hour. The method was first developed for high throughput SNP analysis, but is also suitable for medium throughput SNP typing or sequencing of shorter stretches of DNA. Pyrosequencing technology has previously been utilized successfully for mtDNA sequencing and autosomal STR- and Y-STR analysis of forensic samples in our laboratory. The pyrosequencing data from the DNA-tagged material from each bank note is sent to Trace Tag International (TTI) in UK, where a database is used for identification of the unique code and the serial number of the sequence. The code is thereafter submitted to 3SI security systems, in Belgium, which identifies the customer (bank, security company, or others), the location, and the installation of the unique DNA tag.

Successful analyses of unique DNA tags by pyrosequencing analysis, and the subsequent identification through TTI and 3SI, show that tracing bank notes back to specific cash in transit boxes or ATM machines and their staining device is an efficient way to conquer crime. This labeling system and identification assay will thus provide an asset to overall security and has the potential to be applicable on a larger scale in a near future. The system can also be applied for invisible labeling of a large variety of additional items of value such as paintings, brand clothing, passports, documents, perfume, and much more. This will allow increased security in general and possibly prevent crimes. In addition to the possibility to actively trace valuable items or cash back to owners or banks, the information about DNA labeling can deter attacks, e.g., cash in transit vehicles.

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**Security Research, DNA Analysis, Pyrosequencing**

## **A83 Who Needs Gold?**

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The goal of this presentation is to explore the capabilities of various polymerases to overcome inhibition and generate short tandem repeat (STR) profiles from degraded, low template DNA samples encountered in forensic cases. Nuclear DNA extracted from evidence, which has been exposed to adverse environment conditions, may be degraded and the amount obtained may be lower than necessary for obtaining complete STR profiles. This research investigates a combination of extraction methods, various enzymes, and amplification protocols which can overcome the difficulties associated with amplification of low amounts of degraded DNA which may also contain inhibitors.

This presentation will impact the forensic science community by offering other options regarding enzymes used in polymerase chain reaction (PCR). Use of these enzymes and optimal amplification conditions can yield high quality human DNA profiles from low amounts of degraded DNA which may also contain inhibitors.

Forensic scientists are constantly looking for ways to attain complete human DNA profiles from less than ideal biological samples, particularly from low template DNA. DNA extracted from items of evidence which have been subjected to adverse environmental conditions can be degraded or inhibited in a way that could negatively impact the PCR reactions. Scientists in the past have changed amplification conditions in order to increase PCR efficiency which is crucial to the success of DNA analysis.

Cigarette butts retrieved from crime scenes are sometimes less than ideal forensic evidence. Temperature, humidity, and soil are some of the factors which can cause degradation of biological fluid deposited on cigarette butts. Tar and nicotine, a few of the components of cigarettes, can also act as inhibitors, as can humic acid in soil. The concentrations of these ingredients can adversely affect the amplification process. In addition, saliva deposited on cigarette butts that have been exposed to detrimental environmental conditions may yield lower than optimal amounts of DNA. DNA extracted from these items is also often degraded and contains inhibitors. Amplification of low template, degraded DNA can result in allelic dropouts, peak imbalance, and low intensity peaks.

This research explores the use of various polymerases during the amplification process of low template, degraded DNA samples which may also contain inhibitors. Although AmpliTaq Gold® is the polymerase of choice for obtaining human DNA STR profiles from forensic samples; there are other enzymes available in the scientific community. This study uses various polymerases and optimal amplification conditions to increase the efficiency and specificity of the PCR reaction, which in turn can yield better quality DNA profiles from low amounts of degraded or inhibited evidence samples.

The first part of this study focuses on amplification with polymerases other than the commonly used AmpliTaq Gold® polymerase to determine if other enzymes can yield similar or better STR profiles from an optimal amount of DNA which is neither degraded nor contains possible inhibitors. The second part of the study is conducted with DNA extracted from environmentally insulted cigarette butts. This includes depositing known amounts of saliva from various donors on cigarette butts, exposing them to a range of temperatures, submerging the cigarette butts in water and burying them in soil. These conditions are carried out over varying lengths of time. Various extraction procedures are used and the amplification conditions, including the amount of enzymes, are optimized to obtain high quality STR profiles.

Complete DNA profiles were obtained using 0.1ng of uncompromised DNA and AmpliTaq Gold®, Ex Taq™, and Diamond Taq™ polymerases. Although all alleles were present, some of the alleles were below threshold parameters when 0.1ng of DNA was amplified with DFS Taq polymerase. Attempts to amplify 1.0ng or lower amounts of uncompromised DNA with Titanium® Taq and PrimeSTAR® HS DNA polymerases yielded either partial profiles or no profiles. Amplification parameters for these reactions were as recommended by the manufacturer of the kit used in this research.

While some of the compromised cigarette butts yielded full STR profiles, most extracts gave partial profiles when the recommended parameters of the amplification kit and the AmpliTaq Gold®, Ex Taq™, and Diamond Taq™ polymerases were used. When extraction procedures and amplification parameters, including the enzyme concentration, were optimized, more of the compromised DNA extracts yielded complete STR profiles.

This study indicates that by employing polymerases not commonly used in analyzing forensic evidence, analysts could potentially produce human STR profiles from low template, degraded DNA which may also contain inhibitors.

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**Polymerase, Inhibition, Degradation**

## **A84 Multiplex Amplification of Deletion/Insertion Polymorphisms: A New Kit on the Block**

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The goal of this presentation is to detect deletion/insertion polymorphisms from evidence samples which have been subjected to various adverse environmental conditions. Nuclear DNA extracted from these compromised body fluids can often be degraded and low in amount. Extracted DNA samples may also contain substances that inhibit polymerase chain reaction (PCR) used for the amplification of genomic DNA. Short tandem repeats (STRs), due to their high discriminatory power, is currently the method of choice for human identification. However, detection of STR loci requires large fragments of nuclear DNA, and commercially available kits used to detect STR loci from forensic samples do not always yield complete profile from degraded or inhibited samples. This research discusses a combination of the extraction methods and short deletion/insertion polymorphisms (DIPS or Indels) which can help analysts acquire additional information from body fluids different from that obtained with only the STR amplification kits.

This presentation will impact the forensic science community by introducing deletion and insertion polymorphisms that can be derived from

degraded and inhibited evidence samples. Currently, forensic community uses STR profile from genomic DNA for identity. Addition of the deletion and insertion polymorphisms along with the sex determining locus, Amelogenin, would greatly enhance the capabilities of the scientists and help them obtain more genetic information from compromised samples.

Forensic scientists are constantly looking for ways to obtain complete DNA profiles from less than ideal biological samples. Some of the procedures to achieve this are to use different types of extraction techniques and more sensitive amplification kits. Saliva samples deposited on cigarette butts easily become less than ideal biological samples due to the environment where cigarette butts are often discarded during the commission of a crime. Temperature, humidity, and soil are only some of the factors which can hasten the process of degradation. Amplification of these types of samples may show allelic dropouts, peak imbalance, and low intensity peaks.

Tar and nicotine, some of the components of cigarettes themselves, can act as inhibitors. The concentrations of these ingredients can also adversely affect the amplification process. In addition, saliva samples deposited on cigarette butts that are exposed to detrimental environmental conditions may yield lower than optimal amounts of DNA, and the DNA in these extracts may be degraded and/or contain inhibitors.

One of the ways to improve DNA amplification when faced with degraded and inhibited samples is to change the size of the desired amplicons. The Investigator DIPplex kit takes advantage of short amplicons which are more likely to be amplified in degraded DNA samples. The short amplicons in this kit makes the DIPplex kit an ideal vehicle for analyzing degraded forensic samples such as body fluids deposited on cigarettes and then exposed to various adverse environmental conditions.

DNA from cigarettes butts, exposed to unfavorable environmental conditions, was extracted with various techniques and subjected to amplification using commercially available kits for detection of short tandem repeat polymorphisms. The same extracts were also amplified using the DIPplex kit in order to detect deletions/insertions in the genomic DNA.

Although the STR polymorphism and deletion/insertion polymorphisms cannot be directly compared, an assessment of the profiles can be easily made. It is concluded that the DIPplex kit is a highly sensitive and useful tool when amplifying degraded DNA samples. This assay can yield more identifying information from degraded samples than using only STR amplification. A combination of STR amplification and the deletion/insertion polymorphisms would give the forensic analysts more capabilities to analyze compromised samples.

#### **STR, Insertion/Deletion, Polymorphisms**

### **A85 Identification and Secondary Structure Analysis of a Region Affecting Electrophoretic Mobility of the STR Locus SE33**

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After attending this presentation, attendees will understand the difficulties in designing suitable primer binding sequences to amplify the SE33 locus due to a highly polymorphic region outside of the repeat region which, if amplified, affects the electrophoretic mobility of the amplification product.

This presentation will impact the forensic science community because the SE33 locus is listed among the highly recommended loci to be included as part of the expanded CODIS core loci and because of the expectation that this locus will increase international compatibility to assist law enforcement data sharing efforts.<sup>1</sup>

Concordance between new and existing STR kits is of paramount importance when querying historical data stored on National DNA Databases or when sharing information across international borders. As such, one of the most important aspects of any multiplex STR development project is a comprehensive investigation into the impact any changes to existing primer sequences may have on comparison of new and existing results. SE33 is one of the most informative markers in forensic use due to its high power of discrimination. During the course of developing a multiplex STR kit several SE33 primer designs were screened with one primer pair yielding a high frequency of discordant alleles when compared to the AmpFISTR® SEfiler Plus™ PCR Amplification Kit. This discordance was mostly specific to samples of African descent with an estimated frequency of 5.1% and was a result of a mobility shift of approximately +0.84nt. The sequence analysis of the affected alleles revealed that the only difference from the wild type sequence was a SNP outside of the SE33 repeat but within the amplicon of this particular set of experimental primers. In total, three different SNPs were all within 9nt of each other, each of which could cause the mobility shift individually. A computer model generated with the Mfold software predicted a region of secondary structure that encompassed the SNPs. This secondary structure was a stem-loop structure and the SNPs affecting the electrophoretic mobility of the amplicon fell within the stem portion of the structure. In order to characterize this region further the wild type SE33 sequence region was cloned into plasmid DNA. Site directed mutagenesis on this DNA revealed that mutations within the stem portion affected the mobility of the amplicon whereas mutations introduced immediately outside or within the loop portion of the stem-loop structure did not affect the mobility of the amplicon. Thermostability measurements using an oligonucleotide containing either the wild type sequence or sequences containing each one of the three SNPs demonstrated that the oligonucleotides containing the SNPs had significantly lower Tms when compared to the wild type sequence. These experiments strongly suggest that the polymorphic region contains a secondary structure that, when disrupted due to the presence of a variant SNP, results in mobility shift relative to the wild type sequence. To overcome this problem, the SE33 primers used in the final configuration of the multiplex STR kit avoided the amplification of this polymorphic region yielding in turn results highly concordant with the SEfiler Plus™ Kit.

#### **Reference:**

<sup>1</sup> Harris et al., Expanding the CODIS core loci in the United States, *Forensic Sci Intl.*, (2011) *in press*.

#### **SE33, Stem-Loop, STR**

### **A86 Proximity Ligation RT-PCR (PLiRT-PCR) for the Forensic Detection of Spermatozoa**

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After attending this presentation, attendees will understand the protein detection method of proximity ligation real-time PCR (PLiRT-PCR) and how this assay could be used for the forensic detection of spermatozoa.

This presentation will impact the forensic science community by offering a potential alternative to microscopic identification of spermatozoa. PLiRT-PCR is an inexpensive, sensitive, fast, and amenable to automation technology that could contribute to the reduction of sexual assault backlog. The reproducible sensitivity of this assay could also promote the extension of the time since intercourse collection interval to support the advancements in DNA typing.

Identification of seminal fluid from sexual assault evidence is standard practice in forensic biology laboratories, though procedures for processing these samples may vary. Generally, for the presumptive indication of semen, practitioners use an alternate light source as an enhancement tool

followed by testing for Seminal Acid Phosphatase. If positive, the next step may be to test for Prostate Specific Antigen (PSA/p30) with commercially available immunochromatography kits. PSA has been identified at very low levels in other body fluids, thus a positive PSA result may not be considered by all practitioners to be confirmatory for seminal fluid. The only undisputable confirmatory test for the presence of semen is the microscopic observation of spermatozoa.

However, microscopic observation of spermatozoa can be extremely time-consuming. Automated sperm searcher systems decrease the time spent on a single sample; still, these systems can only process samples one at a time. Furthermore, the cost of fluorescent microscopes and automated sperm searching technology is a large financial commitment for a laboratory.

Sexual assault samples are a large contributor to the backlog that many laboratories face. The time, cost, and limited automation capabilities of microscopic observation limit the reduction in this backlog. Forensic laboratories would greatly benefit from the implementation of a faster, cost effective, and amenable to automation method for the identification of spermatozoa. PLiRT-PCR has the potential to be the technology that meets these needs.

PLiRT-PCR is a molecular assay that enables detection and quantitation of a target protein. Using antibody probes that are specific for the target, a representative DNA molecule that can be detected by real-time PCR (RT-PCR) is generated if the antigen is present. The amount of signal from this surrogate amplicon is indicative of the amount of protein in the sample. It is a powerful and highly sensitive assay that combines the specificity of an immunological reaction with the sensitivity of PCR. If a protein only present on spermatozoa could be successfully targeted with PLiRT-PCR, it could serve as a confirmatory assay for the presence of sperm.

Several additional aspects make the PLiRT-PCR assay compatible with forensic laboratories. First, thermocyclers and RT-PCR machines are commonly found in crime laboratories and thus would not be an added cost. Also, PLiRT-PCR minimizes sample consumption as it is sensitive down to femtomolar ranges. Finally, PLiRT-PCR minimizes the chances of sample switching and has the potential to be fully automated and incorporated into a robotic platform because there is only one tube transfer during the whole procedure, which occurs just prior to the final RT-PCR step.

As a proof of concept study, and to evaluate its potential for forensic use, a PLiRT-PCR assay for PSA was developed. Results showed that the PLiRT-PCR assay was able to detect PSA in a 1:5,000,000 dilution of a one year old semen sample. In contrast, 1:10,000 dilutions of this sample yielded negative results with a commonly used forensic immunochromatographic PSA test. Low levels of PSA were also detected in saliva and blood samples with the PLiRT-PCR assay, though this is expected due to the sensitivity of the assay. This presentation will discuss the proof of concept study and the preliminary results obtained with a PLiRT-PCR assay targeting a potential semen specific protein. Sensitivity, specificity, and time necessary for the assay will be addressed. A molecular method for the detection of semen could far surpass the capabilities of microscopic detection in terms of sensitivity, speed, cost, and automation.

#### **Spermatozoa, Sexual Assault, PLiRT-PCR**

## **A87 Investigations on the Use of Tissue-Specific MicroRNA Markers to Determine the Wound-of-Origin of Bloodstains**

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After attending this presentation, attendees will have an understanding of the limitations of bloodstain pattern interpretation in certain case circumstances and the potential use of tissue-specific microRNA assays to determine the relationship of discovered bloodstains to the homicide under investigation.

This presentation will impact the forensic science community by revealing a new approach to correlate bloodstains with injuries. The method offers a way to test suspects' alibis which cannot be accomplished with current methods. The presentation will additionally impact the forensic science community by demonstrating that molecular markers can provide information on the circumstances surrounding a crime.

The body of a homicide victim is oftentimes removed from the primary scene by the perpetrator and disposed of elsewhere. The location of the murder then becomes an important fact to establish in the investigation. Knowing where the murder took place can assist investigators in identifying suspects. Murders often result in significant bloodshed which can allow investigators to establish the location of the murder based on bloodstain pattern interpretation. However, the circumstances of other homicide cases are such that little blood is shed or even discovered due to the nature of the injuries or the act of cleaning by the perpetrator. Additionally, the suspected murder scene is often a place where the victim is known to have a history of physical activities—sometimes the suspected murder scene is the victim's residence. These circumstances can complicate investigations; because if small amounts victim's blood are found at the victim's residence or at a place where the victim visits, then the question becomes whether the bloodstains are related to the homicide or the result of some prior accidental injury. Unfortunately, current forensic methods used to correlate bloodstains with injuries are greatly limited when dealing with trace amounts of blood or bloodstains that have uninformative patterns.

However, in several cases the authors have been able to correlate bloodstains with particular wounds based on the cellular composition of the stains. In each of these cases, the victim's injury was a fatal gunshot wound to the head, and the body was found at a site away from where the homicide was thought to have occurred. However, several bloodstains from the victim were discovered at the defendant's house which was the suspected homicide scene. The defendant stated the blood was unrelated to the murder—the victim had previously cut his finger as a result of an accident. The size, shape, and distribution of the bloodstains were consistent with the defendant's alibi. However, each of the bloodstains contained visible pieces of tissues. These tissues were sectioned and stained for histological examination and were identified microscopically as brain. The presence of brain tissue in each of the bloodstains indicated the blood was shed as a result of head trauma. The finding refuted the defendant's alibi and was pivotal in the resolution of the case. Unfortunately, the finding of discernible pieces of tissue in bloodstains is rare. However, the authors hypothesized that trace quantities of wound-track cells are present in evidentiary bloodstains. However, the minute quantity of cells would render the histological approach impractical. The detection of these cells requires a method that is sensitive and specific. Sensitivity and specificity are properties of current forensic DNA typing methods; therefore, this

research investigated a molecular approach to correlate bloodstains with injuries.

The developed a PCR-based technique to detect trace amounts of wound cells in bloodstains is reported. In this proof-of-concept study, the laboratory rat was used as a model to investigate the use of tissue-specific micro-Ribonucleic Acid (miRNA) markers to distinguish bloodstains originating from different wounds. Specifically, the miRNA species, Rn\_miR-124a\_1, as a marker for rat brain tissue was studied. The basic procedure for the miRNA assay consisted of the following steps: (1) extraction of total miRNA from simulated head wound bloodstains using a rat blood brain mixtures using QIAGEN's miRNeasy mini kit; (2) synthesis of cDNA from miRNA with QIAGEN miScript Reverse Transcriptase Mix; (3) amplification of the target miR124a-1 with Taq polymerase and oligonucleotide primers from QIAGEN miScript Universal Primer, and miScript Primer Assay; and, (4) identification of the miR124a-1 cDNA using the QuantiTect SYBR Green PCR Master mix fluorescence detection with the Rotor-Gene Q Real-Time PCR Detection System.

Preliminary studies included the optimization of the detection assay and the evaluation of the specificity of the marker. Additionally, a procedure for the collection of bloodstains for use by this assay, and the stability of the marker under different environmental conditions was examined. Proof-of-principle was achieved by the ability to distinguish bloodstains produced by a gunshot wound to the head versus bloodstains produced by a gunshot wound to the chest with use of the assay. This research illustrates that molecular markers can reveal information about the circumstances surrounding the deposition of biological evidence. This research stems from limitations encountered with current forensic methods, and the use of this approach may enhance the successful resolution of forensic investigations and the administration of justice.

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**Tissue-Specific MicroRNA, Bloodstains, Wounds**

## **A88 A Multichannel Microdevice for Rapid Forensic DNA Analysis**

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After attending this presentation, attendees will understand the development and functionality of a system developed for integrated microfluidic forensic DNA analysis.

This presentation will impact the forensic science community by demonstrating the advantages of using a microfluidic system for DNA analysis, including reductions in analysis time, sample and reagent volumes needed and concurrent time reduction that could impact processing of future forensic DNA samples.

STR typing is the accepted gold standard for human identification and is now successfully employed in forensic, civil, and military laboratories. Although highly successful and reliable, the process typically requires 8-10 hours to complete under routine conditions, employs large sample volumes, costly reagents, and is labor-intensive. Additionally, samples are susceptible to contamination as they are exposed to the environment at multiple points during sample processing. Transitioning sample processing and analytical methods to the microscale format will permit automation,

miniaturization, and integration providing the end user a system capable of expedited, cost-effective analysis in a closed system that reduces sample handling and possible contamination.

Previously, a system capable of fully-automated processing and analysis of STR loci directly from buccal swab samples was presented. The system utilized a single, integrated glass microfluidic chip, and encompassed liquid DNA purification, PCR amplification, and electrophoretic separation and detection of STR loci. Although capable of detecting 16 loci in under 75 minutes, the techniques involved were not sufficiently robust for field analysis and only allowed for analysis of one sample at a time, warranting improvements to the system. Additionally, the microchip substrate was made from glass using standard microfabrication methods which are cost-ineffective from a mass production perspective.

The work presented here highlights improvements to the integrated system. The transition to plastic microchips for multiplexed integrated STR analysis is described, allowing for more cost-effective, single-use (disposable) chips. With the improved system, expedited purification of DNA from crude samples is performed and a mixture of DNA and PCR reagents (commercially-based) are guided into chambers on a device capable of multiplexed analysis for PCR. Rapid amplification of 16-18 STR loci is achieved through use of an IR laser for non-contact heating and a non-contact method for temperature sensing. A six-fold reduction from conventional amplification time is demonstrated while still achieving STR profile quality required for forensic interpretation. Simultaneous amplification of multiple samples in the multichannel microdevice will be presented, demonstrating the capability for increasing sample throughput. Following PCR, precise fluidic control allows for movement of the amplified product into the separation domain of the device. Electrophoretic separation of the amplified fragments is performed with five-color fluorescence detection using an improved detection system capable of multiplexed detection. Single-base resolution is achieved during a separation that consumes <12 minutes, a three-fold time reduction from conventional separation and detection processes. A software analysis system, interfacing between the raw data output and the interpretable profile, allows for automated and accurate allele calling of samples processed using the integrated system from multiple donors. An overview of the functionality of the integrated instrument capable of accepting the multichannel microfluidic device will be presented, with data supporting the capability of the microfluidic system for rapid, automated, end-to-end genetic analysis for human identification.

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**DNA, STR, Microfluidics**

## **A89 Development of a Novel Human Mitochondrial DNA (mtDNA) Amplification Method for Use With Illumina® Next-Generation Sequencing Instrumentation**

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After attending this presentation, attendees will gain understanding of the current methods used for library preparation using the Illumina® next-generation sequencing (NGS) platform. Additionally, attendees will learn of a novel method, developed in our laboratory, which enables researchers to bypass the recommended laborious and costly Illumina® library preparation method. This novel method allows researchers to utilize PCR to generate amplicons that can be sequenced directly. A high-fidelity TaKaRa™ enzyme with proofreading activity is used so that DNA is amplified accurately and efficiently. Data will be presented that illustrates

the advantages of using TaKaRa™ versus a traditional polymerase enzyme such as Applied Biosystems® AmpliTaq Gold®.

This presentation will impact the forensic science community in terms of mtDNA sequence analysis. It is apparent that the field is moving in the direction of employing next-generation sequencing technologies for forensic mtDNA analysis. However, these techniques are often expensive, and laborious. A method to increase the efficiency and cost effectiveness of the Illumina® next-generation sequencing workflow, enabling the technology to be used more readily in the forensic laboratory has been developed. Laboratories wishing to adopt this novel method will no longer need to prepare libraries for sequencing by fragmenting gDNA, repairing ends and adding A overhang, ligating adapters and selecting for high quality DNA of interest. This method incorporates Illumina® adapters into the amplicons during PCR. Laboratories that are not quite ready to adopt next-generation sequencing technologies can still benefit from this amplification strategy. The TaKaRa™ high-fidelity enzyme produces significantly higher concentrations of amplicons during PCR of both pristine and compromised sample types than Applied Biosystems® AmpliTaq Gold® DNA polymerase. Use of the TaKaRa™ enzyme may enable laboratories to obtain mtDNA sequence data from difficult samples when AmpliTaq® Gold does not produce results.

Challenging forensic DNA samples, including bones and hair, often contain DNA that is degraded and/or is present in very low amounts. In order to obtain a reliable DNA profile, mitochondrial DNA (mtDNA) analysis is often utilized on these sample types. Studies employing newly emerging DNA sequencing technologies have been designed to interrogate amplified targets down to the single molecule level. While these technologies are capable of producing large quantities of usable sequencing data, they are laborious and peripheral instrumentation can be costly. For example, typical library preparation for the Illumina® GAIIx platform includes DNA fragmentation (often using an expensive Covaris® DNA shearing instrument), end repair and addition of a single A overhang, adapter ligation and DNA selection. Additionally, multiplexing experiments that maximize the use of flow cell space require an expensive Paired-End Module (PEM) fluidics system coupled to the Illumina® GAIIx. The novel method was developed using forensically relevant sample types for human mtDNA amplicon generation for single-read DNA sequencing on the Illumina® GAIIx, which enables laboratories to obtain next-generation sequencing data using familiar protocols without the additional instrumentation described above. This method includes traditional PCR amplification of target DNA with TaKaRa™ high-fidelity DNA polymerase with 3' to 5' exonuclease proofreading activity. A high-fidelity enzyme was chosen in order to reduce misincorporation of bases during amplification which may have an impact on NGS sequencing results downstream. Flow cell adapter sequences and multiplexing index tags are included on the 5' end of the mtDNA hypervariable (HV) region-specific primers and are incorporated into the amplicon during PCR. Specifically, the amplification primers were designed with multiplexing tags directly 5' of the target specific primer sequence so that resulting sequences can be parsed by tag using Sequencher® software that contains a specific parsing algorithm. This amplification strategy produces equal or higher concentrations of amplicons than the current protocol employed in forensic laboratories. Further, these amplicons can also be sequenced using Sanger methods without any apparent hindrance from the extended primer sequences. Thus, this method enables forensic laboratories to adopt one mtDNA amplification protocol for multiple downstream sequencing technologies. Additionally, this library preparation proves to be more efficient and more cost effective than methods recommended by Illumina®.

#### **Sequencing, MtDNA, Amplification**

## **A90 Method Development for Analyzing SNPs Associated With Stature**

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The goal of this presentation is to demonstrate to attendees how SNPs (Single Nucleotide Polymorphism) can be analyzed to predict stature using biological evidence.

This presentation will impact the forensic science community by demonstrating that long bones are no longer needed to predict stature, instead more commonly seen biological evidence samples have the potential to be analyzed to predict stature.

The field of forensic molecular photofitting provides methods for the prediction of physical characteristics by the analysis of associated genetic markers. The prediction of physical characteristics presents scientists with the opportunity to provide investigators with reliable investigative leads and may prevent innocent people from being convicted. Several genetic markers for physical characteristics have already been thoroughly researched by the forensic community including ethnicity, red hair color, and eye color; however, researchers have been less successful with characteristics including stature, body weight, hair color (other than red), and age. Researchers have identified many potential genetic markers associated with stature; however, only limited populations studies have been performed to determine if a true correlation is noted between genotype and adult stature. To provide a predictive range from samples of unknown origin, the additive effect of multiple genetic markers related to stature needs to be evaluated. This study aimed to develop and evaluate a method that could be easily adapted by forensic DNA laboratories that is designed to specifically detect single nucleotide polymorphisms (SNPs) known to have a strong association with adult stature. The SNPs focused on in this study included two confirmed variants associated with stature, rs1042725 within HMG2 and rs6060369 within GDF5, as well as two other variants found in regions or hotspots within the genome that have been associated with stature, PTCH1 (rs10512248) and BMP2 (rs967417). The SNaPshot Multiplex Kit, a primer based single-extension assay which analyzes up to ten SNPs in one reaction, was used to analyze HMG2, GDF5, PTCH1 and BMP2. The method development using the SNaPshot Multiplex Kit involved designing and optimizing general primers and SNaPshot primers for HMG2, GDF5, PTCH1, and BMP2. A sensitivity study was then performed to determine the optimal input of template DNA and to compare the full and reduced volume SNaPshot reaction. Following the method development, the PTCH1 and BMP2 height associated SNPs were successfully incorporated into a SNaPshot assay. The optimal input of template DNA for BMP2 was found to be 2.0ng for both the full and reduced volume SNaPshot reaction while PTCH1 had an optimal input of template DNA of 1.0-1.5ng for the full volume reaction and 2.0ng for the reduced volume reaction. All subsequent reactions were performed using the 2.0ng input of template DNA with the reduced volume SNaPshot reaction. Additionally, the data produced from a stature test pilot study of both males and females ranging from 196-149cm implicated the A allele as the height increasing allele for the PTCH1 SNP rs10512248, which was previously unknown. Lastly, the combined predictive power of only PTCH1 and BMP2 successfully predicted height from known samples with an error rate of 10.05cm. With the analysis of additional SNPs associated with stature, the combined predictive power of SNPs has the potential to produce an error rate similar to, if not better than, the current gold standard anthropological methods for height estimation. The availability of this type of assay for height prediction would eliminate the need for long bone and

extend the application for height prediction to more commonly analyzed biological evidence samples including saliva, semen, and blood.

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**Molecular Photofitting, Stature, Single Nucleotide Polymorphism**

## **A91 Internal Validation of a Real-Time Quantitative Polymerase Chain Reaction Assay for Human Mitochondrial DNA**

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The goal of this presentation is to inform the attendees of an internal validation performed on a human mtDNA real-time quantitative PCR assay at the University of North Texas Center for Human Identification. Attendees will gain a practical working knowledge of the assay's design, chemistry and performance. In addition, attendees will learn of this assay's potential for improving the work-flow efficiency and success rate of mtDNA sequence analysis.

This presentation will impact the forensic science community by exploring ways in improving human mtDNA sequence analysis.

An internal validation was performed on a human mitochondrial DNA (mtDNA) real-time quantitative PCR (qPCR) assay following standard 8.3 of the "Quality Assurance Standards for Forensic DNA Testing Laboratories." The purpose of this internal validation was to demonstrate that the developed method performed as expected and is suitable to be used at the University of North Texas Center for Human Identification (UNTCHI). The internal validation studies conducted include: (1) precision and reproducibility; (2) sensitivity; (3) inhibition: and, (4) known and non-probative evidence samples. Data collected from 18 separate qPCR runs demonstrate this assay has a high degree of precision and reproducibility evidenced by consistent cycle threshold values of the quantification standards and controls. The mtDNA target in the lowest quantification standard was detected in each experiment indicating the assay's sensitivity of detection is extremely low at 0.0001 pg/ $\mu$ L or approximately six human mtDNA copies per microliter. The assay successfully produced results at various levels of template mtDNA when challenged with various concentrations of three different PCR inhibiting compounds. Finally, reportable mtDNA sequence data were obtained for forensic casework sample types, including whole blood and skeletal remains, which had been tested with the human mtDNA qPCR assay. The successful completion of this validation study demonstrates the suitability of the human mtDNA qPCR assay for use in forensic casework and identification of human remains. Using this assay can assist an analyst in determining: if a sample contains sufficient human mtDNA to proceed with downstream sequence analysis; the amount of sample to be used for mtDNA amplification; and, if a sample with inhibitors requires dilution or additional purification measures.

The University of North Texas Center for Human Identification is an accredited laboratory which performs nuclear and mitochondrial DNA analysis. MtDNA sequence analysis is especially useful for challenging samples, such as telogen hairs, teeth, and older skeletal remains where the amount of genetic material and its quality vary greatly and often fail to produce nuclear DNA typing results. Implementation of a sensitive quantitative assay for mtDNA will improve work-flow efficiency and increase success rate of these sample types. The amount of mtDNA used for amplification and the quantity of amplified product for sequencing is critical for obtaining high quality data. Too much product added to the cycle sequencing reaction results in noisy data and too little product

generates low sequence signal. With an optimal amount of product added to the cycle sequencing assay, clean data are obtained. High quality sequence data which exhibits good signal intensity and very little baseline noise is critical for efficient interpretation of data and high throughput sequence analysis. Additionally, if an optimal amount of DNA is added to the amplification reaction, then downstream cycle sequencing procedures can be standardized. Using an optimized quantity of mtDNA in front-end amplification reactions for sequence analysis also preserves precious sample extract. This facilitates judicious use of the amount of sample extract consumed which is a principal concern when analyzing forensic samples. Although several methods have been developed to quantify DNA, real-time quantitative PCR (qPCR) assays offer great advantages such as a high degree of specificity, sensitivity and precision.

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**Quantitative PCR, Mitochondrial DNA, Internal Validation**

## **A92 Advanced Forensic Sample Analysis Utilizing Genome Wide Identification: Complex Mixtures and Copy Number Variants**

*Kevin C. McElfresh, PhD\*, Casework Genetics, 13580 Groupe Drive, Suite 301, Woodbridge, VA 22192*

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After attending this presentation, attendees will have a better understanding of SNP analysis of complex mixtures and the interpretation methods associated with forensic casework. Attendees will also have a basic understanding of the comparison of STR and SNP technology as it pertains to complex mixtures.

The presentation will impact the forensic science community by providing insight and understanding of the uses of Human Genome technology.

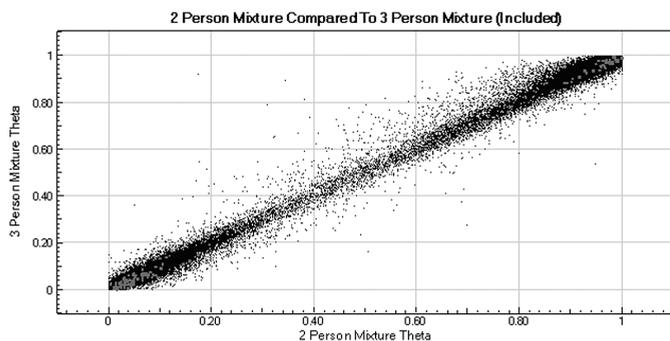
Ultra High Density Single Nucleotide Polymorphism arrays (UHDSNP) routinely used in Genome Wide Association Studies (GWAS) are now capable of analyzing from one million to over five million loci. The net effect of this analytical capability is the effective sequencing of the molecular differences in the human genome thus providing direct assay of the genetic differences between samples. GWAS analyze the SNPs on the autosomes, the SNPs on the X, Y and mitochondrial genomes, as well as the Copy Number Variant (cnv) loci, all in a single reaction and scan. The biggest advantage in adapting GWAS to Genome Wide Identification (GWID™) is the analysis of complex mixtures. In single-source match vs. non-match, GWID over analyzes the samples since the molecular differences are distinct. However, as the quality of the sample and/or the amount of sample decreases or the number of contributors increases, or both, the molecular complexity quickly outstrips the analytical capabilities of systems in which the results are based on secondary measures of actual molecular differences (capillary electrophoresis based sieving methods, i.e., STRs). The UHDSNP system in use at Casework Genetics (Illumina Omni1-Quad): assays 27 SNPs in the mtDNA genome, 139 cnv loci and 2,184 SNP loci on the Y chromosome, as well as 2,738 cnv loci and 24,756 loci unique to the X chromosome in addition to > 900,000 autosomal loci generating >1.1 million sequence points. The principle metric of GWID mixture analysis is the sum of the B allele frequency differences between the samples compared, providing a measurement of genetic similarity or dissimilarity between samples. The smaller the difference in the B allele frequencies, the more genetically similar the samples are. DNA from a series of test samples was initially analyzed as single source material using GWID. DNA from these samples was then used to create mixtures of two or more individuals, analyzed using autosomal GWID, and then re-analyzed using the autosomal and Y chromosome cnv loci. Scatter plots of these data correlate with the results of the autosomal data and provide additional insight into the analysis of complex mixtures. In this study, the samples for mixtures were also reanalyzed and compared to possible contributors from other studies by reassembling the manifest files and initiating a new analysis file. Table 1 provides representative data. Note

that a two person mixture can be included or excluded as being a subset of a three person mixture.

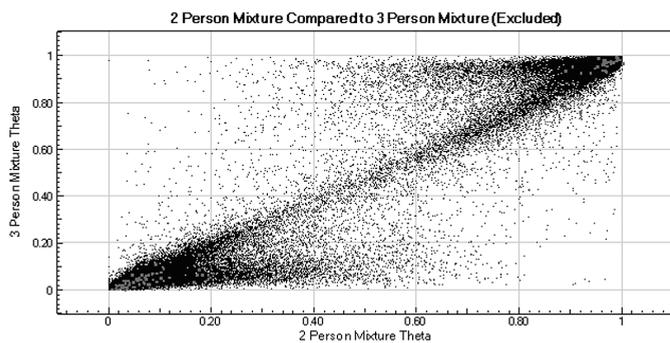
Comparison	$\Sigma f$ B Allele Differences
Buccal Swab : Semen ( single source match )	16,804
Buccal Swab : Semen (single source exclusion)	255,148
Suspect : Victim + Semen Mixture ( 2 person mix exclusion)	177,806
Suspect : Victim + Semen Mixture (2 person mix inclusion)	39,149
2 Person Mixture : 3 Person Mixture (exclusion)	121,029
2 Person Mixture : 3 person Mixture (inclusion)	13,751

**Table 1. B allele frequency differences between mixed and non-mixed samples.**

The scatter plots of all cnv loci, with the Y chromosome cnv SNPs highlighted, mirror the results from Table 1, especially with regard to the analysis of mixtures within mixtures. Figures 1 and 2 are representative of this comparison. The red points are the cnv loci on the Y chromosome. These results will be discussed in light of their application to cases in which challenging samples were processed.



**Figure 1. 2 Person Mixture Compared to 3 Person Mixture (Included).**



**Figure 2. 2 Person Mixture Compared to 3 Person Mixture (Excluded). SNPs, Complex Mixtures, Array Technology**

## A93 Development of a Single Mitochondrial DNA Amplification Strategy for Two Platforms: Next Generation and Sanger Sequencing From the Same Amplicon Library

Groves B. Dixon, BS\*, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723

After attending this presentation, attendees will have learned the basics of how the Roche GS Junior works, the benefits of such an instrument, and how it may be incorporated into forensic casework.

This presentation will impact the forensic science community by demonstrating the applicability of this new and promising technology for forensic casework.

When forensic samples contain limited or degraded nuclear DNA, mitochondrial DNA (mtDNA) analysis is a valuable substitute for short tandem repeat (STR) genotyping.<sup>1</sup> Drawbacks to mtDNA analysis include inferior statistical strength compared with STR genotyping and

interpretational challenges associated with rapid mutation rate and tendency toward heteroplasmy of the mitochondrial genome.<sup>2</sup> Heteroplasmy describes the presence of two or more unique mtDNA types within a single individual, tissue, cell, or mitochondrion.<sup>3</sup> Researchers now consider both length heteroplasmy (mixtures of mtDNA types that differ by indel mutations) and sequence heteroplasmy (mixtures that differ by substitutions) as expectations, rather than exceptions in mtDNA analyses.<sup>4</sup> The pattern of variation of heteroplasmy in the mitochondrial genome is still a matter of debate, making interpretation of similar but distinct mtDNA sequence data of particular difficulty to forensic technicians.<sup>1,5,6</sup>

Massively parallel sequencing (MPS) (also called Next Generation Sequencing, or NGS) technologies have the potential to generate orders of magnitude more sequence data than traditional Sanger sequencing at a competitive cost per base pair sequenced.<sup>7</sup> MPS platforms make use of spatially separated independent sequencing reactions of clonally amplified single molecules. This allows for the generation of thousands of independent sequence reads.<sup>8</sup> Thus, MPS allows greater breadth (the proportion of the genome that is sequenced) and depth (the number of independent sequencing reads taken of each nucleotide position in a region of interest) of sequence data.<sup>2</sup>

To improve the statistical strength and interpretational issues of mtDNA analysis, researchers recommend establishing a profile of the frequencies with which point heteroplasmy occurs at each nucleotide position and expanding the breadth of the mitochondrial molecule that is examined.<sup>9-12</sup> Because they enable significantly higher throughput and sensitivity than traditional Sanger sequencing methods, MPS technologies hold great promise for each of these recommendations.

In this study, the utility of the 454 Roche GS Junior Titanium MPS platform for forensic applications was assessed. The objectives included: (1) to optimize a single amplification protocol that enables forensic crime laboratories to analyze mtDNA using both Roche 454 MPS and Sanger methods; and (2) to assess the ability of the instrument to detect low level variants in mixtures of mtDNA.

The DNA from hair, blood, and buccal samples from twenty individuals was extracted. From these, modified amplicon libraries for use on the Roche GS Junior were generated. Reference sequences for mtDNA hypervariable (HV) regions were obtained for each donor with Sanger sequencing using these modified libraries. Mixtures of mtDNA HV amplicons were prepared in ratios of 95% / 5%, 98% / 2%, and 99% / 1%. These mixtures were sequenced using 454 pyrosequencing technologies to assess the ability of the Roche GS Junior instrument to accurately detect minor variants of mixed mtDNA.

Modified amplicon libraries for an MPS platform can be sequenced using traditional Sanger sequencing. This protocol allows for selective use of a sequencing method based on the quality of the sample. Straightforward exclusions can be interpreted directly from Sanger sequence data; however, in cases where Sanger sequence data provides insufficient resolution for confident interpretation, the analyst can return to the same original amplified library for MPS. Low level variants at mixture ratios of 99% / 1% were detected using the Roche GS Junior. Additionally, an optimized a protocol that allows seamless inclusion of the technology into forensic crime laboratories using current mtDNA testing methodology was developed.

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#### Heteroplasmy, Next Generation Sequencing, Mitochondrial

### A94 Molecular “Eyewitness”: Predicting Phenotype and Geographic Ancestry via SNPs Analysis for Forensic Applications

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After attending this presentation, attendees will be able to illustrate the potential for the prediction of physical traits and geographic ancestry of an individual using SNPs analysis.

This presentation will impact the forensic science community by understanding DNA analysis targeted at inferring the possible ancestral origin and phenotypic characteristics (i.e., hair color, skin color, and eye color) of the possible perpetrator could yield information valuable to the investigators.

Often an STR DNA profile obtained from crime scene evidence does not match identified suspects or profiles from available databases. In such cases, further DNA analyses targeted at inferring the ancestral origin and phenotypic characteristics (i.e., hair color, skin color, and eye color) of the possible perpetrator could yield valuable investigative information. Such a tool would aid in prioritizing suspect processing, corroborating witness testimony, determining the relevance of a piece of evidence to a crime, and ultimately increase the ability to identify individuals related to the crime scene. The completion of the Human Genome Project and the International

HapMap Project have provided the scientific community with a repository of reference information for the human nuclear genome, and efforts such as the 1000 Genome Project continue adding to this wealth of data. Numerous SNPs have been identified as having alleles associated with certain populations and/or correlated to specific physical characteristics.

The method chosen to develop a SNP based assay for ancestry and phenotype prediction is the Single Base primer Extension (SBE). This technique allows for the simultaneous typing of over 30 SNPs. Once an assay is optimized, it is possible to obtain robust results over a broad range of both quality and quantity of genomic DNA template. The sensitivity is in the range of STRs (down to 300 pg) and the method utilizes the same Capillary Electrophoresis equipment typically available in Forensic DNA laboratories.

The SBE technology has been used to develop panels which include 100 ancestry and phenotype markers selected from recent literature. Over 270 DNA samples along with corresponding ancestry/ phenotype survey information, and spectrophotometric skin color data have been collected from anonymous volunteers of varying ethnicity, gender, and age. These DNA samples, along with additional samples of known ancestry (without phenotype data), have been screened with the SBE panels. The genotypes and corresponding known characteristics are being evaluated to assess the predictive value of the candidate SNPs with the goal of identifying the optimal panel of SNPs to efficiently assess an unknown individual’s characteristics. Different statistical approaches are being evaluated for effective ancestry and physical trait inference. *STRUCTURE 2.3* is a population genetics and anthropology software package, based on Bayesian statistics, which was developed to analyze the genetic composition of individuals and populations. It can be used for various purposes including, but not limited to, assigning individuals to populations. While this software is powerful, other tools are being evaluated which are potentially more appropriate for estimating the phenotype and ancestry of a particular individual. These include analysis of molecular variance (AMOVA), multinomial logistic regression, and principle component analysis (PCA). The ideal statistical tool would allow for a more complex model that could appropriately incorporate the ancestry information contained within different types of markers such as mtDNA and the Y chromosome.

The final goal is the selection of the most informative ~ 30 SNPs that will be incorporated into a robust and sensitive SBE assay for ancestry and somatic trait prediction. This analytical tool, utilizing technology currently available in forensic DNA laboratories, could be implemented in a kit form and used on casework as needed. Preliminary results show good correlation between a small set of SNPs and eye color, making blue or brown (light versus dark) eyes highly predictable. Furthermore, PCA analysis shows clear separation between black and red hair, while dark brown, light brown and blond hair are more difficult to separate. Similar results have been obtained with skin pigmentation and ancestry, indicating that developing models for the prediction of skin and hair pigmentation together with ancestry will be a challenging process.

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#### SNPs, Phenotype Prediction, Ancestry Prediction

### A95 A Sensitive Multiplex PCR Based Next-Generation Sequencing Assay for Resolution of Mixtures and Analysis of Forensic Samples

*Cassandra Calloway, PhD\*, Saloni Pasta, PhD, and Christina Parodi, BS, Children’s Hospital Oakland Research Institute, 5700 Martin Luther King Jr Way, Oakland, CA 94609; and Henry Erlich, PhD, Roche Molecular Systems, 4300 Hacienda Drive, Pleasanton, CA 94588*

After attending this presentation, attendees will have a broader understanding of the 454 next-generation sequencing technology and the applications for forensic analysis, specifically for mtDNA mixture analysis.

This presentation will impact the forensic science community by providing an overview of a new, sensitive multiplex PCR assay for analysis of forensically relevant samples including mtDNA mixtures and heteroplasmy using the 454 Next-generation sequencing technology.

Next-generation sequencing (NGS) technologies have proven to be a powerful tool for research and clinical applications and have the potential to revolutionize forensic DNA analysis. NGS technologies offer a high-throughput solution for parallel sequencing of thousands to millions of sequences and can be used for de novo sequencing of small whole genomes or direct sequencing of DNA products generated by PCR. While several NGS technologies are available, the 454 sequencing technology appears to be the most suitable for forensic applications because it can directly sequence 400-500 bp amplicons. The 454 Genome Sequencer is a scalable, highly parallel pyrosequencing system that uses emulsion-based PCR for "clonal" amplification of single DNA sequences. The "clonal sequencing" aspect of this technology allows unambiguous allele resolution and provides for quantitative detection of variants present in less than 1% in a mixture. The 454 sequencing technology has been successfully used to analyze mixtures in clinical samples. Recently, the feasibility of 454 sequencing technology for analysis of mixed DNA samples similar to those encountered in forensic evidence has been demonstrated by sequencing mtDNA and nuclear STR markers. However, each marker was amplified in single-plex and significantly more DNA was required than is typically available in forensic cases. Thus, before this technology can be routinely used for analysis of forensic samples which are often limited and/or degraded, assays which require much less DNA need to be developed for use with NGS technology.

To greatly reduce the amount of DNA consumed for NGS, a multiplex PCR assay for parallel sequencing of mitochondrial and nuclear DNA markers which can be used for analysis of pooled forensic DNA samples has been developed. The use of a multiplex PCR assay for targeted resequencing of multiple mtDNA and STR markers is essential for forensic applications whereby DNA is often limited. Fusion primers containing unique multiplex identifiers (MID) are incorporated in the PCR primers containing target and 454-sequences and used to amplify individual samples for pooling and sequencing in a single run, thereby increasing sample throughput and reducing per run cost. The results from a sensitivity and mixture study demonstrating the utility of this NGS technology for analysis of forensic samples and the 454 sequencing data from heteroplasmic samples will be presented. Preliminary results show that the starting amount of DNA can be significantly reduced by using the multiplex PCR assay, similar to conventional forensic methods.

This multiplex PCR based NGS assay will allow the practitioner a start to finish method for sequencing mtDNA and nuclear STRs in a single run. This system can be used for the resolution of mixed or heteroplasmic samples commonly encountered in forensic cases and would therefore provide forensic laboratories with a more sensitive alternative to standard forensic assays.

**Next Generation Sequencing, mtDNA, Mixtures and Heteroplasmy**

## **A96 Extraction and Identification of Lifestyle Markers During Mitochondrial DNA Testing of Human Hair**

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After attending this presentation, attendees will learn a procedure for the chemical analysis of human hair for lifestyle markers using the discarded fractions from a protocol for mitochondrial DNA sequencing.

This presentation will impact the forensic science community by showing how the fields of microscopic, chemical, and genetic analysis of

hair can interact and be used to determine lifestyle markers of questioned hair samples. This presentation will demonstrate how the gaps between the fields of forensic hair analysis can be bridged by adopting the theme of "One Biologist's Trash is Another Chemist's Treasure."

Forensic hair analysis can be divided into three main areas: microscopic exams, chemical analysis, and genetic analysis. Each of these areas is focused on its own particular questions and analytes, and as such, these areas have developed independently of each other with their own literature, procedures, and practitioners.

The research presented here bridges the gaps between the areas of forensic hair analysis by adopting a theme of "One biologist's trash is another chemist's treasure." Several methods by which discarded fractions from a typical protocol for mitochondrial DNA (mtDNA) sequencing can be subjected to chemical analysis and the results provide information about the lifestyle of the subject, including cosmetic modifications to the hair, use of tobacco, and demographic information such as age and gender. It is hypothesized that any small organic compounds incorporated into the hair (e.g., hair dye components, nicotine/cotinine, integral lipids) will be released and partition into the organic layer during liquid-liquid extractions.

In practice, forensic science laboratories recommend that appropriate hairs should be selected for nuclear DNA (nDNA) analysis during the microscopic examination of the hair. In order to be suitable for nDNA, the hair root must be present. Hairs removed while actively growing (anagen phase) are more likely to yield partial or full DNA profiles. Hairs naturally shed when the hair follicle is dormant (telogen phase) are less likely to yield DNA profiles. Given that many cases when the root of the hair is absent or nDNA is otherwise unavailable (such as in telogen hairs), mitochondrial DNA (mtDNA) analysis can be utilized.

The first phase of a multi-phase project is presented here. Each phase involves three main developmental steps: validation, scale down, and extrapolation. Using literature methods for analytes of interest, the methods have been recreated and shown to be reliable using known standards. The published methods have been scaled down to the level of a typical mtDNA analysis (2cm length of hair) and the methods were extended to yield successful results when explicitly applied to the discarded rinses, washes, and organic layers of a mtDNA protocol.

The first phase of this project is concerned with the discarded fraction from the first step of an mtDNA hair analysis protocol. It is hypothesized that in addition to any residues of mounting media, neutral surface components will be extracted in the xylene wash. This includes surface lipids such as free fatty acids, fatty esters, squalene, and cholesterol. Residues of hair care products such as conditioners and hair gels are also extracted.

The validation step of this project was conducted by creating a standard mixture of surface lipids and relevant components of hair care products in xylene. Large volume injection gas chromatography/mass spectrometry (GC/MS) was used to achieve baseline resolution of all components. Linearity and limits of detection for each standard were determined.

The scale down step was accomplished by ultra-sonicating milligram quantities of hair in xylene and analyzing the extract. Microgram quantities were then analyzed which correspond to single hair fragments whose length is on the order of centimeters.

The first phase of this analysis was extrapolated by ultra-sonicating and analyzing xylene extracts of 2cm hair segments. The xylene extract was analyzed by GC/MS using large volume injection in a programmed temperature vaporizer (PTV).

This procedure will open up new possibilities for information that can be obtained from a hair sample, to include factors such as age (youth versus adult), tobacco use, and hair dyes. These methods could be applied during a typical extraction protocol for mitochondrial DNA and would result in an unknown sample being more fully characterized, while a comparison of a questioned and known sample would be more probative.

**Forensic Hair Analysis, Lifestyle Biomarkers, Mitochondrial DNA Sequencing of Hair**

## **A97 Demonstrating the Efficacy of Ethylene Oxide Sterilization for the Reduction of DNA in Plastics Used for DNA Extraction**

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The goal of this presentation is to describe the comparison of various sterilization methods for their ability to reduce the presence of amplifiable human DNA contamination. Additional studies also evaluated the effects of post-production sterilization on downstream DNA analysis.

This presentation will impact the forensic science community by providing information regarding options for decreasing the risk of DNA contamination, addressing a critical need as DNA typing methods become increasingly more sensitive.

Over the past several years, DNA analysis methods have become increasingly more sensitive with regard to the detection of very low quantities of DNA. Concurrently, laboratories have implemented automated processes to enable higher throughput and increased efficiencies within the laboratory. Many laboratories have moved away from manual extraction methods such as phenol: chloroform extraction in favor of automated bench top or high throughput systems. These systems are favored as they minimize the interaction between the analyst and the sample, reducing the risk of DNA contamination by the operator and the likelihood of sample switching errors. However, this trend has increased the burden on manufacturers of forensic products to ensure that collection devices and system consumables are free of extraneous DNA. This was highlighted in the recent "Phantom of Heilbronn" case in which a female profile, later attributed to contaminated swab collectors, was detected in evidence from numerous serious crimes throughout Germany. A joint publication issued by the ENFSI, SWGDAM and BSAG organizations (*Forensic Science International: Genetics* 4 (2010) 269–270) highlights the need for controls in the manufacture of consumables used for DNA analysis in order to minimize the introduction of human DNA. A number of measures can be introduced at the manufacturing site including manufacturing in a clean room environment, implementing extensive personal protective equipment (PPE) and automating the filling and assembly lines. Additional benefits may be achieved through post-packaging sterilization as a final measure of extraneous DNA removal. A series of studies to evaluate the efficacy of various sterilization methods including gamma irradiation, ultraviolet, electron beam and ethylene oxide (EtO) sterilization for the removal of DNA from plastics used for DNA extraction have been performed. Ethylene oxide is a widely accepted gas phase sterilization technique in the medical industry for the elimination of viable micro-organisms from medical devices and has recently been demonstrated to effectively minimize the presence of amplifiable DNA. In order to evaluate these sterilization methods applicable samples were spiked with extracted DNA and cellular material to mimic conditions of contamination. The spiked samples were provided to various sterilization vendors for treatment and then compared to untreated samples. The efficacy of DNA removal was evaluated using real-time PCR and STR-based detection methods. The studies demonstrated significant reductions in contaminating DNA from samples spiked with extracted DNA or cellular material using dual-cycle ethylene oxide treatment. Gamma irradiation, ultraviolet and electron beam treatment were less effective at reducing the presence of contaminating DNA. Further studies were performed to determine whether the ethylene oxide treatment would result in any deleterious effects on downstream sample processing for samples extracted with EtO-treated plastics. Treated and untreated plastics were used to extract a range of sample types. The extracts were subjected to downstream processing with real-time PCR and STR-based detection methods. Among the comparisons made for data generated from treated and untreated plastics were an evaluation of DNA recovery, profile quality, overall peak height, intra-color balance and artifacts. Ethylene oxide treatment was

demonstrated to significantly reduce the risk of human DNA contamination without detrimentally affecting downstream results.

**Contamination, Sterilization, DNA**

## **A98 The Effects of Ionizing Irradiation on Liquid, Dried, and Absorbed DNA Extracts With and Without Preservatives**

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After attending this presentation, attendees will become familiar with the possible effects of ionizing irradiation on samples of DNA extracts that are shipped or stored in various tube types and absorbed on storage papers.

This presentation will impact the forensic science community by presenting data intended to assist in the decision process of storing DNA extracts at ambient temperatures.

The stability of extracted DNA samples has been and continues to be the subject of much discussion and research. Storage conditions for DNA extracts range from frozen to ambient with ambient storage being the "green" method of choice. In addition to storage methodologies, issues associated with the shipment of extracted DNA samples should be considered. After the events of September 11, 2001, the ways packages are handled during shipping and upon receipt have changed.

This study was designed to examine the effects of various shipping scenarios on extracted DNA samples at two different DNA concentrations: 2ng/μL and 200pg/μL. DNA samples were shipped as: liquid in tubes, dried in tubes, dried with a preservative in tubes, dried stains on FTA paper, and dried stains on 903 paper with or without a preservative used during the drying process. Three different storage tubes were tested: perfluoroalkoxy fluoropolymer (PFA), polypropylene, and medical-grade polypropylene. Control samples of all test materials were held at laboratory ambient temperature ( $\approx 21^\circ\text{C}$ ), and refrigerated at  $2^\circ\text{C}$  to  $8^\circ\text{C}$ . Sets of shipping test materials were exposed to one of the following scenarios: (1) high dosage X-ray irradiation; (2) commercial carrier from Gaithersburg MD to Quantico VA where they received low dose X-ray irradiation and were then returned by way of a commercial carrier to Gaithersburg MD; and, (3) commercial carrier from Gaithersburg MD to Seattle WA and back. All materials are being evaluated at NIST using qPCR quantitation and Short Tandem Repeat (STR) testing. STRs are first evaluated using commercial 16 loci genotyping kits; if the larger STR loci in these kits fail to yield results, samples are evaluated using a commercial "miniSTR" kit.

In scenario one, DNA packs were added to containers of mail and processed at an industrial facility using an x-ray beam generated with energy of 5 MeV and a beam current of 23 mA. The mail containers were either trays of letters or tubs of magazines. The trays and tubs were filled into large metal totes and passed through the x-ray beam four times to achieve the target dose level. The radiation exposure resulted in the packages experiencing temperatures exceeding  $57^\circ\text{C}$  for 20 min to 30 min. In scenarios two and three, packages were shipped at ambient in late April to early May 2011.

The effect on liquid DNA extracts in tubes varied widely, regardless of how they were treated. Even in ambient storage, the samples in medical-grade polypropylene tubes completely evaporated or were greatly reduced in volume. The other tube types were better at maintaining volume but all were completely dried by the high irradiation and/or temperature in scenario one. Because of this volume loss, all "liquid" samples are reconstituted to their original volumes prior to qPCR and genotyping. Full STR profiles were obtained with all ambient stored materials and the scenario two samples.

The results of direct amplification of the samples absorbed on either FTA or 903 papers were variable. Even in ambient storage, no full STR profiles were obtained with either paper at the low DNA concentration. FTA paper yielded partial profiles for the high DNA concentration samples at ambient storage whereas the 903 paper and 903 with preservative generally yielded full profiles.

Quite variable qPCR quantitation STR typing results have been obtained for the highly irradiated samples of scenario one. MiniSTRs yielded degraded profiles for some but not all of these samples, with typing results improving with increasing qPCR-estimated DNA quantity.

**Short Tandem Repeats (STRs), DNA, Ionizing Irradiation**

## **A99 Evaluation of Concordance, Sensitivity, STR Amplification Input, and a True Zero Value for the Qiagen Investigator™ Quantiplex Kit**

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After attending this presentation, attendees will understand the novel aspects of a new DNA quantitation kit, Qiagen's Investigator Quantiplex kit, and learn about the outcome of an internal validation as well as how effectively the kit was used as a predictive screening tool for STR amplification success in a data set with low yield DNA samples.

This presentation will impact the forensic science community by informing it of the evaluation of a novel quantitation kit in a forensic DNA research setting and its ability to predict STR amplification success with a set of low yield DNA samples, thus aiding laboratories who may be interested in implementing a new quantitation kit.

Qiagen's Investigator™ Quantiplex kit, a total human DNA quantitation kit, has a 200 base pair Internal Control, a fast cycling time relative to current forensically relevant quantitation kits utilized, and a novel scorpion molecule containing a primer, probe, fluorophore, and quencher covalently linked for an optimized interaction. In this study, the Investigator™ Quantiplex kit was compared to the Quantifiler™ Human kit for concordance, sensitivity, optimal DNA input for STR amplification reactions, and determination of a true zero value, a value under which no useful STR data is consistently obtained, thus reliably predicting STR failure. Full and half volume reactions were also evaluated with the Investigator™ Quantiplex kit. For this study, buccal swabs were extracted using the Qiagen QIAamp® DNA Blood Mini Kit, quantified with Quantifiler™ Human or Investigator™ Quantiplex kits, and amplified with the ABI AmpF/STR® Identifiler kit using a previously validated reduced volume reaction. Amplified samples were separated on the 3100Avant Genetic Analyzer with a default ten second injection and standard STR analysis parameters. Data were analyzed with GeneMapper® ID v.3.2 using an analytical threshold of 50 RFU. On average, Investigator™ Quantiplex quantitated samples 0.558ng/μl lower than Quantifiler™ Human, but standard deviations were large. When compared to previous studies conducted in the laboratory, Investigator™ Quantiplex is slightly more sensitive than Quantifiler™ Human, and preliminary data in this study suggests Investigator™ Quantiplex may be more robust to inhibitors. Four samples known to contain an inhibitor were undetected using the Quantifiler™ Human kit but were detected by the Investigator™ Quantiplex kit and exhibited quantitation values ranging from 0.043ng/μl to 0.461ng/μl. With the Investigator™ Quantiplex kit, the half volume reaction was found to be analogous to the full volume reaction based on consistency of data and the range of quantitation values detected. Optimal DNA input into reduced volume AmpF/STR® Identifiler amplification reactions was determined to be 2.0ng, as this was the only input with which average heterozygote peak heights were obtained within the desired 1000 – 1500 RFU range. Samples with 0.25ng input DNA or greater resulted in average

peak heights above 200 RFU, a typical stochastic threshold, but substantial standard deviations were observed. Samples with DNA inputs of 0.05ng and 0.10ng exhibited average peak heights above the analytical threshold but below 200 RFU. Allelic dropout was first observed with samples under 0.5ng total DNA input and was more extensive with samples under 0.25ng total input. For the samples with a 0.25ng total DNA input, all allelic dropout was attributed to the same sample. No true zero value was identified in this study, but samples undetected with the Investigator™ Quantiplex were consistently unlikely to result in enough useful data for statistical calculations or upload into CODIS (two alleles and 0.82 complete loci above the analytical threshold on average). Of the undetected samples in the true zero study, 66% yielded no allele calls and 78% yielded only a single allele or none. The Investigator™ Quantiplex kit displays a number of advantages, including time and cost savings, high sensitivity, and potential robustness to inhibitors. Future work should include completion of a reproducibility study with the half versus full volume reactions, and testing of more low level and inhibited samples.

**Investigator Quantiplex, STR Success, DNA Quantitation**

## **A100 Development and Validation of a Multiplex qPCR System for Male and Total DNA Quantification**

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After attending this presentation, attendees will acquire information about the development and validation of a novel multiplex qPCR system simultaneously quantifying total genomic DNA and male human genomic DNA.

The presentation will impact the forensic science community by providing a new total genomic DNA and human male genomic DNA quantification method other than the ABI Quantifiler Duo DNA Quantification kit and Promega Plexor HY kit, assisting other labs in their determination of which kit will most likely fit their current system.

STR analysis, the core technology in DNA identification, requires a defined range of template quantities to produce optimal results. Quantification of both total human DNA and male human DNA in mixed forensic samples provides critical information for the selection of either autosomal or Y STR profiling, and to determine the quantity of extract to amplify. Two commercially available kits commonly used in forensic laboratories to simultaneously measure total and male human DNA are Plexor HY and Quantifiler Duo®. A recently published study compared the two kits in terms of precision, sensitivity and accuracy.<sup>1</sup> The results showed both systems produced linear estimates for DNA quantity over a broad range of input DNA. However, the study also indicated that the Plexor HY kit performed well with low level amounts of DNA, whereas the values for the ratios of total DNA to male DNA were far more accurate for Quantifiler Duo®. One of the most important reasons for the differences in performance between the commercial kits is the Y chromosome target. TSPY is a multi-copy number Y chromosome target used in the Plexor HY kit, with varying copy numbers from 23 to 64 among different populations of males.<sup>2</sup> SRY, used in the Quantifiler Duo® kit, is a single copy target with no copy number variation reported among males. Although increasing the copy number of a target enhances sensitivity, it also results in more variation.

A novel multiplex qPCR method to simultaneously measure total and male only DNA in a more sensitive and accurate manner will be presented. The multiplex qPCR system includes three amplification targets, one of which is located on an autosomal chromosome, and the others are found on the Y chromosome. Three TaqMan probes corresponding to the three targets are labeled with different fluorescence. One of the Y chromosome targets is a single copy gene which is used to quantify male DNA accurately when the male DNA concentration is higher than 16pg/μl. The other Y chromosome target is a multi-copy number gene which was shown to measure as little as 0.32 pg/μL of male DNA. The novel multiplex qPCR system was compared with the Quantifiler Duo® kit and Plexor HY kit regarding accuracy, sensitivity, and precision. Purposeful male and female DNA mixtures and mock evidence samples were measured using the novel Multiplex qPCR system, and the profiles generated with the determined DNA concentrations were assessed. Studies demonstrate that this system overcame the drawbacks of both of the Quantifiler Duo kit and Plexor HY kit as it is more sensitive than the Quantifiler Duo® kit and more accurate than the Plexor HY kit.

#### References:

1. H E. LaSalle, G Duncan, B McCord, An analysis of single and multi-copy methods for DNA quantitation by real-time polymerase chain reaction. *Forensic Sci. Int. Genet.* 5 (2011) 185–193.
2. Repping S, van Daalen SK, Brown LG, Korver CM, Lange J, Marszalek JD, et al. High mutation rates have driven extensive structural polymorphism among human Y chromosomes. *Nat Genet* 2006; 38:463–7.

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#### Male DNA, Genomic DNA, Quantification

### A101 Measurement of a Stochastic Threshold and Development of a Reduced Volume Reaction for PowerPlex® 16 HS and Identifiler™ Plus

*Joseph Ring, BS\*, 9531 Crown Court, Richmond, VA 23294; and Kelly R. Johnson, MS, Brad Jenkins, MS, and Susan Greenspoon, PhD, Virginia Department of Forensic Science, 700 North 5th Street, Richmond, VA 23219*

After attending this presentation, attendees will learn how establishing a stochastic threshold can affect the interpretation of DNA profiles for lower template quantity samples and the resulting statistical analysis. In addition, attendees will learn how stochastic thresholds compare between PowerPlex® 16 HS, Identifiler™ Plus, and PowerPlex® 16.

This presentation will impact the forensic science community by allowing analysts to be more confident in their interpretations of DNA profiles, thus differing stochastic thresholds for commonly used STR multiplex kits can affect both the useful data obtained for forensic samples and the statistical analysis performed. The information gleaned from this study may allow other forensic laboratories in the midst of developing stochastic thresholds to improve their DNA typing result quality.

Validating a stochastic threshold has been deemed necessary by SWGDAM for interpreting STR typing results. The stochastic threshold is the threshold at which the analyst can be confident that if one peak for a heterozygote is above this threshold, then its sister allele will be present and should be at least above the analytical threshold. This increases confidence in homozygous calls made for single source samples and alleles attributed to each contributor in mixture analysis. Promega and Applied Biosystems have released two STR amplification kits (PowerPlex® 16 HS and Identifiler™ Plus, respectively), which claim to improve upon the DNA typing results obtained with their previous kits. Specifically, these kits allege better heterozygous locus balance, which could mean a lower stochastic threshold. To test the validity of these claims, the stochastic threshold was defined for both kits and compared to the PowerPlex® 16 threshold defined previously by the Virginia Department of Forensic Science. The amplification parameters of half-volume reactions (12.5mL) for PowerPlex® 16 HS and Identifiler™ Plus were first modified to closely

mimic full-volume (25.0mL) reactions since typically, reducing the PCR reaction volume greatly enhances sensitivity. A half-volume (12.5ml) reaction with one cycle removed (31 cycles) provided sensitivity with PowerPlex® 16 HS that was comparable to its full-volume reaction. A half-volume reaction with the standard cycle number (28 cycles) provided sensitivity with Identifiler™ Plus multiplex that was similar to its full volume reaction, which was surprising. Stochastic thresholds were determined using half-volume reaction conditions under three injection times for each kit: two, five, and ten seconds for PowerPlex® 16 HS and five, ten, and twenty seconds for Identifiler™ Plus. The stochastic thresholds established using PowerPlex® 16 HS were 180, 320, and 370 rfu for a two, five, and ten second injection, respectively. The stochastic thresholds established using Identifiler™ Plus were 200, 300 and 380 rfu for a five, ten and twenty second injection, respectively. All of the thresholds established were equal to or lower than those for the corresponding injection time using PowerPlex® 16. The average peak height ratios for all three kits were statistically similar. The stochastic threshold was applied to single-source and mixture mock case samples typed using each of the kits and the useful data obtained, both for interpretation and statistical analysis, compared.

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#### PowerPlex® 16 HS, Identifiler™ Plus, Stochastic Threshold

### A102 What's New on the Water Front

*Peter J. Diaczuk, BS\*, and Dominick Bongiovi, BS, John Jay College of Criminal Justice and the CUNY Graduate Center, 445 West 59th Street, New York, NY 11019*

After attending this presentation, attendees will learn about the minimum depth of water necessary to allow a 9mm bullet to ricochet without touching the submerged substrate, and at what angle the bullet has departed the water as a factor of incident angle

This presentation will impact the forensic science community by revealing the dangers inherent in bullet ricochet off of water, will offer the calculation used to make this determination, and will examine the parameters necessary to have a successful water ricochet.

A bullet can ricochet off of many different surfaces. These surfaces may be categorized based upon how the bullet interacts with them. Hard surfaces traditionally remain intact after low angle bullet ricochet because they will not yield to the bullet's impact. Materials such as concrete, steel or automobile glass fall into this category. Bullet ricochets off of hard unyielding surfaces tend to have ricochet angles that are less than the incident angle. Soft surfaces will not remain intact, but instead will deform or deflect, yielding to the bullet's impact. Materials such as sand, gypsum wallboard, or water are examples of surfaces that fall into this category. Bullet ricochets off of relatively soft yielding surfaces tend to have ricochet angles that are greater than the incident angle. When a bullet is fired at either of these materials, there is an angle at which a ricochet will no longer occur, but instead the bullet will either break apart into fragments after hitting the surface, or it may remain intact, to either penetrate (imbed but not exit) or perforate (enter and exit) the material.

This research was a continuation from earlier work involving the study of 22 caliber bullets that were ricocheted off of water. It was discovered that 22 caliber bullets could successfully ricochet off water that was just under half the diameter of the bullet and still not come into contact with the floor of the water tank. This was counter-intuitive, as it was expected that at such a shallow depth, the bullet would scrape the floor of the tank leaving traces of that interaction behind, and that the bullet would take with it some of the paint from the tank's floor. Careful inspection of the tank's floor revealed that no such interaction between the two had taken place, which was confirmed by recovery of the bullets and microscopically examining them for transfer as well. The next obvious question was whether larger bullets, such as 9mm, could also ricochet off shallow water and not contact the floor of the tank.

The witness panel set-up was down range and was used to determine if the bullets were in stable or de-stable flight and to generate a measuring point for angle calculations. The same water tank from the 22 caliber work was used for these experiments. High-speed photography was used to aid in determining the location of bullet impacts to the water. Trigonometry was applied for the calculation of ricochet angle. Incident angle was recorded using an angle finder directly affixed to the barrel of the firearm. Using this method, the angle at which 9 millimeter full metal jacket bullets did not ricochet at all (the critical angle) was determined to be approximately eight degrees. At angles greater than eight degrees, the bullets entered the water and remained submerged. Shots were then fired at an incident of two degrees into the water tank. The water depth was incrementally lowered until the bullet finally scraped along the floor of the tank. Recovered bullets, examined microscopically, confirmed the interaction had taken place. Not until the water level was lowered to less than the diameter of the bullet (0.36 inches) did the bullet hit the tank floor when the incident angle was only two degrees.

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#### **Ricochet, Bullet, Water**

### **A103 Statistical Firearm Correlation Analysis**

*Matthew J. Bohn, PhD\*, Analytic Services, Incorporated, 1330 Inverness Drive, Colorado Springs, CO 80910; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

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After attending this presentation, attendees will learn about the mathematical techniques of auto- and cross-correlation as applied to images from tool marks, more specifically with the markings left on shell casings as they are ejected from a weapon. The correlations will be followed by a statistical analysis that can aid a forensic examiner in comparative analysis. This analysis will enable a quantitative score with confidence intervals when comparing images of tool marks.

This presentation will impact the forensic science community by demonstrating mathematical techniques to make quantifiable statements regarding the confidence of comparative analysis.

Cross-correlation is a mathematical technique that compares the similarity of two sets of data and is often used in pattern recognition algorithms. The cross-correlation is calculated by shifting one data set with respect to another, multiplying the two data sets and summing the result. The technique is insensitive to amplitude and background and is invariant under translation. Because of these features, the cross-correlation is a powerful technique that can be used to quantify the similarity of two samples. Once the similarity of the evidence is quantified, the data can be presented at court using statistics. Quantifiable comparative analysis will strengthen the evidence presented at trial because the evidence is not solely dependent on the opinion of the forensic examiner, but based on quantifiable statistical techniques. Ultimately a national database of shell casings and bullet rifling could be developed and searches made to find and make linkage between forensic evidence found at a crime scene and previously collected data.

A firearms examiner examined and recorded all of the data used in this experiment. First, the examiner observes a shell casing under 40x magnification and notices unique markings that can be exploited using cross-correlation. Several images of this area are recorded of this shell casing and of shell casings from a different weapon of the same make and model. Using a Graphical User Interface (GUI) developed in MatLab, the examiner selects the region of interest in the image. The region of interest is then cut out of the image and the color information is converted to grayscale. The grayscale image is then normalized with a zero mean. Typically, the examiner can accurately align the horizontal striations to the pixels in the image when the photograph is made; however, if any rotations are needed, they can be accomplished by the software. Since the striations are aligned horizontally, average pixel intensity is calculated for each row in the selected area. This reduces the dimension of the problem to 1-D; the average pixel brightness across the striations. A normalized cross-correlation can be calculated in order to compare the similarity of the

striations. Normalization is a crucial step in comparing cross-correlations because it provides a means of calibrating one "yardstick" versus another — otherwise the comparisons would be meaningless. A useful method for normalizing the cross-correlation is to divide the cross-correlation by the square-root of the auto-correlations. The auto/cross-correlation between photos of the same shell casing should be nearly one; whereas the shell casings from different firearms are expected to have cross-correlations significantly less than one. A statistical significance can be determined by comparing the means of the correlations of the same shell casings versus the correlations of the different shell casings use a z-score and a student's t distribution, since there are few images to compare. The null hypothesis is the difference in the means of the correlations. A p-value can be calculated based on the means, standard deviations, and number of samples. The null hypothesis that the two data sets have the same means can then be either accepted or rejected based on a predetermined significance level.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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#### **Cross-Correlation, Firearms, Comparative Analysis**

### **A104 The Application of UPLC/MS/MS to the Analysis of Smokeless Powders and Gunshot Residue Samples**

*Jennifer L. Greaux, BS\*, and Bruce R. McCord, PhD, Florida International University, Department of Chemistry, 11200 Southwest 8th Street, University Park, Miami, FL 33199; and Danielle R. Lincoln, 1000 Holt Avenue, Box 1770, Winter Park, FL 32789*

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After attending this presentation, attendees will understand the fundamentals of ultra-high performance liquid chromatography coupled with tandem mass spectrometry, the advantages of these separation and detection techniques, and their forensic application to gunshot residue analysis.

This presentation will impact the forensic science community by providing an alternative method that is quick, reliable, and sensitive enough for analyzing GSR samples collected in cases involving firearms. The method can also be used as an investigative tool by law enforcement personnel when trying to identify shooting suspects, particularly in situations where inorganic primer residues are unavailable or missing.

Ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) is being proposed as an alternative technique for the analysis of gunshot residue (GSR) and smokeless powder samples. When a gun is fired the primer and smokeless powder in the cartridge combust forcing the bullet out of the weapon. A release of vapors and particulates are deposited onto the shooter's hands and clothing. Generally referred to as gunshot residue, these particulates contain a mixture of organic and inorganic compounds that can be collected and analyzed to determine whether or not a person has fired a weapon. However, this research focused on the detection of organic GSR and examining compositional differences between brands and lots of smokeless powder. Identifying differences between powders can be useful when trying to link a suspected shooter to a specific weapon and/or ammunition. Live-fire residue samples were also collected and analyzed to test the usefulness of the method on field samples. It is desired to have a method that is quick, reliable, and sensitive enough for analyzing GSR samples collected in cases involving firearms.

In this project, a previously developed UPLC/MS/MS method was applied to the analysis of smokeless powders and gunshot residue samples. UPLC is a newer technique that offers increased efficiency and separation speeds when compared to traditional HPLC. This is possible because the system can accommodate smaller particle columns and higher backpressures which help to minimize band spreading and decrease analysis times. The method involved the reversed-phase separation of 20 different smokeless powder additives and reaction products on a C18

column. For detection, both parent and daughter ions were monitored by tandem mass spectrometry in order to accurately identify the individual components. Simultaneously, positive and negative ESI was used along with negative APCI in the same run to detect all of the relevant compounds. Optimized analysis times were under eight minutes with a gradient of 10%-73% organic at a flow rate of 0.500mL/min. To confirm the presence of each chemical, several parameters were monitored: retention time, MS time, and specific parent-to-daughter transitions.

For smokeless powder analysis, organic additives including nitroglycerine, diphenylamine, nitrodiphenylamine, and ethyl centralite, were extracted from various smokeless powder samples using methylene chloride. An aliquot was removed from the supernatant following extraction and evaporated to dryness under a stream of nitrogen gas. This extract was then reconstituted in the HPLC eluent. For the live-fire residue samples, the swab used for collection was cut and extracted with acetone in a centrifuge tube. The extract was evaporated to dryness and reconstituted in solvent. Other procedures were also tested in order to obtain higher recoveries of the organic compounds. These results demonstrate that organic GSR can be used as an investigative tool by law enforcement personnel when trying to identify shooting suspects, particularly *in situations where inorganic primer residues are unavailable or missing.*

#### **GSR Analysis, Ultra-high Performance Liquid Chromatography, Tandem Mass Spectrometry**

### **A105 Bullet and Cartridge Case Signature Identification Using Topography Measurements and Correlations: The Unification of Microscopic and Mathematical Comparisons**

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After attending the presentation, attendees will understand the methods in both microscopic comparison and identification of bullets and cases, combined with 3D surface topography measurement and correlations. The results of blind testing of bullets fired from ten consecutively rifled barrels and ten consecutively manufactured slides will be presented.

This presentation will impact the forensic science community by building onto current firearm identifications based on image comparisons using optical comparison microscopes. Because ballistic signatures are geometrical micro-topographies by nature, the main objective is to demonstrate the usefulness of surface topography measurement techniques for firearm identification. These results provide an objective mathematical validation of identifications that is in harmony with the results of optical comparison microscopy employed by an experienced firearms examiner.

**Methodology:** A 2D and 3D Topography Measurement and Correlation System was developed at NIST for certification of NIST Standard Reference Material (SRM) 2460/2461 Bullets and Cartridge Cases. Based on this system, a prototype system for signature measurement and correlation of fired bullets has been recently developed at NIST for bullet identifications. The 3D topography data of the land engraved areas (LEAs) of fired bullets are captured by a commercial Nipkow disc confocal microscope. The LEAs were processed by the “edge detection” method to determine the “striation density” by which the surface area with low striation density on the LEA could be masked out from correlation. The

modified 3D micro-topography data on the remaining “valid correlation areas” are compressed into a 2D profile which represents the 2D ballistics signature of the LEA. A correlation program using two methods has been developed for matching the paired profile signatures: the “CMS” (Consecutive Matching Striae) method used by many firearm examiners and the  $CCF_{max}$  (cross correlation function maximum) method developed by NIST based on analysis methods in surface metrology.

Cartridge cases that comprised test fires from ten consecutively manufactured pistol slides, fifteen unknown cases, and five “persistence study” cases examined and measured. The cases were microscopically examined, and the results were later confirmed as accurately associating all of the questioned cases back to the correct pistol slide sources. A Nipkow disc confocal microscope was used to gather the 3D topography data from the breech face area of each case. The software applied the cross correlation algorithm to quantify the similarity between two cases.

**Results:** A set of 20 known-matching bullets fired from ten consecutively manufactured barrels (two bullets from each barrel) were tested. Their 3D topography images were captured by the confocal microscope at NIST, and correlated by the prototype ballistics identification system using the cross-correlation function maximum ( $CCF_{max}$ ) as a correlation indicator. The correlation result was excellent: correlation values of all ten pairs of known-matching bullets scored highest on all correlation lists, yielding a correct identification rate of 100%. For the 60 pairs of matched LEAs (each bullet includes six LEAs), correlation values of matching LEAs scored highest on 59 out of 60 correlation lists, yielding a correct identification rate for individual LEAs of 98.3 %.

An additional set of 15 unknown matching bullets fired from the same set of ten barrels was blind tested. These bullets were correlated with the 20 known-matching bullets mentioned above. Both the CCF and CMS method were used and showed excellent correlation results. When using the CMS method, one matching pair did not meet the selected CMS criterion (3X) for a “match,” and 29 out of 30 pairs of matching bullets were correctly identified, yielding a correct identification rate of 96.7%. When using the CCF method, all 30 pairs of matching bullets scored at the topmost position on their respective correlation lists, yielding a correct identification rate of 100

Cartridge cases that comprised test fires from ten consecutively manufactured pistol slides, fifteen unknown cases, and five “persistence study” cases were microscopically examined and the results were later confirmed as accurately associating all of the questioned cases back to the correct pistol slide sources.

Using statistical analysis from the known match and known non-match correlations, a baseline cross correlation function ( $CCF$ ) was established to identify matches. Based on the  $CCF$  results, and a statistical analysis of the match and non-match case scores, each of the 19 of the 20 unknown cases were correctly identified to the slides that it came from. One case was not identified back to its original slide and had an “inconclusive” scoring. However, this one case was identified to other unknown cases that were correctly identified to the same slide.

#### **Firearm, Topography, Comparison**

### **A106 Reflection Confocal Microscope Systems in the Forensic Laboratory — Problems Encountered in Bullet and Cartridge Case Examinations**

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After attending this presentation, attendees will better understand the factors which define the capabilities and limitations of confocal microscopy

in the forensic examination of bullets and cartridge cases. They will also understand how to select confocal instruments to fit their needs and the important factors in setting up those instruments for optimal use in the laboratory.

This presentation will impact the forensic science community by helping practitioners select and use incident light confocal microscopes for firearms examinations in the laboratory. Consequently, it should help in decision-making about the purchase of instruments and avoid the waste of limited resources as a result of improperly choosing equipment or using non-ideal settings of the instruments.

In December of 2009, funding was received to develop incident light confocal microscopy as a means determine surface topography of fired bullets and cartridge cases. Attempts have been made to determine to what extent impression markings as revealed by confocal microscopy can be used to individualize firearms and whether they can be interpreted in the same way as random striation markings on fired bullets and cartridge cases. Unexpected and challenging issues were encountered early in the work with confocal imaging on curved surfaces such as bullets and cartridge case firing pin impressions. Consequently, it became necessary to evaluate and, if possible, solve these problems before further work could continue. Theoretical issues, instruments, and optimal settings necessary to optimize confocal microscopy for the forensic examination of curved surfaces have been studied. The issues encountered, the scanning mechanisms and conditions determined to be optimal, and the instruments evaluated and ultimately selected for further research are reported here.

Based upon prior work, proposals were submitted to NIJ for funding to develop applications of confocal microscopy for firearms evidence examination. It was believed that the technique showed very significant promise in the forensic laboratory. Early in the research it was found that noise and the consequent lower quality of feature height measurement was directly related to the angle of the surface to the optic axis, and to other factors of instrument design. Evaluation of the instrument design element proved difficult due to limited information available from the manufacturers. However, both theoretical considerations and experimental results demonstrated the importance of numerical aperture and lens design on the quality of data obtained. It was determined that optimal magnification at the imaging plane is based on the consideration of both the maximum resolution available and the desired magnification (and resolution) necessary for the work at hand. In some instances optimal magnification is less than the maximum magnification available. While manufacturers' claims of vertical resolution initially appeared to greatly exceed theoretical limits, actual results obtained reveal that the digital image processing used in confocal microscopy does significantly improve on conventionally accepted limits of vertical resolution. After evaluating instruments from variety of manufacturers it was determined that two instruments from Zeiss MicroImaging, LLC, were well suited the continuing research needs of the research.

It has been determined that when carefully chosen and set up, confocal microscopes can yield the quality of data necessary for continued research, and can remain potentially valuable operational forensic laboratories.

#### **Confocal Microscopy, Firearms Examination, Instrument Selection**

### **A107 Detection of Ammonium Nitrate and Ammonium Nitrate Mixtures in Soil**

*Tammi Green\**, Albany State University, 504 College Drive, Albany, GA 31705; *Candice Bridge, PhD*, and *Jesse D. Brown, BS*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and *Michael J. Salyards, PhD*, 45 High Street, Sharpsburg, GA 30277

After attending this presentation, attendees will learn a minimal preparation methodology to rapidly identify ammonium nitrate and

ammonium nitrate mixture particles while in the presence of various soil matrices.

This presentation will impact the forensic science community by providing a methodological model for analytical processes to identify ammonium nitrate mixtures that is conducive to an in-theatre environment.

Homemade explosives (HME's) are commonly encountered in theatre, in both pre- and post-blast scenarios. In post-blast scenarios, the burned and unburned particles can settle onto the surrounding soil or sand. It can be difficult to physically separate residual explosive particles from soil for instrumental analysis. Several types of explosive mixtures can be used in improvised explosive devices (IEDs). One of the more commonly used HMEs is ammonium nitrate (AN) because it is readily accessible. While AN can be used alone as an explosive, it is generally mixed with a fuel, commonly referred to an ammonium nitrate mixtures (ANM).<sup>1</sup> It can be difficult to identify these fuels which can be a key identifier for the bomb builder, especially in post-blast scenarios. With the increase of soil samples being sent to forensic labs to be analyzed, this study will help analysts by giving them a methodological model for extraction and analytical processes that is conducive to an in-theatre environment.

The objective of this study was to determine if AN and ANM can be identified in different soil matrices using the following in-theatre hand-held instrumentation: Raman Spectroscopy, Infra-red (IR) spectroscopy, and an ion-trap gas chromatograph – mass spectrometer (GC-MS). The more commonly identified fuels used in this study were: powdered aluminum, powdered sugar, spics, and diesel fuel. Ammonium nitrate was powdered and mixed with the powdered fuel to simulate the close contact necessary to create a significant blast. This was not necessary to do for the ammonium nitrate mixed with diesel fuel because the fuel is absorbed into the AN pill providing the a similar close contact. Each ANM was analyzed and compared to the analysis of the individual components to determine if each fuel could be identified in the presence of the AN. Subsequently, these ANM were mixed with soil samples at predetermined concentrations and analyzed to determine if either the AN or the ANMs were readily identifiable. The soil samples matrices were silicon band sand, red clay, and humus (garden-top) soil.

The resulting spectrum or chromatogram of the individual components, ANMs, and soil mixtures were compared by a Pearson correlation with a Student's t-test to determine if samples were significantly different or similar (~1900 pair-wise comparisons made). Based on research thus far individual fuels can be identified in the presence of AN at a concentration of 10% w/w. For the ANMs, the hand-held IR instrument had the ability to identify residual components (the fuel), which when analyzed had a higher correlation value with the individual fuel than the mixture itself. When the ammonium nitrate mixtures were mixed in the sand, high correlation values ( $R_2 = \sim 0.8$ ) were achieved between ANM in sand and the pure ANM. Similar correlation coefficients for the mixture and the individual components were achieved for the Raman spectroscopy analysis. The GC-MS analysis did not provide any identifiable peaks for AN nor the fuels except for the diesel fuel at 1mg/ml and a 5mg/ml concentrations. Current results suggest it will be possible to identify AN and the fuels in a soil matrix.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

#### **Reference:**

<sup>1</sup> Beveridge, A.D.; Development in the Detection and Identification of Explosive Residue, *Forensic Science Review*, **1992**, 4(1), 18,32

#### **Ammonium Nitrate, Homemade Explosive, Soil**

## A108 Forensic Soil Analysis of New Jersey State Parks Using a Combination of Simple Techniques and Multivariate Statistics

Jennifer Bonetti, BS\*, Cedar Crest College, 18 York Place, Kingston, NY 12401; and Lawrence Quarino, PhD, Cedar Crest College, Department of Chemistry & Physical Science, 100 College Avenue, Allentown, PA 18104

After attending this presentation, attendees will realize that soil evidence is not utilized to its fullest potential in forensic science laboratories and that probative analysis of samples may not involve expensive or complicated instrumentation.

This presentation will impact the forensic science community by serving to provide a method of analysis for soil evidence that provides meaningful results, yet is neither time nor labor intensive.

Soil evidence is found in a large number and variety of criminal cases, yet it is often overlooked in forensic laboratories. Although there are a number of published techniques for testing soil evidence at an analytical scientist's disposal, it seems as though labs without access to a trained forensic geologist are left floundering when it comes to gathering useful information from soil evidence. Recently, emerging techniques have become more and more complicated leading to time and labor intensive methods that are simply not practical for the average crime laboratory. For this study, two simple yet effective methods, particle size distribution and pH measurements, were combined to generate a quick and easy method that can still be used to discriminate between samples from different locations by applying multivariate statistics such as a Principle Component Analysis (PCA).

Five samples at 50 feet intervals from one another were obtained for this study from 12 state parks distributed evenly across the state of New Jersey. The method employed for the particle size distribution was a modification of the method outlined by the Health and Physics program at the University of Nevada, Las Vegas<sup>1</sup>. Small aliquots (1.0-1.8g) were taken from each sample for the particle size distribution. These samples were treated with sodium acetate, hydrogen peroxide, sodium citrate-bicarbonate buffer with sodium dithionite, and sodium hexametaphosphate to remove all cementing and organic materials from the samples before using a wet sieving technique with mesh sizes of #10, #35, #60, #120, and #230. The Kolmogorov-Smirnov test, a nonparametric statistical test, was utilized to identify statistically significant differences between state parks based solely on the particle size distribution data. This curve-comparison test showed less than 35% of park to park comparisons to be indistinguishable (determined by a p-value greater than 0.05).

The pH measurements were carried out in triplicate in both water and 1M CaCl<sub>2</sub> using 1.0 grams of soil in 10mL of liquid and analyzed with an Accumet AB15 pH meter. The pH measurements for soil in water and soil in 1M CaCl<sub>2</sub> were analyzed separately using a Student's two-tailed t-test without assuming equal variance. In water, four of 66 park to park comparisons were not statistically significant while in CaCl<sub>2</sub>, five of 66 park to park comparisons were not statistically significant. Only one park to park comparison was indistinguishable using pH measurements in both water and CaCl<sub>2</sub>.

Using a 2D and 3D Principle Component Analysis (PCA) as well as a 2D and 3D Linear Discriminate Analysis (without prior PCA preprocessing), the 12 state parks could be viewed as clusters and discriminated. These results suggest that for general forensic comparisons, there is no need for complicated, time-consuming, and expensive methods. With the correct statistical analysis, simple conventional methods can be utilized and yield extremely meaningful results.

### Reference:

1. Johnson WH. Soil Particle Size Analysis. UNLV Health Physics Program Laboratory Operating Procedure. 1996:1-16.

Soil Analysis, Particle Size Distribution, Multivariate Statistics

## A109 Storage and Handling Procedures for Using Soil Molecular Biology as Trace Evidence

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After this presentation, attendees will understand how soil sample handling and storage can alter soil microbial community fingerprints using capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) and fatty acid methyl ester (FAME) analysis.

This presentation will impact the forensic science community because it presents some fundamental knowledge for using soil molecular biology as trace evidence.

The use of soil as trace evidence has been well documented for many of its chemical and physical properties. However, there is less understanding when it comes to soil biochemical properties. One method to explore soil biochemistry is to extract microbial DNA and fatty acids to make a fingerprint. This method is currently in the development stage. This study looks at the effects that storage and handling of soil has on the microbial fingerprint made by soil microbial DNA and fatty acids.

In this project four soil types from Nebraska that vary in soil texture were tested. Each sampling location contained three plots. From these plots, 20 soil cores were collected from a depth of 0cm to 5cm three different times over a one year period. After each collection the soils were sieved and placed in sealed plastic bags in their storage conditions (-80°C, -20°C, 4°C, air dried, oven dried, and freeze dried). As a control, one soil sample from the collection had the microbial community DNA and fatty acids extracted within 36 hours; this became the "fresh" sample to which the storage samples would be compared. Soil was collected at the same location two weeks after the initial collection to identify if rapid changes in the soil microbial community itself exist; to determine if it is possible to go back to a crime scene and recover a similar microbial profile. Two methods have been chosen for the analysis of the soil microbial DNA and fatty acids. The microbial DNA is analyzed by capillary electrophoresis single-stranded conformation polymorphism (CE-SSCP) to form a nucleic acid fingerprint. In theory, CE-SSCP is well suited for forensic use, but is a newer method and still requires detailed testing. The microbial fatty acids are analyzed as fatty acid methyl esters (FAME) to form a lipid-based fingerprint. FAME is a robust and highly used protocol that will support CE-SSCP data.

Thus far, the results from FAME analysis show soil stored at -80°C and -20°C show no significant difference from the fresh sample. The other storage methods (4°C, air dried, oven dried, and freeze dried) showed significant difference when compared to the fresh sample. Samples taken two weeks after initial sampling have shown significant differences in microbial fingerprint compared to fresh samples. These results determine the best way to store soil samples when using soil microbial biochemical molecules is at either -80°C or -20°C, as to not change the microbial fingerprint. It also demonstrates the soil microbial community can change rapidly, possibly making it difficult to develop a robust method when using the soil microbial community for a fingerprint when several weeks have passed between crime and investigation. Results from genetic analysis will be presented.

Single-Stranded Conformation Polymorphism, Fatty Acid Methyl Ester, 16S rDNA

## A110 Elemental Analysis and Comparison of Bulk Soil Using LA-ICP-MS, $\mu$ XRF, and LIBS Methods: An Inter-laboratory Study

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After attending this presentation, attendees will better understand the use of elemental analysis methods for the analysis of bulk soil samples. Elemental analysis of soils has many applications in the environmental, forensic, and geological sciences. The provenance or origin of soil transferred to objects such as shoes or tires can be useful in a criminal investigation and elemental profiles can be used to discriminate soil samples originating from different geographic regions and also associate soil samples originating from the same source.

This presentation will impact the forensic science community by comparing standardized and validated elemental analysis methods for bulk soil analysis. Thirteen laboratories have participated in a round robin exercise, and the results will be presented here.

Laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS), micro X-ray fluorescence ( $\mu$ XRF), and laser-induced breakdown spectroscopy (LIBS) were used in this study since all three techniques produce similar analytical figures of merit. LA-ICP-MS and  $\mu$ XRF methods are established elemental analysis instrumental techniques that are currently available in many forensic laboratories. A LA-ICP-MS method was recently optimized for analysis and comparison between different soil samples in an environmental forensic application<sup>1,2</sup> and LIBS produces sensitivity equal to or better than that of  $\mu$ XRF (especially in the low mass range). LIBS has recently attracted the interest of analytical chemists and forensic laboratories as a simpler, lower cost alternative to the more established analytical methods. In developing a LIBS method, there are many parameters to consider, including laser wavelength, spectral resolution, sensitivity, and matrix effects. The first LIBS method using a 266 nm laser for forensic soil analysis has been also reported by our group and presented.<sup>3</sup>

A soil analysis round-robin test was organized by Florida International University and distributed amongst some of the members of the Elemental Analysis Working Group (EAWG). The aims of this first soil round robin (EAWG-RR5) were: a) to evaluate the inter-laboratory performance of the three methods in terms of accuracy (bias), precision (relative standard deviation, RSD) and sensitivity using standard reference materials (SRMs); b) to evaluate the newly released NIST SRM 2710a, which supersedes 2710; and c) to evaluate the utility of LIBS as an alternative technique to LA-ICP-MS and  $\mu$ XRF for bulk analysis of soils.

Samples were submitted to thirteen laboratories: six used LA-ICP-MS, five used XRF and two used LIBS. Four samples were sent to each laboratory for analysis, consisting of standard reference materials: NIST SRM 2704 Buffalo River Sediment, NIST SRM 2710 Montana I Soil, NIST SRM 2710a Montana I Soil, and NRC Canada PACS-2 Marine Sediment Reference Material. For LA-ICP-MS, one of the samples was used as the single-point calibrator. For  $\mu$ XRF and LIBS, participants were asked to construct a calibration curve from four additional samples with known concentrations of the elements of interest. Each sample and standard was homogenized in a high-speed ball mill and pressed into pellets. Participants were instructed to measure the following elements: <sup>7</sup>Li, <sup>25</sup>Mg, <sup>27</sup>Al, <sup>42</sup>Ca, <sup>45</sup>Sc, <sup>47,49</sup>Ti, <sup>51</sup>V, <sup>55</sup>Mn, <sup>88</sup>Sr, <sup>137</sup>Ba, <sup>206,207,208</sup>Pb (LA-ICP-MS); Ti, Cr, Mn, Fe, Cu, Sr, Zr, Pb ( $\mu$ XRF); Ba, Cr, Cu, Fe, Li, Mg, Mn, Pb, Sr, Ti, Zr (LIBS). For both LIBS and  $\mu$ XRF, the choice of appropriate spectral lines was determined by the user, optimizing for linearity, sensitivity, and precision.

Results for both LA-ICP-MS and  $\mu$ XRF were generally consistent for most elements, resulting in good intra-laboratory precision (< 8 % RSD for

LA-ICP-MS; < 20 % RSD for  $\mu$ XRF) and low bias (< 10% for LA-ICP-MS; < 35 % for  $\mu$ XRF), which are important characteristics for forensic comparison of soils. Linear calibration curves were also obtained for both  $\mu$ XRF and LIBS. Results for LIBS showed good precision (< 15 %) and bias (< 15 %) for most elements. Some problem elements have been identified and are discussed. Limits of detection for trace and minor elements were in the 0.01 – 1 ppm range for LA-ICP-MS and 1 to 200 ppm for LIBS.

Unlike some other matrices such as glass, soil can be spiked with internal standard(s) and calibration standards can be easily created, allowing for quantitative analysis using an external calibration curve. The result of this first soil round robin study bodes well for future method development in the characterization and forensic analysis/comparison of bulk soil composition.

### References:

1. L Arroyo, T Trejos, P.R. Gardinali, and J.R. Almirall, Optimization and Validation of a LA-ICP-MS Method for the Quantitative Analysis of Soils and Sediments, *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2009, 64(1), 14-25.
2. L Arroyo, T Trejos, T Hosick, S Machemer, JR. Almirall, and PR Gardinali, Analysis of Soils and Sediments by Laser Ablation ICP-MS: An Innovative Tool for Environmental Forensics, *J. of Environmental Forensics*, 2010, 11(4), 315-327.
3. SC Jantzi and JR. Almirall, Characterization and forensic analysis of soil samples using Laser-Induced Breakdown Spectroscopy (LIBS), *Analytical and Bioanalyt. Chem*, 2011, 400(10) 3341-3351.

### Soil, Elemental Analysis, LA-ICP-MS, $\mu$ XRF, LIBS

## A111 The Potential Effects of Differential Transfer and Persistence on Forensic Soil Comparison

Andrew M. Bowen, MS\*, Stoney Forensic, Incorporated, 14101-G Willard Road, Chantilly, VA 20151

After attending this presentation, attendees will understand how physical properties of mineral grains such as size, density, and shape could potentially affect their differential transfer to and persistence on evidence. The implications of this with respect to appropriate selection of soil comparison methodology will be discussed.

This presentation will impact the forensic science community by introducing a phenomenon that is well established in the geological literature and has the potential to significantly impact forensic soil comparisons, but has yet to be addressed in the forensic science literature or by many published forensic soil comparison methods.

A tremendous variety of methods have been advocated for forensic soil comparison in the forensic science literature. These include comparison of soil color, particle size distribution, bulk chemical composition, pH of water extracts, isotopic composition, pollen content, enzymatic activity, mineralogy determined by polarized light microscopy, SEM/EDS, or x-ray diffraction, FTIR spectroscopy, UV-Vis spectrophotometry, microbial DNA profiling, analysis of organic components by GC/MS, and many more. While a handful of these methods involve analysis of soil particles of one type or another by microscopic methods, the majority of the published methods involve analysis of bulk soil properties.

Despite the common use of bulk methods for forensic soil comparison, there are some reasons why these methods may not be appropriate in some forensic scenarios (particularly those involving very small sample sizes). Sedimentary geologists have put a significant amount of effort into understanding the behavior of different mineral grains in sediments that are transported and deposited by wind and water. They have developed mathematical models that describe the behavior of mineral grains having different size, density and shape being transported and deposited by a variety of fluids. A garnet sand grain with a density of 4.0 g/cm<sup>3</sup> would

settle in water at the same rate as a quartz sand grain (density of 2.65 g/cm<sup>3</sup>) roughly one and a half times larger. This phenomenon produces many sediment samples containing coarse grains of light minerals along with fine grains of heavy minerals. Shape is important primarily for platy minerals like muscovite and biotite, which sort with coarser fractions of other minerals of similar size and density. Given that mineral grains with different size, density, and shape behave differently during transport and deposition in fluids, they may well behave differently during transfer from a source to an evidence item and may have different persistence on evidence after transfer. It is expected that coarser grains, denser grains, and more equant grains are less likely to be transferred to evidence, and less likely to persist on evidence, than their finer, less dense, and platy counterparts. If this is in fact the case, it has the potential to change the modal mineralogy and particle size distribution of soil evidence relative to its source, particularly in cases where the amount of soil transferred is quite small. While it is common practice for forensic soil comparison to be performed on a limited size fraction of soil, fractionation due to differences in density and shape are unaccounted for in most published methods and have the potential to produce false negatives during comparison of bulk properties.

Methods based on microscopic analysis of the soil particles themselves (e.g., minerals, pollen) enable analysts to directly observe the particles and determine whether the questioned and known samples have particle assemblages consistent with each other. Both the identities of the particles (mineral types, pollen taxa) and their character (mineral varieties, state of preservation of pollen) can be observed and compared along with their relative abundance. These methods have the potential to be more robust to the differential transfer and persistence of minerals, especially if research is conducted that provides insights into expected trends, such as an increase in platy minerals and decrease in dense minerals in a given size fraction on evidence relative to source soil. Methods based on microscopic analysis of soil particles described in the literature include optical mineralogy, automated SEM/EDS analysis (QEMSCAN<sup>®</sup>), SEM analysis of quartz surface texture, palynology, mycology and cathodoluminescence of minerals.

With the exception of an article discussing changes of particle size distribution before and after transfer to the sole of a shoe, virtually no research has been conducted in the area of how transfer and persistence issues might affect forensic soil comparison. Additional research should be conducted to determine the effects of differential transfer and persistence of different mineral types in forensic scenarios. The results of such research would likely provide insights into the appropriate selection of methodology for conducting forensic soil comparisons.

**Soil, Minerals, Transfer and Persistence**

## **A112 Development of a Pigment Classification Scheme by Raman Spectroscopy**

*Christopher S. Palenik, PhD\*, and Skip Palenik, BS, Microtrace, Limited Liability Company, 790 Fletcher Drive, Suite 106, Elgin, IL 60123-4755*

After attending this presentation, attendees will understand a broad overview of the development of a procedure for systematically identifying pigments in samples of forensic interest by Raman spectroscopy.

This presentation will impact the forensic science community by understanding how pigments in paint are overlooked in paint evaluation samples. The presentation will discuss results of several years of research on pigment identification. The presentation will also present a scheme for approaching and conducting pigment identification.

Colorants (pigments and dyes) are everywhere. In theory, they seem like an obvious material to exploit as forensic evidence; yet beyond the macroscopic color that colorants impart to a material, the identity of individual pigments or dyes are largely ignored in forensic casework. The reason for this is the small size and low relative concentration of pigments in a given application. Successful efforts have been made to characterize

select pigments in specific forensic applications (by XRF, FTIR, PLM and microchemistry, for example); however, each of these approaches has been subject to a particular limitation that has prevented the widespread application of pigment evidence to forensic investigations.

The development of Raman microspectroscopy has opened a new avenue for the possibility of identifying pigments in a consistent and reliable manner. The art community has embraced this technology and has made great strides in developing Raman spectroscopy as a practical analytical tool for pigment identification; however, the requirements of the art and conservation disciplines differ somewhat from those of the forensic laboratory. To this end, a program of research in the analysis of pigments by Raman spectroscopy with the aim of developing a practical approach for forensic scientists to exploit pigment evidence in casework has been conducted. These results have applications for both comparative forensic examinations as well as developing investigative leads, the latter of which could assist with sourcing paint samples.

In this research, samples were selected from a physical pigment reference collection of over 1,100 pigments. Samples were selected to represent as many organic and inorganic pigments as possible. When multiple samples of a given pigment existed, samples with stronger provenance were selected. Each pigment sample was analyzed by Raman spectroscopy, under varying conditions to obtain the best possible spectrum. In total, over 250 different pigments were examined and a database of Raman spectra was established.

Due to the fact that pigment samples are not always uniformly named by manufacturers (despite Colour Index naming conventions) and that samples have varying provenance, pigment identity was verified by several orthogonal methods. For example, Raman spectra were checked for consistency by comparing to other samples of the same pigment. Spectra were also compared to other chemically related pigments to ensure that chemically related pigments were spectroscopically related. All pigments were also elementally analyzed to ensure that the elemental data was qualitatively consistent with published compositions. Pigments with varying polymorphs were verified or identified by powder x-ray diffraction. Ultimately, any impure pigments or those with conflicting data that could not be resolved were withdrawn from the collection.

The Colour Index, maintained by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists, provides a basic level of chemical grouping in their classification approach; however, it was established that the chemical groups specified by the Colour Index were not sufficient in scope to provide the level of classification necessary to take advantage of the level of data in a Raman spectrum. As such, a new program of chemical groups was devised (drawing from many literature sources, as well as our own knowledge) that was consistent with the spectroscopic data. Major peaks from the pigment reference spectra were then used to spectrally sort pigments into the defined chemical groups. The result was the development of a forensic pigment identification scheme that permits identification of the majority of pigments in modern commercial use through a flow-chart type approach. The scheme is expandable to accommodate other pigments that are not currently in the collection. Pigments with strong fluorescence obviously cannot be grouped by this method; however, they are included in the appropriate chemical group.

Ultimately, this identification scheme permits specific identification of pigments (when possible), but also allows an examiner to determine the extent to which a pigment can be identified or grouped. For example, pigments within certain chemical groups have indistinguishable Raman spectra, which mean that a pigment can only be identified to a certain level (e.g., certain diarylide pigments). In other cases, pigments can be identified as a single specific pigment. The benefits of such an identification scheme will be illustrated through the analysis of a small group of paint samples.

**Pigment, Raman Spectroscopy, Paint**

## A113 Development of a Field Method for the Identification of the Hallucinogenic Herb *Salvia Divinorum* Using ATR-FTIR

Rebecca L. Mead, BS\*, Elise R. Chom, MS, and J. Graham Rankin, PhD, Marshall University, Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701

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After attending this presentation, attendees will have an increased understanding of the need for an in-field test for the identification of *Salvia divinorum*. They will also become familiar with the use of ATR-FTIR to identify *Salvia divinorum* in the field.

This presentation will impact the forensic science community by presenting a new method of identification of *Salvia divinorum* that can be used as a simple in-field presumptive test that does not require the extraction of salvinorin A prior to analysis.

*Salvia divinorum* is a hallucinogenic plant that is smoked or chewed as a substitute for marijuana. The short-lived “high” is a result of the chemical salvinorin A, which is a component of *Salvia divinorum* that has not been found in any other species. Currently, *Salvia divinorum* and/or salvinorin A is illegal in twenty-two states and is regulated in eight additional states. Several countries have also outlawed or regulated *Salvia divinorum* and/or salvinorin A. With the recent increase in regulation of *Salvia divinorum* across the country, there is a need for a quick and simple preliminary test for *Salvia divinorum* that can be used in the field.

Currently, *Salvia divinorum* is not identified in crime laboratories. Instead, salvinorin A, the active component, is extracted from the plant material and analyzed using gas chromatography/mass spectrometry and thin layer chromatography. However, no in-field tests have been identified to aid in the quick presumptive identification of *Salvia divinorum* or salvinorin A. Previous studies have shown that no color test can definitively identify *Salvia divinorum* or salvinorin A, and *Salvia divinorum* does not possess unique physical characteristics that can be used for identification like marijuana. While confirmatory tests are sufficient for identification once the seizure reaches the laboratory, the lack of a field test will result in either too many or too few samples being sent to the lab for analysis. Also, all techniques currently used for identification require an extraction step, thus identifying salvinorin A rather than *Salvia divinorum* itself. Developing an in-field presumptive test to aid in the identification of *Salvia divinorum* will allow law enforcement to determine if vegetation found in the field is possibly *Salvia divinorum*. ATR-FTIR is a simple technology that does not require any sample preparation. Within minutes, a trained law enforcement officer with a portable FTIR instrument can determine if vegetation could be *Salvia divinorum*. In the laboratory, FTIR spectroscopy would provide a category A confirmatory test for identification that would not require an extraction as currently used methods do.

This study addresses differences and similarities in the infrared spectra of *Salvia divinorum* and more than thirty other species of *Salvia* as well as common herbs that may be confused with *Salvia divinorum* and extract enhanced products of *Salvia divinorum*. Additionally, as part of the validation of this method, the top and bottom of the leaves were compared to determine if a difference is present that would affect the leaf’s identification. Salvinorin A is also being analyzed to determine if FTIR spectroscopy can be used to identify the pure substance. Finally, a portable FTIR instrument is being used to ensure the results found are comparable with those obtained with a research grade bench ATR-FTIR spectrometer.

***Salvia Divinorum*, Plant Identification, ATR-FTIR**

## A114 Using Analytical Techniques to Distinguish Illicit and Over-the-Counter Drugs in Trace Evidence

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After attending this presentation, attendees will understand the difference between the data obtained from analytical techniques utilized to identify over-the-counter and illicit drugs.

The presentation will impact the forensic science community by providing an in depth comparison of techniques which can enhance the analysis of data obtained from crime scenes.

The illegal drug trade consists of the manufacturing, distribution, and sale of controlled substances. The most recent annual data from the Federal Bureau of Investigation (FBI) show that 12.2 percent of more than 14 million arrests in 2008 were for drug violations, the most common arrest crime category. Additionally, the abuse of common over-the-counter pain medication drugs has also escalated in the past decade. The drugs with the highest dependence or abuse levels were marijuana, prescription pain relievers, and cocaine. Many of these drugs are considered “controlled substances” that have a legally recognized potential for abuse. Officials responding to crime scenes (e.g., firefighters, police, and hazmat teams) are often ill equipped to handle the ever growing magnitude of drugs that are encountered at crime scenes. These samples generally are collected and sent to crime labs for extensive analysis which can be time consuming and impede the criminal justice system. However, recently several analytical techniques have been developed which can be used in a portable mode. These techniques can be used to identify white powders which are otherwise indistinguishable based on color, texture, and odor. The present study examines the different analytical techniques that can be used to identify the unknown compounds frequently encountered by first responders in the field. The two analytical techniques for chemical identification which can be used in the portable mode include Fourier transform infrared spectroscopy (FTIR) and Raman Spectroscopy. While infrared spectroscopy utilizes infrared radiation to probe the chemical structure of the drug/compound, Raman spectroscopy utilizes a monochromatic light source (e.g., a laser) focusing onto a sample and analyzing the resulting scattered light. In both cases, the radiation interacts with the bonds of the compound producing a unique spectral fingerprint of the drug. The identity of the compound is determined by comparing spectra against a database of FTIR or Raman spectra of known spectra present in a library. In this study, different drugs (Aspirin, Acetaminophen, Caffeine, and Excedrin) were tested and compared with common household materials, such as talcum powder, using the two different analytical methods. Specifically, data was compared data from the above mentioned techniques for Excedrin and its individual components: caffeine, acetaminophen, and aspirin. Raman spectroscopy had the advantage of providing sharper peaks for symmetric bonds in aromatic compounds and those that contain double bonds, while FT-IR showed stronger peaks for asymmetric hydroxyl and carbonyl groups. In addition, fewer peaks of interest in Raman spectra may facilitate mixture analysis. Even though both techniques probe the structures using molecular vibrations, they did differ in the information that they provide. In conclusion, it was found that these two methods are also easy to use, give quick results, and are now available in a portable form which can be used for detection at crime scenes.

**OTC Drugs, Illicit Drugs, Analytical Techniques**

## A115 Development and Validation of a Quantitative Method for Identification of Synthetic Cannabinoids Using GC/MS

Jennifer L. Rehme, BS\*, Amanda Heeren, MS, and J. Graham Rankin, PhD, Marshall University, Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701

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After attending this presentation, attendees will have a solid grasp on the properties and characteristics of synthetic cannabinoids and the current trends in use and regulation of the drugs, as well as how to effectively analyze samples to determine the type and quantity of synthetic cannabinoid used in the herbal matter tested.

Due to the increasing abuse of these herbal incense products, this presentation will impact the forensic science community by informing attendees about new developments in the battle against the use and abuse of synthetic cannabinoids as well as a method that has been developed to quantitate the synthetic cannabinoids in each product tested in a quick and accurate way.

These products often have names that are ambiguous or suggestive drug references. While labeled as "Not for Human Consumption" and purportedly for use as incense only, these are a part of a growing trend to use as a way to get around the legality of using marijuana but still obtain a high. These products are laced with chemicals known as synthetic cannabinoids, named for their structural similarity to the psychoactive ingredient in marijuana. The structural similarity allows them to bind to the same receptors in the brain and other organs, giving the user the same effects of marijuana. These products have been seen in the market since at least 2004 when they were quickly banned by the military. Since then, a number of states have followed suit and begun banning these products.

With the recent temporary scheduling of five of these compounds in March 2011 by the DEA, there has been a need to find a method that accurately and quickly determines the type and quantity of synthetic cannabinoid in each seized product. The method needs to have the ability to separate out the components as to allow the analyst to distinguish synthetic cannabinoids from other components such as Vitamin E, which is often added to try to disguise the synthetic cannabinoid's presence. The fact that Vitamin E is easily separated from the synthetic cannabinoids by gas chromatography negates any attempt at disguising the active ingredients. Gas chromatography/mass spectrometry (GC/MS) has been previously shown to be a good method for the qualitative analysis of the synthetic cannabinoids. However, quantitative analysis has been limited until now due to the limited availability of reference standards and that most states do not require quantitative analysis for seized drugs. This presentation will describe a method used to obtain accurate results using a GC/MS and will show how the synthetic cannabinoids in such products can be quantitated using this method.

In order to develop a quantitation method, various synthetic cannabinoid standards were obtained from commercial sources and subsequently analyzed by gas chromatography/mass spectroscopy. The reference standards analyzed include the following: JWH-018; JWH-073; JWH-200; JWH-250; and ( $\pm$ )-CP 47,497 (C8) homolog. Using tetracosane as an internal standard and a standard curve, the data obtained was applied to the analysis of various brands of herbal incenses obtained from Huntington area head shops in 2010 and a service station in Kentucky in 2011. GC/MS data is presented for all reference standards as well as for all samples analyzed.

**Synthetic Cannabinoids, Herbal Incense, JWH Compounds**

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## A116 Target Compound Ratio Analysis of Medium Petroleum Distillates

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The goal of this presentation is to present initial results of a quantitative method developed to differentiate between medium petroleum distillates (MPD) and residues of MPDs. The method is an extension of a method developed for the differentiation of kerosene samples as neat liquids and residues using Gas Chromatography/Mass Spectroscopy with a target compound ratio analysis method. Comparison to determine if a sample is from a specific source is a problem in the application of fire debris analysis. The long-term goal of the project is to develop a uniform statistical method to determine degrees of similarity between any two ignitable liquid residues in fire debris analysis.

This presentation will impact the forensic science community by providing a methodology and a statistical basis for declaring similarities between any two medium petroleum distillate samples as required by the courts and the National Academy of Sciences Report.

Petroleum-based products are the most commonly used accelerants in the United States. Most of these products are readily available to the public and are flammable. Gasoline is the most often encountered ignitable liquid, followed by kerosene and medium petroleum distillates. Medium petroleum distillates as classified by the American Society for Testing and Materials (ASTM) E1618 include paint thinners, dry cleaning solvents, and charcoal lighters. Medium petroleum distillates have a boiling point range of 120 to 240°C and contain C<sub>8</sub> to C<sub>13</sub> compounds, which include normal and branched alkanes, some cycloalkanes, and some aromatics. Differences between manufacturing processes should provide variation between medium petroleum distillates, which is often seen in reference collections because of the lack of homogeneity within the class of MPDs. This research was performed to determine the key components and peak ratios of medium petroleum distillates to provide an analytical and statistical basis for the differentiation between these products.

High resolution GC/MS data was analyzed using target compound ratios from key compounds found in medium petroleum distillates. Compounds eluting off of the non-polar polydimethylsiloxane column generally elute in order of increasing boiling points. Therefore, peak ratios comparing closely eluting compounds are less affected by preferential evaporation in the course of a fire.

Previous research performed on kerosene and gasoline has established a number of compounds that can be used to distinguish kerosene and gasoline samples from different sources. In this work, seventeen compounds have been identified from mass spectra and retention times in medium petroleum distillates as possible candidates. From those compounds, sixteen ratios were calculated and compared for reproducibility within ignitable liquid and significant differences between liquids. Evaporation and burn tests were also performed to test the robustness of the ratios under conditions that may occur during an actual fire.

Several "green" paint thinners and solvents have been analyzed which are listed as non-flammable. The composition of these newer products, although not likely to be used by an arsonist, may occur as incidental liquid residues in fire debris.

A database of gasoline, kerosene, and MPD analyses is being developed from a large number of samples in each class and a variety of different sources, which will be used to establish statistical criteria for comparison between two different samples with a probability of error.

**Medium Petroleum Distillates, Fire Debris Analysis, GCMS**

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## **A117 Ignitable Liquid Residue Distribution in Pour Patterns as Affected by Substrate Type and Ignitable Liquid Class**

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The goal of this presentation is to present the effects of substrate type on the migration of ignitable liquids in a pour pattern during a fire and to present the best place to collect an evidence sample from in each case.

This presentation will impact the forensic science community by providing fire investigators with an expanded knowledge of the primary areas to sample from a suspected ignitable liquid pour pattern on various substrates in order for the fire debris analyst to obtain the best results in the lab.

Collection of fire debris evidence from a fire scene most commonly falls on the shoulders of the fire investigator in charge of the scene. The sample is then sent to the lab to be analyzed by a fire debris analyst for the presence of ignitable liquid residues. For the best chromatographic results, the evidence samples must be collected from an area of the pour pattern suspected to contain the highest concentration of ignitable liquid residue. The question is whether it is best to collect from the center of the pour pattern, the edges of the pour pattern, or somewhere in between.

One factor to consider is whether the substrate the ignitable liquid was poured onto has any effect on the prime area to collect the sample from. Carpeting, for example, can wick the ignitable liquid away from the original pour pattern diluting the ignitable liquid over a larger area. Some newer synthetic carpets can also self-sustain combustion beyond the edge of the original pour pattern leaving a completely unrelated pattern. Sampling from the edge of this pattern could potentially give negative results. Also, different types of wood may absorb the ignitable liquid allowing for a deeper burn pattern while others may resist it allowing the ignitable liquid to spread farther and burn faster with little effect on the substrate.

An experiment was designed to test the concentrations of ignitable liquid residues in different specified areas of pour patterns post-burn. A circular pour pattern representing a central dump of the ignitable liquid was tested, as well as a line pattern representing a trail pattern. Substrates were allowed to burn to 70% completion and were extinguished with water. Multiple samples were collected at designated areas across the pattern. Any volatile ignitable liquid residues present were collected by passive headspace analysis on activated charcoal strips and submitted to analysis by GC/MS. Total ion chromatograms for each sample were analyzed qualitatively and quantitatively. The ratio of the total peak area to that of the internal standard, 3-phenyltoluene, was calculated and compared.

Initially, the results have shown that higher concentrations of ignitable liquid residues can be found toward the center of the pour patterns than toward the outer edges under these conditions. This would suggest that the center of a pattern would be the best place for fire investigators to sample from for the best results. Differences in relative concentration of ignitable liquid residue due to substrate, actual pour pattern, and class of ignitable liquid will be presented.

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### **Fire Debris Analysis, Pour Pattern, Ignitable Liquid**

## **A118 Effects of Various Substrate Types on E1618 Pattern Classification of Ignitable Liquids Present in Fire Debris**

*Amanda Heeren, MS, J. Graham Rankin, PhD, and Jared Vititoe, BS\*, Marshall University, Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701*

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After attending this presentation, attendees will gain an understanding of how substrate type affects the ASTM E1618 classification of ignitable liquids present in fire debris samples. Attendees will also gain an understanding of the methods and techniques currently used in the analysis of debris collected from fire scenes.

This presentation will impact the forensic science community through a discussion of the potential troubles associated with the analysis of specific burned problem substrates, such as misinterpretation and misclassification of ignitable liquids present.

Charred and uncharred substrates of various types will be compared and ignitable liquids, if present, classified according to ASTM method E1618. This presentation will benefit the fire debris analysis community by informing analysts and researchers of the potential of common fire debris substrates to produce products that may interfere with the GC/MS analysis and E1618 classification of ignitable liquid residues in fire debris.

Due to the extraction procedure for ignitable liquid residues, compounds present in the substrate, such as various polymers and glues, may co-adsorb to activated charcoal strips with the ignitable liquid residues present in fire debris. These interfering products will also be present on the chromatograms generated during analysis. Additionally, pyrolysis and combustion products generated from the burning of the substrate may potentially affect the chromatographic analysis of ignitable liquids. As non-wooden substrates (carpet/padding 52%, fabric/paper 11%, and vinyl flooring/plastics 10%) constitute a large percentage of the substrates submitted as fire debris samples to a crime laboratory, it is important for fire debris analysts to be aware of the difficulties such substrates may cause in ignitable liquid analysis.

In this study various fire debris substrates were selected for E1618 analysis of the ignitable liquids gasoline, kerosene, and charcoal lighter fluid. These ignitable liquids are classified as gasoline, heavy petroleum distillates, and medium petroleum distillates, respectively. The substrates chosen were yellow pine, carpet, and carpet pad, which after charring to approximately 50% by weight with a propane torch to simulate burned fire debris, were analyzed with 10 $\mu$ L spikes of 50%, 75%, and 90% ignitable liquid evaporates. Additionally, uncharred substrates were also spiked with 10 $\mu$ L of each of the ignitable liquid evaporates. Un-spiked samples of each substrate, charred and uncharred, were also analyzed as substrate blanks. Ignitable liquid vapors were then concentrated according to the ASTM E1412 method on activated charcoal strips with carbon disulfide as the eluting solvent. 3-phenyl toluene was added to carbon disulfide as an internal standard.

It was experimentally concluded that a substrate may affect E1618 classification. For yellow pine, several notable terpenes were present in the uncharred blanks (1S- $\alpha$ -pinene, 5.6 min;  $\beta$ -pinene, 6.2 min; 1,5-dimethyl-1,5-cyclooctadiene, 7.0 min); however, these peaks were present but effectively buried in the samples that were un-charred and spiked with ignitable liquids. In the charred samples, the terpene chromatographic peaks were severely diminished, nearly undetectable, a phenomenon reasonably explained by the volatility of terpenes. For carpet, several minor precursory products were present in the substrate blanks, mostly olefins from the carpet fibers (such as dodecene, 8.6 min). However these peaks were insignificant in the ignitable liquid spiked samples. Carpet pad had very few detectable compounds in the substrate blanks. These few peaks were present in minor concentrations and easily masked in the baseline of the spiked samples. With kerosene the ignitable liquid pattern was found to shift to the lighter end of the chromatogram for both yellow pine and carpet pad, a phenomenon not observed with the other ignitable liquids or in the

other substrates. With yellow pine the entire chromatographic pattern was observed shifting approximately one carbon lower (heavier components not recovered) in comparison to the neat ignitable liquid. This phenomenon did not change the classification of kerosene as a heavy petroleum distillate (C<sub>8</sub>-C<sub>20+</sub>) in this substrate. The relative abundance of the normal paraffins was reduced relative to the branched and cyclic hydrocarbons, as has been reported previously for yellow pine. Thus, during analysis, analysts should be informed and remain cognizant of the substrate being analyzed as it may affect their interpretation and E1618 classification of the results.

**Fire Debris Analysis, ASTM E1618, Substrate Effects**

## **A119 New Gas Chromatography-Positive Chemical Ionization Tandem Mass Spectrometric Method for the Detection of Methylenedioxypropylvalerone (MDPV), 4-Methylmethcathinone (Mephedrone), and 4-Methoxymethcathinone (Methedrone)**

*Lindsey E. Hume, BS\*, Marshall University, Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701; Randall D. Fornshell, BS, and Timothy P. Rohrig, PhD, Sedgewick County, Regional Forensic Science Center, 1109 North Minneapolis Street, Wichita, KS 67214; and J. Graham Rankin, PhD, Marshall University, Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will be familiar with a new approach for the analysis and identification of common “bath salts” ingredients utilizing GC-MS/MS with chemical ionization.

This presentation will impact the forensic science community by providing a definitive method for the identification of synthetic cathinones which are becoming increasingly popular among recreational drug users and are rapidly being regulated by state and national governments.

MDPV, mephedrone, and methedrone are some of the most common compounds currently seen in products being marketed as “bath salts.” They are structurally similar to the scheduled stimulants cathinone, and methcathinone. Therefore, the synthetic cathinones, including mephedrone, methedrone, and MDPV, are also structurally similar to each other. As a result, they have similar fragmentation patterns, often with poor molecular ions, when characterized by traditional GC-MS methods utilizing electron ionization. In this study, different GC-MS techniques, including electron and chemical ionization and single and triple quadrupole (QQQ) mass spectrometry, were evaluated in order to determine the most definitive method for identification of synthetic cathinones. Here, it is proposed that GC-MS/MS with chemical ionization in multiple reaction monitoring (MRM) mode can unequivocally detect the presence of and identify the studied compounds.

The method was also developed with the designer drug market in mind. As has been demonstrated recently with the synthetic cannabinoids, “bath salts” have also shown the tendency to have variable contents as bans on certain compounds are enacted. Most often, “bath salts” are marketed as a “legal high,” so changing the active compounds in accordance to the new laws is common. As these new compounds arrive on the market, and are also likely regulated, forensic analysis will need to be able to detect them in addition to those currently controlled. This method was developed so that any synthetic cathinones can be run, responses can be optimized, and the appropriate MRM ions can be added to the method with minimal adjustments to facilitate rapid analysis.

Electron ionization, with both single and triple quadrupole analysis, gave very similar spectra for mephedrone and methedrone. Chromatographic peaks also had poor shapes. MDPV exhibited a low-detail fragmentation pattern, but a characteristic peak at *m/z* 126 was detected, due to the presence of the pyrrolidinyll group on the alkyl chain of the compound, that helped distinguish it from the other investigated

compounds. Because of similar fragments in single quadrupole analysis for the other two drugs, fragments chosen for tandem mass spectrometry also produced similar patterns. Chemical ionization significantly increased the intensity of the protonated molecular ion relative to the nearly nonexistent molecular ion produced with electron ionization. When triple quadrupole analysis was paired with chemical ionization, unique mass spectra were also observed through the selection of the protonated molecular ion as the precursor ion at Q1. Additionally, chromatographic peaks were clean and lacking the shoulder present in electron ionization analyses.

Compounds that could potentially interfere with the compounds of interest were also subjected to the method. Compounds studied for possible interference included amphetamine, caffeine, cathine, ephedrine, ketamine, phentermine, alprazolam, benzylpiperazine (BZP), clonazepam, cocaine, codeine, diazepam, heroin, hydrocodone, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), oxycodone, pseudoephedrine, trifluoromethylphenylpiperazine (TFMPP), cathinone, inositol, and phenethylamine. No interference was observed, though peaks were occasionally present in the chromatogram. Future studies should be performed to optimize compound-specific conditions for the analysis of other synthetic cathinones in order to confirm the broad application to this class of drugs.

**Bath Salts, Designer Drug Analysis, Cathinones**

## **A120 Ignitable Liquid Residue Source Elimination by Molecular Weight Estimation**

*John J. Lentini, BA\*, Scientific Fire Analysis, Limited Liability Company, 32836 Bimini Lane, Big Pine Key, FL 33043*

After viewing this presentation, attendees will understand a simple method for excluding a suspected source of ignitable liquid residue using basic chemical fundamentals.

This presentation will impact the forensic science community by utilizing this straightforward method of analysis for fire debris analysts in order to provide additional useful information to fire scene investigators.

The literature of fire debris analysis is replete with descriptions of attempts to match an ignitable liquid residue to a suspected source. Many different methodologies have been suggested for making comparisons, and more recent attempts have focused on chemometrics such as principal components analysis. Because gasoline is the single most commonly used accelerant, most of the efforts at identifying the source of ignitable liquid residues have focused on gasoline. In environmental forensics, other methods have been used to identify the source of hydrocarbons spills. Two-dimensional gas chromatography-mass spectrometry and the analysis of biomarkers have been successfully applied. Unfortunately for fire debris analysts, the quantity of extract is often far too small to be analyzed using the same methods available to environmental chemists.

Most forensic science laboratories today follow ASTM E1618, *Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry*. This methodology is, and historically has been, designed to allow for classification of ignitable liquid residues into any number of recognized classes. The 2010 edition of the standard recognizes eight classes of ignitable liquid: gasoline; petroleum distillates including de-aromatized distillates, isoparaffinic products; aromatic products; naphthenic-paraffinic products; normal alkane products; oxygenated solvents; and others-miscellaneous. Most of these classes of ignitable liquid residues can be further subclassified as light, medium, or heavy. Gasoline has no subclasses, and oxygenated solvents are only characterized as light or medium.

Making distinctions within a class, or finding the source of an ignitable liquid residue are outside of the scope of ASTM E1618. The standard states, “This test method is intended to allow identified ignitable liquids to be characterized as belonging to one of the classifications. Distinguishing between examples within any class may be possible, but such further characterization is not within the scope of this test method.”

Two case reports will be presented. In each case, an attempt was made to connect a suspect to a fire scene by comparing ignitable liquid residues. In one case, a plastic milk jug was found a few blocks from the scene, and was suspected as being the container used to carry a heavy petroleum distillate (HPD). In the second case, medium petroleum distillate (MPD) found in two samples of fire debris from the scene was believed to have come from the same source as medium petroleum distillate found on the suspect's shoes. In both cases, analysis of the average molecular weight (expressed as carbon number range) revealed that the suspected source was heavier than the extract from the scene.

When exposed to a fire, ignitable liquid evaporates, and its average molecular weight necessarily increases. No predictable experience will cause the average molecular weight of the fire-exposed liquid to decrease. If the suspected source of an ignitable liquid, which was not exposed to the fire, exhibits a higher average molecular weight than the residue extracted from samples collected at the scene, the suspected source can be conclusively eliminated.

Using this straightforward method of analysis, fire debris analysts will be able to provide additional useful information to fire scene investigators.  
**Accelerant, Ignitable Liquid, Identity of Source**

## **A121 Analysis of a 100-Year-Old Alleged Opium Sample: Quality Assurance and Legal Issues**

*Thomas M. Blackwell, BS\*, Drug Enforcement Administration, 99 Tenth Avenue, Suite 721, New York, NY 10011*

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After attending this presentation, attendees will better understand the importance of best laboratory practices and quality assurance in everyday work and how this can drastically affect legal implications in drug analysis from a criminal standpoint as well as a business perspective. A comparison of best laboratory practices and quality assurance measures from 1887 and today will also be addressed.

This presentation will impact the forensic science community by emphasizing and addressing proper documentation, quality assurance practices of accepted methodologies, bias, and the importance of reviewable data as they pertain to our current legal system. In today's world of accreditation and transparency, quality assurance is more important than ever. Analysts and managers struggle with a continued question, "What's more important, quality or quantity?" The answer to this question is simply both. Quantity and quality must be weighed together to ensure efficiency and functionality; however, picking one over the other may result in the lack of reviewable data or accepted practices that have no validation. It is critical for a reviewer to be able to reconstruct an analysis or be able to know how an analyst arrived from "point A" to "point B."

This presentation will demonstrate the incongruities associated with the analysis and legal status of imported opium in 1887 as three different "morphia" (or morphine) content results were reported for one sample. Handwritten reports detailing little to no information were supplied with no analytical data or description of tests performed to support the quantitative results. This practice indicates that the analyst/law officers were often taken "at their word" over 100 years ago.

In 1887, opium was legally imported into the U. S. from China as long as the morphine content was above 9% in an "unprocessed" or "crude" opium sample. This type of "smoking" opium had a peculiar flavor and was largely used in Hong Kong. However, Chinese immigrants living in the United States favored a different class of crude opium known as "Patua" which had a morphine content of less than 7% and, thus, was illegal in the United States.

Opium was often imported as crude or processed opium and subsequently appraised by the U.S. Customs House. Part of the appraisal processes involved the analysis of morphine content. Initial assays conducted in 1887 indicated that morphine was present at a level of 11.30%, rendering the shipment legal in the United States. Subsequent

purchase of this opium at auction by sellers of Persian opium objected to the classification as crude "Patua" opium, since they argued that this class of opium rarely, if ever, contained more than 7% morphine. The importation of this "illegal" opium would be a detriment to their legitimate sales. They, therefore, submitted a formal complaint to the Customs House who, in turn, ordered a reanalysis. The sample was reanalyzed (2x) by Customs House analytical chemists later that year who reported the morphine content to be on average 4.1% (separate analyses of 4.05% and 4.25%). This new result caused the U.S. Customs House Department to launch an investigation. The larger value of 11.30% was subsequently dismissed upon conclusion of the investigation.

A U.S. Marshall conducting research in the 19<sup>th</sup> century records of the Bureau of Customs found a case file that included an envelope of alleged opium. This led to a request by the National Archives in 1993 requesting that DEA analyze the sample. Analysis of the sample utilizing gas chromatography/flame ionization detector (GC-FID), gas chromatography/mass spectroscopy (GC/MS), and infrared spectroscopy (IR) of the sample indicated that the alleged opium contained morphine (75%) and codeine (4.5%). Typically, the naturally occurring alkaloids of an opium poppy (morphine, codeine, thebaine, noscapine, and papaverine) are required to be present in a substance deemed to be opium. Yet, it is unclear if this was a requirement in 1887 due to the lack of reviewable data. Interestingly, the reported morphine content in 1887 was 4.1% (re-analysis), yet modern GC-FID analysis of the sample indicated that codeine was present at 4.5%. So the question arises, was codeine being erroneously identified and quantitated in the 1880s instead of morphine?

Assuming that morphine was the correct analyte being tested, why was there such a large disparity in quantitative values (11.30% vs. 4.1% vs. 75%)? Best laboratory practices and proper quality assurance practices such as: clear and concise documentation; reviewable data; analytical schemes; chain of custody; and, sampling protocols, which are critical in today's forensic science arena, could have alleviated much of this disparity. All of these issues are routinely depicted on popular television shows and by current real world events surrounding forensic laboratory closures in various states. Although a number of handwritten notes surfaced from the 1887 opium sample, no data relating to the chemical testing of the morphine could be located.

**Morphine, Opium, Quality Assurance**

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## **A122 The Characterization and Discrimination of Pink and Red Nail Polish Lacquers – A Preliminary Study**

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After attending this presentation, attendees will learn about the most discriminating analytical sequence for pink and red nail polish lacquers when they are recovered as physical evidence.

This presentation will impact the forensic science community by providing information about the chemical compositions of the studied nail polish lacquers as well as about their between-sample discriminations.

Suppose that a nail fragment is recovered from an upper garment seized from an individual suspected of having raped a woman. On the fragment a colored layer is observed and it is assumed to be nail polish lacquer. What is the best approach to examine this colored substance?

The goal of this study was to detect the different components for the characterization of nail polish samples, to evaluate the discriminating power of the available methods, and to identify an appropriate analytical sequence.

One hundred ten pink and red-colored nail polish lacquers of different common brands were collected. The samples were examined using visual and microscopical (unassisted eye observation followed by stereomicroscopy) observation, reflectance visible microspectrophotometry (MSP), Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy, and elemental analysis with scanning electron microscopy/energy dispersion spectroscopy (SEM-EDS).

The results indicate that color was the most discriminating feature. However, although most of the red and pink samples studied could be easily distinguished based on their shades, there were several instances when there was difficulty in determining whether a sample was red or pink. Microspectrophotometry proved to be the most discriminating technique. Out of 5,995 possible pairwise comparisons, 52 pairs could not be differentiated according to their MSP spectra. It was observed that some samples were difficult to distinguish by MSP but could be differentiated by visual examination and vice versa. Therefore, it was confirmed that visual examination and microspectrophotometry are complementary methods. The presence, distribution, and colors of sparkling (or glitter) particles played an important role as discriminating features.

On the other hand, infrared spectroscopy provided confirmation that all the samples of our dataset were nitrocellulose-based substances. Though the presence of additional absorption bands allowed for observing further discriminations, infrared analyses discriminated 43 pairs of the 52 indistinguishable with MSP. Raman spectroscopy (using a near-infrared laser source at 785nm) allowed for the detection of the main pigments contained in the analyzed samples. For red colored samples, C.I. Pigment Red 57:1 (C.I. 15850:1) was the most commonly encountered, while for pink samples the most frequently detected pigment was C.I. Pigment White 6:1 (C.I. 77891). The latter was the anatase form of titanium dioxide. The 785nm laser line, which was the only wavelength used, was not successful in detecting the mixtures of pigments as they were described on the sample bottles. Therefore, low discriminations were observed with the Raman technique. In some cases, it was possible to discriminate pairs that could not be distinguished using MSP and FTIR. Such discriminations were possible by means of the consideration of the presence (or absence) of additional Raman bands along with those of the main pigment. Elemental analysis with SEM-EDS allowed for obtaining a commonly observed profile based on silicon, sulfur, titanium, magnesium, aluminum, and phosphorous. Some differentiations of X-ray spectra were possible on the basis of a higher intensity of the sulfur signal as well as the occasional presence of other elements (such as bromine or tantalum).

To conclude, this preliminary study indicated that the following analytical sequence was the most appropriate one for the characterization and discrimination of pink and red nail polish lacquers: stereomicroscopy, MSP, FTIR spectroscopy, and Raman spectroscopy. This sequence allowed for the observation or detection of the following properties: color observation and sparkling particles, color measurement, binder, plasticizers and additives, and organic pigments.

**Nail Polish Lacquers, Trace Evidence, Microscopy**

## **A123 GSR Analysis: Correlating Quantities of Trace Metals (Pb, Sb, Ba) by ICP-MS to the Presence of GSR by SEM**

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After attending this presentation, attendees will have a better understanding of the trace metal (Pb, Ba, and Sb) quantities identified by the Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) that correlate to the presence of gunshot residue (GSR) and associated particles reported using the Scanning Electron Microscope – Electron Dispersive

Spectrometer (SEM-EDS). Attendees will also understand the effect that time and physical activity have on the quantities of trace metals associated with gunshot residue found on a person's hands after shooting a firearm.

This presentation will impact the forensic science community by highlighting the importance and validity of using the ICP-MS as a means to quantify trace metals associated with GSR by demonstrating a correlation between quantities of metals to the presence of GSR by SEM-EDS, thereby allowing the crime lab a greater throughput for GSR cases. The data gathered in this study from the SEM also has the potential to substantiate a timeline that shows the degradation of GSR evidence over time.

Traditionally, the Scanning Electron Microscope (SEM) has been used for the analysis of gunshot residue evidence because of its ability to identify gunshot residue particles elementally and morphologically. Some disadvantages of using only the SEM in gunshot residue analysis include low throughput ability and a lack of quantitative ability. For agencies with a high volume of cases which involve GSR kits, the Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) is a viable response to these disadvantages. The sensitivity, precision, and accuracy make it an ideal candidate for analyzing GSR evidence from the hands of people suspected of having been around a gun when it has been fired. Literature reports the ICP-MS limits of detection for Pb, Ba, and Sb to be 0.1µg/L, 0.02µg/L and 0.05µg/L respectively. The use of both the ICP-MS and the SEM will allow the agency higher throughput and an ability to quantify trace metals on a person's hands while maintaining the ability to conclusively identify GSR particles.

GSR kits, consisting of both SEM particle lifts and swabs, were taken at zero, one, two, four, and six hour intervals post-firearm discharge. All subjects continued their general laboratory and office duties and kept a record of those daily activities. Two sets of data were generated using two different calibers of weapons (a .22 caliber semi-automatic and a .44 caliber revolver) in order to achieve minimum and maximum quantities of gunshot residue. Particle lifts were taken from the right palm, right back of the hand, left palm, and left back of the hand of the participating shooters. These lifts were then followed by sample swabs of 5% nitric acid (two swabs per region of the hand) as well as one set of control swabs taken from a region of skin that should not have come into contact with GSR during a shooting, such as the covered back of the shooter's calf or shoulder.

Quantities of metals from the ICP-MS were calculated from each sample from the GSR kits and these quantities were compared to results obtained from the sample area using the SEM. Quantitation values of the previously mentioned GSR kits were separated by region of hand, as well as element, and then averaged for practical application. In order to provide minimum quantities that correlate to the presence of GSR and related particles, only .22 caliber results were used in the calculations.

SEM confirmed samples were categorized based on the particles found. Four classes were used to distinguish the samples: round Pb-Ba-Sb particles (gunshot residue), non-round Pb-Ba-Sb particles, round lead particles, or negative. Trend lines were then created to display particle behavior as a function of time. A negative correlation was observed between the amounts of GSR or GSR related particles and the length of the time intervals, while negatives had a positive correlation.

**ICP-MS, Gunshot Residue, Metals Analysis**

## **A124 Wikis in Criminalistics: An Example of Constructive Alignment and Collaborative Knowledge Building**

*Shirly Berends-Montero, PhD\*, Netherlands Forensic Institute Academy, Laan van Ypenburg 6, The Hague, 2497GB, NETHERLANDS*

After attending this presentation, attendees will understand the concept of constructive alignment and will recognize at least one tool for collaborative knowledge building.

This presentation will impact the forensic science community by creating awareness about the optimization of the process of teaching/training-learning forensic science at all levels and all fields, through the use of pedagogical and didactical models.

For some time, forensic sciences have been experiencing a paradigm shift in which ideas and protocols used for many years are thought to be obsolete and logically incorrect. Worldwide, individuals and organizations active with forensic sciences are discussing the optimal approach to the different phases of the forensic process with “a sound scientific foundation and justifiable protocols.”<sup>1</sup> As a consequence, knowledge is being created, modified, and applied at a rapid rate. As proposed in the model of personal and social knowledge-building by Stahl (2000), the initial ideas of an individual about a problem ought to be crystallized in words and made public in order to engage in the cycle of “social” knowledge-building.<sup>2</sup> Through feedback, argumentation and negotiation, the formalization of the created collaborative knowledge is possible and can be followed by its integration into an individual’s comprehension.

These processes or phases in the above mentioned model are recognizable in actual situations within the forensic sciences. Collaborative Knowledge Building (CKB) becomes ideal in many learning situations within a forensic science educational program. Integrating CKB into the curriculum provides the student/trainee with a realistic environment of multidisciplinary teamwork far from the individualism taught in the lower levels of formal education. Active learning takes place when using CKB while engaging in the dynamism of social networking. The involvement of the student/trainee in such an activity can begin while learning the same principles that are under fire. Stimulating the forensic science student/trainee to learn and defend these principles with sound argumentation against the scrutiny of peers seems not only a good idea but a necessity.

Just like with other approaches to active learning, the essence of the constructive alignment theory, the instructor/trainer doesn’t have the central role in knowledge transfer, but takes diverse roles as organizer, planner, coach, and facilitator of the learning process.<sup>3</sup> In addition, the instructor/trainer can turn to different CKB technologies such as the use of wikis, discussion boards, and weblogs to integrate in the course design. These tools offer other advantages for both teaching/training-learning situations and developing new knowledge, among others the ubiquity, asynchronicity, and the possibility for anonymity while using the tool. In addition, under quality control, they can become reference material for professionals in the field.

Criminalistics is a course given within the first year of a two-year master’s program in forensic science at the University of Amsterdam. The students come from different bachelor’s programs in the area of biology, chemistry, physics, mathematics, and computer science. To unify the group is almost an impossible mission. Fortunately, for the basic principles in forensic science, only the general scientific critical thinking level is needed; the content knowledge can be built with time. However, doing this with such a variety of students becomes a difficult task when it is desired that the student’s motivation remains high during the activities. Wikis are said to be useful in expanding community involvement and interest, because wikis grow and evolve as a direct result of people adding material to the site.<sup>4</sup> For that reason, a wiki was chosen to be part of the criminalistics course. The content of the wiki was infrared spectroscopy and its applications to forensic science for identification and comparison of different samples (adhesive tapes, explosives, fibers, documents, drugs, and car paint). Each team had to work on one sample type and review a second type. This activity was designed to align the course to the learning outcome, “Indicate the steps necessary to select a type of analysis method to address a specific research question.” The assessment of the activity was divided into two parts, the wiki itself (CKB) and a quiz (content). The students found the activity useful in learning about the subject and considered the team work beneficial. On the other hand, there was correlation between the results from the quiz and the results from the part of the final exam that assessed the above mentioned learning objective.

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## Constructivism, Education, Training

### A125 Detection of Designer Cathinones in “Bath Salts” Using Ion Mobility Spectrometry

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After attending this presentation, attendees will gain insight into the utility of ion mobility spectrometry for the detection of novel designer stimulants that have gained popularity as legal highs.

This presentation will impact the forensic science community by expanding the current detection menu of ion mobility spectrometers to include synthetic cathinones thereby improving the field detection of these stimulants.

Recent news and law enforcement reports indicate that there is increasing concern regarding the abuse of designer stimulants being marketed as components of several legal products.<sup>1,2</sup> Of these products, the so called “bath salts” have taken prominence and have been readily available over the internet through European websites and in various local gas stations, smoke shops, and tattoo parlors. The active stimulants found in these products are several derivatives of cathinone which is a central nervous system stimulant. These chemicals have been reported to provide euphoric effects that are similar to drugs such as cocaine and the phenethylamine class of drugs which include methamphetamine and methylenedioxymethamphetamine (MDMA). The increased prevalence of these “legal highs” has led some states to ban the sale of these substances though the chemicals are currently not regulated by the controlled substances act.<sup>1</sup> There is very little research on the detection and analysis of these chemicals mostly due to their recent rise in popularity. The Drug Enforcement Administration (DEA) reported the characterization of Methylenedioxypyrovalerone (MDPV) using various spectrometric and mass spectrometric methods.<sup>3</sup> There is a need for a rapid and robust analytical method that can serve as a screening method for these chemicals. Ion mobility spectrometers (IMS) are widely used in airports and other high security areas to detect trace levels of illicit substances such as explosives and drugs of abuse. It will be demonstrated that several synthetic cathinones such as mephedrone, MDPV, and naphyrone can be detected with an ion mobility spectrometer. All five target compounds included in this study produced characteristic peaks in the positive ion mode and the reduced mobility for each peak is used as the qualitative measure of the ion peak produced. A majority of the compounds produced single reproducible ion peaks while the fragment and dimer peaks found were concentration dependent. Assigning accurate masses to the peaks can be achieved by coupling a mass spectrometer to an ion mobility spectrometer and this is being investigated further. The discussion includes data on the limits of detection and limits of quantitation of these compounds. The overall limit of detection for all five target compounds was below five nanograms. Current detection menus of IMS instruments include detection parameters for amphetamine and its derivatives. The data presented will demonstrate the differentiation of the cathinones from these compounds. A discussion

on the resolution of these compounds in a bench-top IMS instrument and future studies to improve and expand the current stimulant detection menu will also be presented. Currently studies are being conducted to test several bath salts and possible interfering substances to validate the IMS method for the different compounds of interest.

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#### Designer Stimulants, Ion Mobility Spectrometry, Bath Salts

## A126 The Effects of Containment System Selection for the Storage of Surrogate Continuation Aids

*Katlynn Beltz, BS\*, Florida International University, 11200 SW 8th Street, Room CP344, Miami, FL 33199; and Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand the steps taken to determine the optimal containment system for storing various detection canine surrogate continuation aids. The determination of the proper containment system is a critical step in determining both the effects of containment on the odor availability of the surrogate continuation aid and the ease at which the aids become contaminated. Headspace analyses using solid-phase microextraction (SPME) coupled with gas chromatography with either electron capture or mass spectrometry detection was used to identify and quantify the odorant emanating from the secondary containment system over a series of weeks. Headspace analyses of the surrogate continuation aids were also performed to determine if cross-contamination of the surrogate continuation aids held within the containment vessel was also occurring.

This presentation will impact the forensic science community by reducing the current gap in knowledge in the field, allowing for reliable system of training aid containment to be implemented in the field. Maintenance of the integrity of the surrogate continuation aid odors is imperative to ensure standardization of training, increasing the reliability of the canine to detect the various illicit odors.

The goal of this study is to determine the optimal containment system for storing various detection canine surrogate continuation aids. Selection of the proper storage system is necessary for the maintenance of potency, efficacy, and functional integrity of canine surrogate continuation aids as cross-contamination of the surrogate continuation aids are always of great concern. Currently canine handlers and trainers use a variety of containment systems (glass, plastic, cloth, etc.) for surrogate continuation aid storage; however, an in-depth and systematic study is required to determine the optimal containment system, taking into consideration different factors that potentially play important roles in the potency and efficacy of the surrogate continuation aids.

In order to establish a consistent and optimal practice among canine handlers, law enforcement agencies and other allied parties formed the Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG) which has produced a series of best practice guidelines covering different aspects of canine and orthogonal detectors. While SWGDOG has identified several areas of continued research, the effects of containment system on odor availability and the development of methods to monitor the levels of contamination of surrogate continuation aids have

been identified as critical research topics. Both of these critical research topics have been addressed in this research study.

The determination of the proper containment system is a critical step in determining both the effects of containment on the odor availability of surrogate continuation aids and the ease at which the aids become contaminated. Three levels of containment have been identified for the proper and adequate storage of a canine surrogate continuation aids. The primary level of containment should deliver a known and controllable amount of odor to the atmosphere, for example through selective permeation. The secondary level of containment encloses the surrogate continuation aid in primary containment. Requirements for secondary containment include: airtightness, sufficient size to hold the surrogate continuation aids, portability, and have no adverse effect on the surrogate continuation aid. The final, tertiary level of containment must be airtight, sufficiently large to hold the surrogate continuation aids in secondary containment, and portable.

Airtightness tests were performed on the secondary containment systems to initially screen viable options since permeation out of the secondary containment system will be reduced if the system is found to be airtight. Once potential secondary containment systems were selected, a cross-contamination study was performed to determine which secondary containment system demonstrates the least permeation of the volatile odorants out of the containment system. Volatile odorants selected for testing included the surrogate continuation aids found in the International Forensic Research Institute (IFRI) Prototype Surrogate Explosives Kit as these results are needed to advance the technology of Controlled Odor Permeation Systems (COMPS) and a standardized training kit. Headspace analyses using solid-phase microextraction (SPME) coupled with gas chromatography with either electron capture or mass spectrometry detection was used to identify and quantify the odorant emanating from the secondary containment system over a series of weeks. Headspace analyses of the surrogate continuation aids were also performed to determine if cross-contamination of the surrogate continuation aids held within the containment vessel was also occurring. Permeation rate comparisons, through gravimetric analyses, were made between various containment media to determine if the containment vessel affects the effective life-span of the aid.

From the small scale studies completed, the canning jars demonstrate the best performance for being the optimal secondary containment system; however, these jars have metal lids that potentially form rust over time and may add unwanted odor to the surrogate continuation aids. To abate this concern, we have expanded the selection of containment systems beyond ones currently used in the field for testing.

Maintenance of the integrity of the surrogate continuation aid odors is imperative to ensure standardization of training, increasing the reliability of the canine to detect the various illicit odors. This study reduces the current gap in knowledge in the field, allowing for reliable system of surrogate continuation aid containment to be implemented in the field.

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#### Detection Canines, Storage, Explosive Surrogate Continuation Aids

## A127 Comparison of Aggregating Agents for Surface-Enhanced Raman Analysis of Benzodiazepines

*Erika L. Doctor, MS\*, Florida International University, CP 175, 11200 SW 8th Street, Miami, FL 33199; and Bruce R. McCord, PhD, Florida International University, Department of Chemistry, University Park, Miami, FL 33199*

The goal of this presentation is to show attendees the applicability of surface enhanced Raman spectroscopy to the analysis and detection of trace

quantities of benzodiazepines. The optimization of various parameters of this technique as well as the limits of this method will also be discussed.

This presentation will impact the forensic science community by showing a technique that has better selectivity and sensitivity than current immunoassay screening techniques for benzodiazepines.

Benzodiazepines are commonly prescribed medications for anti-anxiety and anti-depression. The effects these compounds have on the central nervous system such as drowsiness, amnesia, confusion, and impaired coordination have made these drugs prominent in the commission of drug-facilitated sexual assaults. There has been a significant increase in the prevalence of these types of drugs in case submissions. The target detection limit for these compounds in biological samples is 50ng/mL, which is well below therapeutic concentrations.

Surface-Enhanced Raman spectroscopy (SERS) has previously been shown to detect trace quantities of compounds, such as nicotine, in aqueous solutions. This technique has the advantage of overcoming the low sensitivity and quenching the unwanted fluorescence effects seen with conventional Raman spectroscopy. SERS spectra are obtained by applying a compound of interest onto a SERS-active metal substrate such as colloidal metal particles or metal films. In this case, the colloidal particles are spherical gold nanoparticles in aqueous solution.

To further increase the enhancement of the SERS signal, aggregate solutions are used. These agents are salt solutions which cause the nanoparticles to amass and form hot-spots which increase the signal intensity. Chlorine salts generally provide the greatest enhancement for two reasons. The chlorine ions displace the stabilizing agent to cause aggregation and they affect the ionic strength of the surrounding solution changing the surface charge of the substrate, therefore increasing the signal intensity. While a single aggregating salt will affect a substrate the same, it has different effect on the signal of various analytes. Aggregating agents must be assessed for each individual drug to determine the optimum aggregating agent for a range of benzodiazepines.

Aqueous colloidal dispersions of gold spherical nanoparticles were prepared using a modified Lee Meisel 1% sodium citrate reduction method. Particle size and shape were confirmed with an average size of approximately 30 nm. Diluted benzodiazepine and metabolite samples were prepared in 10% methanol. Four aggregating agents were compared for enhancement of spectral characteristics.  $MgCl_2$ ,  $CaCl_2$ ,  $NaCl$ , and  $KCl$  were chosen and prepared at a concentration of 1.67 M. Aggregate solutions were added to colloidal dispersions followed by the addition of a range of benzodiazepine concentrations (1ng/mL – 1000ng/mL) and SERS spectra were obtained.

It was found that each aggregate had different enhancement effects on each individual drug. Overall  $MgCl_2$  provided the lowest limit of detection, 2.5ng/mL, and linearity over a range of concentrations for a variety of drugs chosen. It was also observed that generally the higher the chlorine ion concentration, the higher the SERS intensity observed. Lastly, the cations of the aggregating solutions had an effect on the SERS signal. The smaller the cation the higher the intensity produced.

This method has shown the applicability of SERS for the detection of trace quantities of benzodiazepines in aqueous solutions as well as the optimization of the technique over a wide range of compounds. This technique can be adapted for use in the detection of trace benzodiazepines in toxicological samples such as urine. SERS is more specific than currently used immunoassays giving spectral information about the compound present. Also, this technique has more sensitivity than immunoassays and in the case of benzodiazepines such as lorazepam that have poor cross-reactivity, the drug can be detected.

**Benzodiazepine, Surface-Enhanced Raman Spectroscopy, Drug Chemistry**

## A128 Preparation of Molecularly Imprinted Monolithic Polymers as the Stationary Phase for Liquid Chromatography

Yuanwei Gao, BS\*, and Seongshin Gwak, BS, Sam Houston State University, 555 Bowers Boulevard, #1528B, Huntsville, TX 77340; and Jorn C.C. Yu, PhD, Sam Houston State University, Box 2525, College of Criminal Justice, Sam Houston State University, Huntsville, TX 77341

After attending this presentation, attendees will learn the theory of MIP, preparation of MIP, and the practical use of MIP in analytical instrumentation such as high performance liquid chromatography (HPLC).

This presentation will impact the forensic science community by showing how molecularly imprinted monolithic polymers (MIMPs) were prepared in three different types of materials including polyetherether ketone tubing, polymer sheathed fused silica tubing, and empty stainless steel columns by *in situ* polymerization with thermal initiation. Attendees will be able to learn how different materials impact the practical use of MIMPs in an analytical column.

The preparation of MIPs usually consists of three steps. First, the functional monomers and template (target) molecules are mixed and interacted by either non-covalent or covalent bonding. Then, the functional monomer aggregation follows in order to allow the formation of the alignment in the presence of cross-linkers. In other words, imprinting of template molecules in a polymer is achieved by the polymerization of the functional monomers and cross-linkers in the presence of template molecules. Finally, the imprinted templates are removed from the polymer to produce binding sites, which are specific to the template molecules. These binding sites on MIPs have a permanent memory of the template molecules in terms of complementary size, geometry, and orientation of functional groups. These specific binding sites can selectively recognize the target molecules, even in a complex sample solution. It has been demonstrated that MIPs have molecular recognition property as a biomimetic recognition layer in enzyme-linked immunosorbent assay (ELISA), chemical sensor systems, selective molecularly imprinted solid phase extraction (MISPE), and in HPLC with a chiral stationary phase.

Molecularly imprinted monolithic polymers (MIMPs) were first studied in the early 1990s. This type of MIPs can be prepared directly in a column or a capillary. Therefore, the process is relatively simple and it can reduce the amount of template molecules consumed during the preparation of MIPs. In this work, MIMPs using (-)-pseudoephedrine as a template were prepared inside of capillaries or columns by *in situ* polymerization. The idea was to prepare a MIP stationary phase for chiral separation of methamphetamines. In order to identify an optimal polymerization condition for the preparation of MIMPs, the back pressure of each polymer was monitored by a liquid chromatography (LC) pump under an isocratic flow condition. The separation of (-)-pseudoephedrine from its stereoisomers including (+)-ephedrine, (-)-ephedrine, and (+)-pseudoephedrine were tested in different mobile phases and flow rate conditions using LC equipped with a tandem mass spectrometer (MS/MS). The final polymerization condition for the preparation of MIMPs was determined using a polymerization mixture with (-)-pseudoephedrine as the template, methacrylic acid as the functional monomer, and ethylene glycol dimethacrylate as the cross-linker. Concentration ratio of template, functional monomer, and cross-linker was 1:3:27. The porogenic solvent mixtures which include toluene and 1-dodecanol, and cyclohexanol and 1-dodecanol were used with the ratio of 1:9.5 and 1:9.3, respectively. The back pressures of MIMP columns were monitored with flow rates from 0.01 to 0.15 mL/min isocratically. The MIMP column prepared with toluene as porogen showed relatively lower backpressure than the one with cyclohexanol. Although the separation of (-)-pseudoephedrine from its stereoisomers has not yet been achieved, the next step is to maximize the selectivity of MIMP by optimizing the polarities of the mobile phases. Once the optimal condition is determined, MIMPs may be prepared in narrower inner diameter tubings (such as nano-LC columns) to maximize

the advantages of using MIMPs for chiral separation in advanced LC systems.

**Forensic Science, Molecularly Imprinted Polymer, Chiral Separation**

## **A129 ASTM Classification of Ignitable Liquids and Residues by Chemometric Techniques**

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The goal of this presentation is to establish a methodology with known error rates for the identification and classification of ignitable liquids in fire debris samples.

This presentation will impact the forensic science community by introducing an objective method to be applied to ignitable liquid and pyrolysis product classification in fire debris analysis. The methods investigated are intended to provide statistical support for current laboratory practices.

Models were developed to assign ignitable liquids to the ASTM classes based on the total ion spectrum (TIS) for each sample from a library of ignitable liquid gas chromatography-mass spectrometry (GC-MS) data and a library of pyrolysis product GC-MS data. The first step of model development was to reduce the dimensionality of the data through the use of principal components analysis (PCA) to construct a set of latent variables. This was followed by linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA) based on the PCA scores. Cross-validation was performed by randomly selecting 20% of the samples from each class for a test set. The remaining 80% of the samples were used to develop a model for classification by LDA and QDA. Classifications for the test set were generated from the model and evaluated by comparison to the Technical Working Group for Fire and Explosions (TWGFEX) Ignitable Liquid Reference Collection (ILRC). The cross-validation steps were repeated 100 times with a new test set being selected and classified each time. Total correct classification percentages were calculated as the sum of the cross-validation tests. All models developed on ignitable liquid and substrate library data were applied to fire debris samples.

The LDA and QDA models were developed to classify samples as: (1) ignitable liquid or substrate; and, (2) by ignitable liquid ASTM class or substrate. Models in both categories included: (a) all ASTM E1618 classes; (b) all ASTM classes other than miscellaneous and oxygenates; and, (c) all ASTM classes but combined the classes of isoparaffinics and normal alkanes as well as petroleum distillates and naphthenic paraffinics. Although the samples within the training and testing sets performed well, fire debris samples were found to have a significantly lower correct classification rate. This was attributed to the presence of pyrolysis products that led to incorrect classifications.

The influence of pyrolysis products on correct classification rates presented a major challenge in this research and has led to consideration of additional methodologies. Other methods under investigation to increase the correct classification rate of these samples include partial least squares discriminant analysis (PLS-DA), soft independent modeling of class analogy (SIMCA), and method of normalized coordinates.

This work was supported by the National Institute of Justice, Office of Justice Programs, award 2009-DN-BX-K227. The content of this publication does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred.

**Chemometric, Fire Debris, Gas Chromatography-Mass Spectrometry**

## **A130 The Use of Canine Field Testing to Optimize the Operating Parameters of a Non-Contact Collection Device for Human Scent**

*Jessica S. Brown, BS\*, Florida International University, 11200 SW 8th Street, CP 344, Miami, FL 33199; and Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand the significance of utilizing the optimal operating settings of dynamic airflow devices used for the non-contact collection of human scent, and the impact they have on canine response when discriminating individuals.

This presentation will impact the forensic science community by highlighting the importance of collecting human scent from crime scenes or articles of evidence utilizing the optimal collection time and airflow speed of the human scent collection system to provide sufficient scent for canine discriminating purposes. In addition, this will encourage the standardization of human scent collection protocols for law enforcement officials.

The collection of human scent from crime scenes can aid investigators in determining whether a suspect/victim was present at a particular location. The tool often used to correlate a person scent to a specific location is a human scent discriminating canine which is trained to detect the scent of an individual excluding all other scents. The collection of human scent can be performed by several different methods: swiping, passive collection, and non-contact collection. Swiping of an object allows for the rapid collection of human scent onto a sorbent material; however, this method of collection introduces the potential of destroying important evidence, such as fingerprints. Passive collection of human scent is a lengthier process which involves placing a sorbent material near an object for a prescribed period of time resulting in the transfer of human scent from the object to the material. Lastly, non-contact collection utilizes a dynamic airflow to draw human scent away from an object and onto a sorbent material. The non-contact collection of human scent is a quick process leaving any potential evidence intact for further forensic analysis.

The Human Scent Collection System (HSCS) is a device which was created for the non-contact collection of human scent from objects and/or locations. Once collected onto a sorbent material, human scent can be presented to a human scent discriminating canine for matching purposes (e.g., scent identification line-up or tracking/trailing individuals). The HSCS is a lightweight, cylindrical device that provides a dynamic airflow, drawing human scent onto a 4"x4" cotton gauze pad. Features such as a digital screen, battery operation, pre-set collection times, and airflow speeds make the HSCS easy to use. The HSCS offers two collection time settings of 30 seconds (default) and 60 seconds, as well as three airflow settings of low, medium (default), and high. These programmed settings aid with standardizing scent collection between individuals or agencies and can be easily reproduced.

Field trials were conducted to determine the optimal operating parameters of the Human Scent Collection System. The responses from human scent discriminating canines were used to gauge which combination of time settings and airflow speeds would allow for sufficient human scent collection for tracking/trailing purposes. A total of 11 canine teams were used for testing. To summarize the design of the field trials, a target walked a path leaving a scent trail at a test site. Subsequently, scent from the hands and saliva of the target was collected (onto a cotton gauze pad) utilizing the HSCS. Once all targets produced scent trails and their scent samples were collected, canine teams were taken to the start of the scent trail and a corresponding scent sample would be presented to the canine for matching purposes. Canine response to the scent sample was evaluated based on three responses: (1) did the canine begin to trail; (2) the canine's first decision; and, (3) the canine's second decision. Canine response to human scent, collected using the HSCS, indicated that the optimal operating parameters were 60 seconds collection time and a high airflow speed.

**Human scent, Canines, Scent Collection Device**

## **A131 Avian Olfaction in Forensic Context: A Preliminary Analysis of Naturally Occurring Volatile Organic Compounds Associated With Feathers From Procellariiforms**

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After attending this presentation, attendees will have a better understanding of avian olfaction as another form of biological detection within a forensic context.

This presentation will impact the forensic science community by providing a foundation of work to understand avian olfaction.

Biological detection, particularly using canines, is in high demand because of its proven detection capabilities for a variety of forensic specimens including drugs and explosives. This work lays the foundation for a potential alternative means of biological detection through avian olfaction. Little is known about the behavioral and physiological sensitivity of birds to a wide range of odorants, and specifically, whether olfactory behaviors can be tuned and directed for applied uses as is the case with canines. A preliminary foundation for this work entails an instrumental evaluation of naturally occurring volatile organic compounds (VOCs) of feather samples to identify naturally occurring chemicals to further the understanding of the types of compounds that are present in their environment prior to explore their potential use in the detection of forensic odorants.

The instrumental evaluation of these biological samples collected from the field is conducted using solid-phase microextraction in conjunction with gas chromatography/mass spectrometry (SPME-GC/MS) utilizing Gould's petrel feather samples. These birds belong to the species of seabird in the Procellariidae family. They have the largest olfactory bulbs among all birds and thus represent an extreme class with a potentially high olfactory capability. They rely on olfactory cues both for foraging and navigation, and are thought to use individual-specific olfactory cues for rapidly and accurately relocating their home burrow when returning to the colony at night during the breeding season. The importance of olfaction in birds remains a matter of controversy. However, experimental behavioural studies regarding the use of olfaction in birds have found evidence of olfactory capability in birds of a variety of species of procellariiformes that use the olfactory sense in several different functional contexts including orientation, reproduction, and even for some social aspects such as individual recognition and mate choice. Virtually nothing is known about the behavioral and physiological sensitivity of petrels to either synthetic or naturally occurring odorants, or what types or combinations of volatile organic compounds they naturally encounter. As a first step, the authors are conducting a preliminary analysis of volatile organic compounds found in 84 feather samples collected from Australia. The identification of over 100 compounds has been achieved using heated headspace extraction methodologies with sensitive instrumental detection. A comparison of the levels of the most common occurring volatile compounds (such as pristane) is being studied to understand olfaction in natural processes such as kin recognition. Thus far, significant levels of common forensic odorants such as those previously identified for drugs and explosives have not been observed. The importance of understanding avian olfaction in a natural habitat is a crucial first step in the exploration which may lead to potential future use of these animals as another form of biological detection for forensic purposes like national security that has been affected due to the increased threats of violence. Often times these threats are carefully hidden from human detection. To address this need, this investigation is a laboratory effort to develop science-based solutions that can be effectively

deployed and used in a variety of real-world settings such as the detection of explosives.

**Olfaction, Forensic Context, Feathers**

## **A132 Micro-Absorption Spectroscopy as a Non-Destructive Tool for Forensic Analysis**

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The goal of this presentation is to illustrate a new method for forensic analysis on the micron scale that combines absorption spectroscopy and confocal microscopy. Results on the characterization of representative samples, including sensitivity and linear dynamic range of micro-absorption measurements will be presented.

This presentation will impact the forensic science community by illustrating a method that uses spatially resolved absorption spectroscopy on a micron scale. It may improve trace analysis of samples that are obtained in micron size with minimal sample preparation.

The ability to investigate samples at a micron level with non destructive probes is a key factor in forensic studies. Fluorescence probes employing confocal or other geometries are available; however they generally require labeling and are limited by photo-bleaching and quenching. On the other hand, micro-spectroscopy based on absorption measurements provides a convenient label free way for characterizing an unknown material. A new experimental approach for micro-absorption spectroscopy is developed and applied to the investigation of fluid and solid samples relevant to forensics. By exploiting the spatial variation of the intensity due to Beer-Lambert's law ground state absorption spectra with a spatial resolution better than 1.4 micron in the lateral and 3.6 micron in the axial direction is measured. A confocal detection system is employed to probe and spectrally resolve the attenuation of a white light beam in the axial direction. It enables the measurement of absorption spectra of biological assemblies at the single cell level and small samples with a thickness of few microns. Confocal absorption microscopy is nondestructive and is capable of collecting both spatial and physical information based on light absorption by microscopic structures.

The quantities of samples often obtained in crime investigations are minute. Micro-absorption spectroscopy enables measurements on smaller sample volumes and with rapid acquisition time on the timescale of seconds. To examine the sensitivity of the technique, absorption spectra of nanoliter solutions in micro-capillaries with a pathlength of 50 microns are investigated. Through measurements of the transmitted intensity in calcein dye solutions at fixed pathlengths, it is established that the absorbance varies linearly with concentration over the range from 0.1 to 2 mM. Preliminary results indicate detection limits of better than 0.1mM in a sample volume of less than a picoliter.

The technology has been used to analyze micro-fibers and can be employed to distinguish the fibers from the known and suspected material at the molecular level. Another important aspect that is addressed is to acquire a spectrum of an individual hair, which is difficult using a Raman spectroscopic probe as it may lead to damage of the sample. As an application to the analysis of biological samples at the single cell level, the visible absorption spectrum of hemoglobin in a single live red blood cell (diameter ~ 7 microns) is measured under physiological conditions. Spectroscopic changes due to heme degradation under pathological conditions are investigated. Variations in the composition of inhomogeneous samples (e.g., thin films) can be determined from spatially resolved absorption spectra. Extensions of the micro-spectroscopic method to the ultraviolet and infrared regions of the spectrum will be discussed.

**Micro-Absorption Spectroscopy, Nano-Liter Samples, Non-Destructive**

## A133 Vacuum-Based GSR Recovery

Jessica A. Motl, BS, 4312 Homestead Circle, San Angelo, TX 76905; Stephen S. Houck, BS, William M. Davis, PhD\*, and Ashraf Mozayani, PhD, PharmD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054

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After attending this presentation, attendees will learn about newly available apparatus that enhance the collection of GSR from fabric using standard vacuums. Specific attention will be made to the quality control aspects of this evidence collection technique.

This presentation will impact the forensic science community by showing how Vacuum-Based GSR Recovery technique is known to be well-suited in the recovery of GSR from fabric.

Gunshot residue (GSR) is formed from primer detonation. The aerosol produced in the conflagration produces particles, upon condensation, composed of lead, barium, and antimony and the various combinations of those elemental oxides. These particles can deposit on the hands, face, and clothing of the shooter and/or any bystander in close proximity to the event. Current methods of testing surfaces for the presence of GSR include using a stub with double sided carbon tape that is then analyzed using scanning electron microscopy coupled with energy dispersive X-Ray analysis (SEM/EDX).

Testing clothing for GSR presents several unique challenges. These include the GSR particles becoming lodged within the weave of the fabric and the inability of current GSR testing methods to analyze the entire article of clothing for GSR in a non-random fashion. Current methods are inadequate due to the clothing providing such a large testing area and stubs losing stickiness. Vacuuming fabric presents an intuitively obvious solution to collecting residues of any kind. Custom-built devices have been reported in the literature but owing to limited capabilities in most forensic laboratories these may not be an adequate solution. A unit comprised of a nozzle, filter canister and vacuum adapter was recently purchased. The configuration of the device allows for easy cleaning and filter pore-size variability.

Preliminary particle recovery was performed using barium silicon oxide ( $\text{BaO}\cdot\text{SiO}_2$ ).  $\text{BaO}\cdot\text{SiO}_2$  is a suitable model for GSR in that it can be applied to a substrate via an atomized spray. Particles are typically less than 10 microns. Additionally, large quantities of particles are deposited using a suspension  $\text{BaO}\cdot\text{SiO}_2$  in water. To ensure that no carryover was present, before testing any clothing with the attachment, the entire set up, complete with a new filter, was tested by pulling room air through the assembly for five minutes. The filter was then analyzed on the SEM. The filter had to produce negative results before being the assembly could be used for testing and analysis. Rinsing the assemblies with water proved to be insufficient in removing whereas sonication followed by rinsing eliminated carry-over as measured in these experiments.

Testing clothing exposed to GSR was then done. A lab coat and a long sleeve shirt were worn independently by an individual as shots were fired from a handgun. The first test, on the right sleeve of the lab coat from the elbow down to the cuff, illustrated that the vacuum filter did pick up GSR, the stub was able to pick up GSR from the filter, and the SEM was able to analyze the GSR from the stub. This initial test timed-out when the SEM reached 50 particles (a particle density of 0.83 particles/mm).

The left sleeve of the lab coat, from the elbow down to the cuff, was vacuumed using the attachment, and the same area was subsequently stubbed. These two stubs were analyzed on the SEM and the results illustrated that the filter did condense the GSR found on the sleeve. The vacuum filter and subsequent stub for the left sleeve of the lab coat displayed 50 particles (0.80 particles/mm and 22 particles (0.23 particles/mm) respectively.

The right sleeve was tested in the same manner described for the lab coat (from the elbow to the cuff), with the results showing eight particles for the vacuum filter and one particle for the stub. The inside of the front panels of the lab coat were vacuumed and the outside of the panels were

subsequently stubbed. The vacuum filter timed out at 50 particles (2.59 particles/mm<sup>2</sup>) and the stub displayed 45 particles (0.48 particles/mm<sup>2</sup>).

It should be noted that the same assembly was used for all of the above measurements. Each cleansing was successful at removing any GSR that may have adhered to the plastic portions of the unit. A new filter was placed in-line for each recovery.

In summary, testing of the vacuum filter attachment produced positive results, illustrating that the filter picked up and condensed GSR particles for a less random, more uniform testing method. Although the filters were disposable, the apparatus and cartridges were re-used, increasing the risk of carryover contamination. Cleansing by sonication followed by rinsing and a quality control system effectively illustrated the vacuum attachment can be used and re-used without contamination.

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**GSR, Vacuum, Fabric**

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## A134 Electrophoretic Separation of Drugs of Abuse Using Laser-Induced Fluorescence Detection

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After attending this presentation, attendees will have a better understanding of how amphetamine-related drugs of abuse can be derivatized, separated and detected using the fluorescent tag 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein (5-DTAF) and capillary electrophoresis system with laser-induced fluorescence detection. Also, the effects of various organic modifiers and surfactants on elution and resolution will be discussed.

This presentation will impact the forensic science community by providing an excellent screening method for trace amounts of phenethylamines and other related compounds which are efficacious at low doses due to their readily being absorbed, distributed and metabolized within the human body.

In capillary electrophoresis, a voltage is applied at the distal ends of capillaries filled with an electrolytic solution. These results in compounds being separated based on their mass-to-charge ratios. This method uses sample volumes in the nanoliter range and is capable of detection in the ng/mL range. For this study five commonly encountered drugs and precursors (used in illicit preparations) were investigated: amphetamine; methamphetamine; norephedrine; ephedrine; and, methylenedioxymphetamine (MDMA). These compounds represent both primary and secondary amine moieties as well as three variations to the phenethylamine parent structure. Laser-induced fluorescence is a commonly utilized detection method in electrophoretic separations. This is due to its high sensitivity and specificity despite the short optical path length necessary when using capillary columns. Because compounds which natively fluoresce are rare, in order to utilize this detection method, the analytes of interest must first undergo derivatization. Fluorescence derivatization is the process whereby non-fluorescent analytes are coupled to an additional compound in order to produce an overall fluorescent molecule.

Due to their structure phenethylamine related compounds all have a pKa approximately within the range of 9.0 to 10.0 and migrate at a fairly similar rate. To compensate for this, modifiers in the form of surfactants and/or cyclodextrins were added to the run buffer to provide a pseudo-stationary phase. Organic solvents are also added to affect the equilibrium between the sample and this pseudo-phase. As a result, the individual drugs separate into distinct zones which are then excited by the laser as they pass the detection window on the way to the cathode. The signal from this excitation and subsequent emission is then collected by the detector and converted into an electropherogram for interpretation.

Drug standards were obtained from the International Forensic Research Institute at Florida International University and dissolved in analytical-reagent grade methanol for storage at 4°C. Prior to analysis,

samples were diluted to appropriate concentrations using deionized water. For this method a micellar running buffer comprised of 50mM borate, pH 9.5/30mM Brij-35 was used for the separation of the analytes. A background electrolyte of 50mM borate, pH 9.5, and a derivatization buffer of 0.5M NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>, pH 9.5 were also utilized. Experiments were conducted using a Beckman P/ACE MDQ unit interfaced with a computer utilizing Karat 32 software (version 7.0). The fused-silica capillary was 60.5cm (effective length 50cm) with an internal diameter of 50µm. An argon ion laser was used as an excitation source (488nm) and electropherograms were recorded by monitoring the emission intensity at 520nm. New capillaries were conditioned by thorough rinsing with 0.1M sodium hydroxide, deionized water, and micellar running buffer in series.

**Amphetamines, Capillary Electrophoresis, 5-DTAF**

## **A135 Fast Gas Chromatography Applications in Ignitable Liquids and Drug Identification**

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The goal of this presentation is to highlight applications of fast gas chromatography (Fast GC) in ignitable liquids as well as drug identification while also assessing the use of hydrogen mobile phase.

This presentation will impact the forensic science community by demonstrating the value of Fast GC and hydrogen mobile phases to forensic analysts in units using conventional gas chromatography by demonstrating statistically significant improvements in retention time, resolution, and laboratory supply costs with implementation.

The objective of this project is designed to assist crime laboratories' assessment of new separation techniques and gauge the feasibility of implementation. The first objective of this project is an assessment of the expected gain in resolution and sample throughput in ignitable liquid and drug identification analysis using a combination of Fast GC and hydrogen carrier gases. This area of application is prime ground for realizing the full potential of Fast GC – H<sub>2</sub>. In 2008, a joint project funded by the Midwest Forensics Resource Center between the University of Wisconsin – Platteville (UWP) and the Wisconsin State Crime Laboratory – Madison demonstrated incredible reductions of over 50% in retention times of ignitable liquids from arson debris using the Fast GC. This study also found that the use of hydrogen as a carrier gas more than compensated for resolution losses related to Fast GC. In fact, the more compressible hydrogen carrier produced improvements in resolution for the Fast GC analysis compared to a conventional GC technique using helium carrier gas ( $p < 0.01$ ). Fast GC-H<sub>2</sub> separation of drug identification samples could potentially decrease retention times of straightforward matrices to several tens to a few hundred seconds. In addition to presenting the 2008 results to the AAFS, this presentation addresses the limiting factors of this technique including the scanning rate of the quadrupole mass spectrometer and effects on detection limits of illicit drugs.

Experimental design involves the Fast GC and conventional analysis of one dozen Scheduled compounds including cocaine, tetrahydrocannabinol (THC), heroin, 3,4-Methylenedioxymethamphetamine (MDMA), trifluoromethylphenylpiperazine (TFMPP), lysergic acid diethylamide (LSD), buprenorphine, synthetic cannabinoids, alprazolam, clonazepam, boldenone, and nandrolone. The figures of merit selected for ANOVA ( $p < 0.05$ ) comparisons center on retention times and chromatographic resolution of a master standard containing these twelve compounds. Given that many laboratories may hesitate to modify existing units for Fast GC, the first assessment examines the benefit in simply switching to the less expensive hydrogen mobile phases from the helium mobile phase. The control in this experiment is the conventional GC operating with a standard DB-5, 30 m

column and helium mobile phase. The hydrogen is employed instead, and a data set is generated with resolutions compared to the helium gas. Subsequently, the heating ramp of the conventional GC is increased without altering other variables to assess just how aggressive of a heating ramp can be used to reproducibly equal the conventional use of helium. The second set of experiments uses solely helium while comparing Fast GC and conventional GC in the event that a laboratory is not ready or able to convert to hydrogen carrier but is interested in the Fast GC gains. In this particular case, the data demonstrate the resolution decrease for cost: benefit analysis. Finally, the two variables (Fast GC and hydrogen mobile phase) are combined to assess the maximum benefit.

**Fast GC, Ignitables, Drugs**

## **A136 SWGDOG and ICODD: Latest Dog and Orthogonal Detector Best Practices and Development of an Accreditation Program**

*Jessie Greb, MBA, Florida International University, 11200 SW 8th Street, CP 330, Miami, FL 33199; and Kenneth G. Furton, PhD\*, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand how the establishment of best practices for detection teams is improving interdiction efforts and courtroom acceptance of dog alert evidence as well as the importance of creating an accreditation commission, the International Commission on Detector Dogs (ICODD).

This presentation will impact the forensic science community by providing a better understanding of how SWGDOG best practices and accreditation through ICODD are improving the consistency and performance of deployed detector dog teams and their optimized combination with emerging electronic detectors.

The Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG) has been developed by a membership of respected scientists, practitioners, and policy makers representing diverse backgrounds. SWGDOG has been cooperatively funded by the NIJ, FBI, DHS, and TWSG with general meetings held biannually since 2005. 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.

The approval of each subcommittee best practice document takes six months to complete, including a two 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; Part G - March '09; Part H - Sept. '09; Part I - March '10; Part J - September '10; Part K - March '11); (2) General guidelines for training, certification, maintenance and documentation (April '06) - Publication in FSC October '06; First Revision (September '08); Second Revision (September '09); (3) Selection of serviceable dogs and replacement systems (October '06) Publication in FSC October '08; (4) Kenneling, keeping, and health care (October '06); (5) Selection and training of handlers and instructors (Part A - October '06; Part B - March '10); (6) Procedures on presenting evidence in court (October '06; First Revision Sept. '10); (7) Research and technology (March '07; First Revision September '10); (8) Substance dogs: Agriculture; Arson; Drugs; Explosives; (August '07); Human remains (September '09); Contraband (March '11); Pest (March '11); Currency; Firearms (September '11); (9) Scent dogs: Non-specific Human Scent Wilderness Area Search; Location Checks; Article Search; Scent identification line-ups; Live People in Disaster Environments; Track Trail people based on Last Known Position; Pre-scented Canines Aged Trail;

Live People in Avalanche (Part A - March '07; Part B - August '07; Part C - March '08; Part D - September '08; Part E - September '09; Part F - March '10; Part G - September '10; Part F - March '11); and, (10) Outreach & Education: PowerPoint, branding materials approvals (September '10)

The current success of SWGDOG is being manifested by a shift of some national canine organizations to adopt the approved SWGDOG best practice guidelines. The mission of the International Commission on Detector Dogs ([www.ICODD.org](http://www.ICODD.org)) is to globally improve the performance of detector dog teams through information sharing and implementation of SWGDOG best practice guidelines through voluntary accreditation of certification bodies. Annual ICODD meetings include open discussions of current, pending, and needed best practice guidelines and provide the mechanism for certifying bodies to apply for accreditation through the Accreditation Council made up of representative commission members.

#### **Detector Dog, Best Practices, SWGDOG**

### **A137 Characterization of Legal Highs**

*Melody P.L. Cai, BSc\*, Tracy P.S. Toh, BSc\*, Ying Ying Tan, BSc, and Angeline T.W. Yap, PhD, Health Sciences Authority, 11 Outram Road, Singapore 169078, SINGAPORE*

The goal of this presentation is to demonstrate the characterization of five legal highs that have either been encountered in Singapore or have already surfaced in other countries.

This presentation will impact the forensic science community by establishing the usage of structure elucidation techniques which will enhance the forensic laboratory's ability and confidence in the identification of legal highs.

New synthetic drugs such as the cathinones (e.g., methylene, butylone MDPV) and cannabinoids (e.g., JWH-018, HU-210, CP 47,497) are making their headways into many countries. These synthetic drugs are often termed as legal highs as they are analogues of existing banned drugs of abuse, bearing similar chemical structures and psychoactive properties as their illegal counterparts. These products are often marketed using labels such as "bath salts," "incense," and "botanical specimens." These seemingly licit product names are used as a ploy to deceive the legal forces from their true recreational use. Consequentially, legal highs have become increasingly popular as legal alternatives to illicit psychoactive substances for the drug abusers. This in turn, brings about a high demand for legal high which is further facilitated by their widespread and easy availability on the internet. Labels on the packaging of legal highs sold over the internet are often unreliable indicators of its actual contents. Consumers may be led to believe that the products are legal which makes internet sales of legal highs highly lucrative.

The accessibility of legal highs for recreational usage and the constant emergence of new legal highs have posed challenges to forensic laboratories around the world in their testing and identification. Due to the novelty of these drugs, reference materials of such legal highs are often not available. Literature reports containing detailed analytical data of such drugs are often limited and, in most cases, unavailable as well. These have made their identification extremely challenging. Hence, there is a need for forensic laboratories to constantly identify alternative techniques and develop capabilities to fully characterise these new legal highs.

The objective of this paper is thus to demonstrate the characterization of five legal highs that have either been encountered in Singapore or have already surfaced in other countries. These five include methiopropamine, methoxetamine, and three synthetic cannabinoids. Methiopropamine is a thiophene-based structural analogue to methamphetamine. It possesses similar chemical structure except for the replacement of the heterocyclic moiety for a phenyl group. Another legal high encountered, methoxetamine, is an analogue of ketamine. Being structurally similar, these legal highs are believed to be able to exhibit similar psychoactive properties as their illicit counterparts. As methiopropamine and

methoxetamine are both very new to the drug market, very little information on these two drugs is available.

Synthetic cannabinoids, or "Spice," are examples of legal highs marketed as herbal products. They are easily available over the internet and their popularity as "legal drugs" have rapidly increased due to their reputation of being potent herbal intoxicants and also as "legal" alternatives to the strictly regulated cannabis.

In this paper, several techniques were used in the characterization of these five drugs. Accurate mass analysis was performed using the orbitrap and time-of-flight mass spectrometry while gas chromatography/mass spectrometry (GC/MS), Fourier transform infrared spectrometry (FTIR), and nuclear magnetic resonance spectroscopy (NMR) were employed in structural elucidation. The analytical data and the interpretation of the results obtained will be presented in this paper. The establishment of the usage of such techniques will enhance the forensic laboratory's ability and confidence in the identification of these legal highs.

#### **Legal High, Characterization, Forensic**

### **A138 Development of a Rapid Screening Method for Differentiation of the Traditional and Emerging Amphetamine Type Stimulants (ATS)**

*Omya Fekry, MSc\*, Michael D. Cole, PhD, and Beverley R. Vaughan, PhD, Anglia Ruskin University, East Road, Cambridge, Cambridgeshire CB1 1PT, UNITED KINGDOM*

The goal of this presentation is to make attendees aware of how presumptive test methods can be when applied to Amphetamine Type Stimulants. It provides a programmed approach to drug screening not previously described.

This presentation will impact the forensic science community by giving them the opportunity, for the first time, for law enforcement officers and forensic scientists to screen for new ATS drugs in both the field and the laboratory. This allows for instant informed decision-making concerning which samples to subject to further analytical testing. Prior to this method, such decisions were not possible.

Over the last decade there has been an enormous increase in the number of 'designer drugs' entering the illicit drug market. Many of these compounds are amphetamine type stimulants (ATS's). According to the recently published annual reports by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), in 2008 some 13 new substances were reported; in 2009, 24 were reported, and, in 2010, 41 new substances were described.<sup>1</sup> Alongside this, there have been well-documented decreases in the purity of these drugs in addition to an increase in the complexity of the drug mixtures. This emphasizes the need for a rapid, selective, and sensitive screening technique to be implemented.

In the United Kingdom there has been a move toward rapid action in law enforcement under the Misuse of Drugs Act, 1971 and its amendments involving these new designer compounds. Similar actions occur in the United States. In response to this, the clandestine chemists rapidly shift synthesis to analogues and derivatives of controlled drugs and continue to search for new alternatives to avoid prosecution.

Uncontrolled analogues and derivatives of well-known drugs of abuse are popular due to their diverse range of biological activities.<sup>2</sup> The use of and demand for these designer compounds is becoming increasingly popular due to their internet sales and regular advertisement as "legal highs." In controlling possession of these compounds, the ability to detect new designer drugs, alongside commonly occurring amphetamines in locations other than analytical laboratories, has still not been thoroughly evaluated. Presumptive tests for controlled substances, for example the Marquis reagent for identification of opiates, amphetamines and ring substituted amphetamines, are occasionally used by forensic scientists and law enforcement officers on site. They are heavily used as screening methods in the laboratory setting.<sup>3</sup> The need for a rapid screening method

for commonly occurring drugs, as well as those newly used, is increasingly urgent as the number of drugs on the market increases exponentially.

The method described here uses a two stage approach in order to discriminate between a variety of ATS and precursor chemicals used in the synthesis of drugs of abuse. The initial stage involves the use of a series of seven presumptive test reagents that provide differentiation between compounds dependent on the functional groups present in the target molecules which include 14 examples of phenylethylamines, cathinone derivatives, ring substituted- and beta keto- amphetamines.

The second stage involves the use of thin-layer chromatography to provide further discrimination between the drugs. Samples were chromatographed on silica gel plates, developing the chromatogram in a chloroform-methanol solvent system (9:1 v:v). After development, separated compounds were visualised under UV light (254nm) and subsequently sprayed with 0.5M NaOH following by 1% Fast Black K Salt in distilled water which was applied directly to the plate. A range of colors were obtained with molecules from different classes.

The limit of detection using the presumptive tests lies in the low microgram range. TLC is more sensitive with visualisation of compounds possible between 0.625 and 10µg on plate. Successful validation of the method was performed in the form of blind trial testing after which the contents of the drug mixtures were confirmed by Gas Chromatography - Mass Spectrometry (GC/MS). This method allows non-specialists to make informed decisions concerning whether further laboratory analysis is required on suspected drug seizures.

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ATS, Presumptive Testing, Drug Screening

### A139 Testing AFIS Search Accuracy at the Limits of Minimal Minutiae

*Gary H. Naisbitt, PhD\*, Aaron Hall\*, and Dale Zinn, Utah Valley Forensic Science Program, Criminal Justice Department, Mailstop 286, 800 West University Parkway, Orem, UT 84058*

After attending this presentation, attendees will appreciate how low numbers of minutiae affect the search accuracy of AFIS software.

This presentation will impact the forensic science community by presenting an example of validating AFIS performance prior to conducting searches to establish that the system is performing within its designed specifications and quality control boundaries and the limits of minimal numbers of minutiae will be investigated by taking latent prints images from the AFIS database, manually reducing the number of minutiae to between fifteen and eight minutiae points, then searching a known set of print images of the same finger. A probability of success can be determined by comparing realized search results against the number of known true candidates.

The goal of this project is to test the probability that a fingerprint can be unambiguously identified from as few as eight minutiae points. An AFIS validation protocol was used at the beginning and end of each experimental session to verify the software was working as designed.

Previously, latent print screening results of commercially available AFIS software was found to be only seventy percent accurate when compared to known theoretical outcomes (Robert E Ryberg, AAFS Proceedings 2010). The problem was traced not to rotational orientation, rather to how the print's image was cropped. Cropping near the edge of the

image, even without eliminating minutia, caused a different set of minutiae to be extracted than when the same image was centered in the field (Aaron Hall, AAFS Proceedings 2011). To overcome this source of error, a standardized AFIS image presentation protocol based on centralizing the print image was developed to maximize latent print database enrollment accuracy. This image presentation protocol tests AFIS performance with a set of standard latent print images with known outcomes to assure the AFIS system was performing to its designed specification and within quality control boundaries. Performing this validation protocol before and after a session searching unknown latent prints enables individual fingerprint examiners to validate the performance of their own AFIS system each time they use it.

This validation protocol was used to assure best possible performance in the following study that measured search accuracy when the unknown latent had low numbers of minutiae.

The AFIS database contained thirty rolled and thirty slapped latent print images of the same finger and the AFIS system was validated according to the protocols referenced above. A duplicate latent print image was taken from AFIS database to be the unknown latent print and the search accuracy of each latent print image was determined to be 100% when compared to its known duplicate in the AFIS database. The AFIS minutiae editing tool was used to reduce the number of minutiae to sixteen and the resulting image was searched to produce a list of matching candidate prints that were judged against the known print as either a True or False result. In subsequent trials, the number of the minutiae were reduced incrementally to a minimum of eight and searched as before.

An example candidate list for a single print in which the database contained thirty different prints of the same finger is (minutiae: true candidates): 15:22, 14:18, 13:16, 12:10, 11:4, 10:2, 9:1, 8:0. Several prints of different patterns will be presented.

#### AFIS Search Accuracy, Minimal Minutiae, Validation

### A140 The GC/MS of Salvinorin A in Blood Plasma as Well as an Evaluation of Standard Color Tests for the Presence of Salvinorin A

*Julia A. Garofalo, BS\*, and Thomas H. Pritchett, MS, Cedar Crest College, 100 College Drive, Allentown, PA 18104*

After attending this presentation, attendees will have the foundation for developing a GC/MS method in their laboratory for the analysis of Salvinorin A and its metabolite Salvinorin B in blood plasma samples. The attendees will also learn which of the standard color tests are not capable of identifying Salvinorin A.

The presentation will impact the forensic science community by adding a new method for detecting a drug, Salvinorin A, which is now becoming regulated in many states. It will also impact the community by alerting practitioners of the color tests which are incapable of correctly identifying Salvinorin A.

In recent years, *Salvia divinorum* has become a major focus by state legislatures throughout the United States looking to prohibit the sale of the psychoactive plant. In some states (Alabama, Delaware, Louisiana, Michigan, Missouri, and Ohio) laws have been passed and many other states (Alaska, California, Florida, Iowa, Maryland, New Jersey, New York, Oregon, Pennsylvania, and Texas) are in the process of creating legislation to prohibit *S. divinorum* sales.<sup>1</sup> With the increasing number of states creating legislation making the sale *S. divinorum* illegal, the need for reliable presumptive and confirmatory testing methods is essential. Potential presumptive color tests were evaluated and an extraction method of Salvinorin A from plasma using selected ion monitoring gas chromatography/mass spectrometry for analysis was developed for this research.

Presumptive color tests were conducted to determine if *S. divinorum* vegetation and Salvinorin A standards would produce a positive colored

result. Dried *Salvia divinorum* leaves, enhanced “extracted” leaves in 20x, 10x, and 5x potencies, liquid tincture Shepardress Essence, and standard solutions on Salvinorin A were all tested with multiple color tests that are used to preliminarily determine common illicit drugs. The *S. divinorum* and Salvinorin A solutions did not react with any of the standard color tests: cobalt thiocyanate (cocaine HCl), Marquis reagent (amphetamines), Mecke reagent (MDMA), *p*-dimethylaminobenzaldehyde (LSD), and most notably the Duquenois-Levine test for marijuana. The *S. divinorum* vegetation and the Salvinorin A solutions were also tested with color tests for Vitamin A, another common diterpene, but again no reactions were produced. Additional reagents, like antimony trichloride, designed to detect the lactone were also evaluated and found to be non-effective. Further color tests are currently being conducted along with presumptive oral tests on saliva and these results will also be presented.

The psychoactive compound found in the plant, Salvinorin A, was extracted from spiked 1mL defibrinated sheep plasma samples using a cyclohexane/ethyl acetate (85/15(v:v)) solution. Salvinorin A was detected from 1000 ng/mL down to 10 ng/mL using gas chromatography/mass spectrometry (GC/MS). 17- $\alpha$ -methyltestosterone was used as an internal standard for the study. Both the Salvinorin A and the 17- $\alpha$ -methyltestosterone were determined in the selected ion monitoring mode. The ions selected were 94, 273, 432 m/z for Salvinorin A and 124, 229, 302 m/z for 17- $\alpha$ -methyltestosterone. The underlined ions were selected for the quantification measurements. The major metabolite of Salvinorin A, Salvinorin B can also be detected in the plasma samples. The total run time was 20.67 minutes and the retention times for Salvinorin A and 17- $\alpha$ -methyltestosterone were 14.995 and 11.958 minutes, respectively. The optimized GC/MS assay was evaluated in terms of limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, analytical recovery, and linearity. The preliminary LOD and LOQ for the assay were determined to be 10 ng/mL, and the other optimization parameters are still being evaluated.

#### Reference:

1. Seibert D. The Legal Status of *Salvia divinorum*. The *Salvia divinorum* Research and Information Center. <http://www.sagewisdom.org/legalstatus.html>

#### Salvia, GC/MS, Drug Analysis

### A141 Characterization of Methylenedioxypropylvalerone (MDPV) and Mephedrone in “Legal High” Products by Chemical Color Tests and Microcrystalline Tests With Confirmation by LC/MS

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After attending this presentation attendees will be able to identify methylenedioxypropylvalerone (MDPV) and 4-methylmethcathinone (mephedrone) in legal high products using common forensic chemical techniques including color tests, microcrystalline tests, and liquid chromatography/mass spectrometry (LC/MS). Attendees will also be able to recognize various commercial “legal high” products purchased over the internet, to name the substances they may contain, and to describe their legal status.

The presentation will impact the forensic science community by increasing awareness of the abuse of these products and the drugs they contain.

Over the last two years, new synthetic drugs with properties similar to cocaine, the amphetamines, or ecstasy have become popular due to the fact

that they are legally obtainable and not controlled, in spite of the fact that they may be dangerous or addictive. These include the phenylethylamine derivatives MDPV and mephedrone. A number of states have controlled these substances and the U.S. Drug Enforcement Agency (DEA) has labeled them as a drug of concern. The drugs are sold over the internet as “legal highs” and have been marketed as “bath salts” or “plant food” to attempt to disguise their true use. Additionally, they have been implicated in several deaths.

Five bath salt samples were purchased from various online sources and were sold under various names. In addition, a package labeled “plant food” was submitted for analysis by a law enforcement agency. All samples were packaged and labeled “not for human consumption.” Standards of MDPV and mephedrone were obtained and analyzed along with the commercial products.

Standard forensic color tests produced consistent positive results for MDPV and had inconsistent results with mephedrone. MDPV turned a bright yellow with the Marquis, Mecke, and Froehde color reagents. Cobalt produced a blue color change and Mandelin produced an olive green change when tested with MDPV. Products purchased over the internet labeled as “bath salts” all had similar positive results to the MDPV standard. Mephedrone did not have a color change with any reagents except for Mandelin and these results were inconsistent. The “plant food” did not have positive results with most of the color test reagents.

Gold chloride in phosphoric acid provided specificity for MDPV and mephedrone standards when performing microcrystalline tests. MDPV crystals appeared needle like in shape with a green/yellow tint under polarized light. Mephedrone produced thin needles with magenta, yellow/green color under polarized light. The mephedrone crystals did not form immediately and developed after an hour.

Following screening by color tests confirmatory analysis was performed using liquid chromatography/mass spectrometry with isocratic elution. The elution had a ratio of 10% ammonium acetate and 90% acetonitrile/isopropanol (50:50). The analysis was performed on an aqueous C18 analytical column with a total run time of 10 minutes. Mephedrone and MDPV were chromatographically separated and distinguishable from other drugs based on retention time and mass to charge ratio. The developed method was found to be very sensitive with limits of detection below 10ng/mL for mephedrone and MDPV.

The purchased legal high products were able to be analyzed using the above methods following dilution in the mobile phases. Most legal high products marketed as “bath salts” were found to contain MDPV while the product marketed as “plant food” was found to contain mephedrone. The LC/MS results regarding the bath salts confirm the effectiveness of the MDPV presumptive color tests.

#### Forensic Science, Mephedrone, MDPV

### A142 Detection and Simultaneous Quantitation of $\beta$ -Naphyrone and 3,4-Methylenedioxypropylvalerone by Gas Chromatography-Mass Spectrometry

Gina M. Parada, BS\*, and Thomas A. Brettell, PhD, Cedar Crest College, 100 College Drive, Allentown, PA 10104; Michelle Dawes, MSFS, 2217 Polo Run Drive, Yardley, PA 19067

After attending this presentation, attendees will have a better understanding of the use of Gas Chromatography-Mass Spectrometry (GC-MS) to identify and simultaneously quantify  $\beta$ -naphyrone and 3,4-methylenedioxypropylvalerone (MDPV) in solid-dosage samples.

This presentation will impact the forensic science community by providing a simple method for screening, identification, and quantitation of the target cathinone analogues in illicit powders.

Naphyrone and MDPV are synthetic cathinone analogues which are part of a group of newly emerging “designer drugs” originating in the United Kingdom and have been recently advertised for purchase on a

number of websites. Naphyrone and MDPV, derivatives of pyrovalerone, are psychoactive drugs that act as norepinephrine-dopamine reuptake inhibitors producing stimulant and psychoactive effects related to those of amphetamine and cathinone. Both compounds have been misrepresented as a so-called “legal high.” Naphyrone, the naphthyl analogue of cathinone, bears a close structural resemblance to mephedrone and MDPV. This substituted cathinone contains a phenethylamine core whose alpha carbon contains an alkyl group and beta carbon contains a ketone group. Naphyrone has been known to users under the various street names and sold as “bath salts” and pond cleaner, and have been referred to as a “legal high” that is stronger than cocaine, amphetamine, and MDMA. Published analytical studies on the bath salts reported the presence of naphyrone, cathinones, caffeine, MDPV, flephedrone, mephedrone, butylone, and other constituents.

MDPV, first seized in Germany in 2007, has reportedly been sold as a legal drug alternative and marketed as “bath salts” under various names. Other reports also indicate it is being sold with labels indicating “for novelty use only” and typically sold in 500mg packets on internet sites based in Europe. MDPV has been controlled (Schedule I) in some states.

This study identifies MDPV in a product sold as a “bath salt” not for human consumption. The unlabeled clear plastic baggie contained 0.32 grams of a light tan powdery substance. A small portion of the powder tested inconclusive with two color spot tests, Chen’s and Simon’s reagent. A UV/Vis spectrum recorded at a scan rate of 1200nm/min in methanol with a 1cm cell path length in the range of 200-800nm resulted in a  $\lambda_{\max}$  = 232nm and peaks at 282nm and 315nm. Infrared analysis using attenuated total reflectance (ATR) accessory compared to a MDPV HCl certified standard. The spectrum was collected using 32 scans between 4000  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$ . GC-MS data was acquired using a quadrupole mass-selective detector (MSD). A GC-MS method has been developed and validated where Naphyrone and MDPV can be simultaneously analyzed and quantified using phenyltoloxamine as an internal standard. Calibration curves obtained from a working standard of naphyrone and MDPV exhibited a favorable  $R^2$  value ( $> 0.99$ ) with a linear dynamic range between 900 – 3.00  $\mu\text{g/mL}$ . The most abundant ion,  $m/z = 126$ , present in the mass spectrum of both compounds was used in the SIM mode for quantification. Naphyrone and MDPV were well separated from other related drugs using a 30 m x 0.25 mm x 0.25  $\mu\text{m}$  phenylmethylsilicone capillary column using Helium as a carrier gas with a linear gas velocity of 38 cm/sec. Methanol was used as the solvent and a sample volume of 1 $\mu\text{L}$  was injected in the split mode with a split ratio of 56.4:1. The gas chromatographic oven program was the following: 130°C for 2.00 min, 15°C/minute ramp up to 250°C, and hold for 10 minutes for a total run time of 20 minutes. A retention time optimization study provided the optimum separation conditions. Quantitative analysis indicated the bath salt sample was nearly pure MDPV hydrochloride.

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**Forensic Science, Naphyrone, MDPV**

## **A143 Chiral Separation of Amphetamine Type Substances Using Ion Mobility Spectroscopy-Mass Spectrometry**

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After attending this presentation, attendees will be introduced to a novel technique, Chiral Ion Mobility Spectrometry (CIMS), which may be used for the separation of controlled and non-controlled enantiomers found in amphetamine type substances (ATS). The presentation will highlight both the degree of separation of enantiomers found in amphetamine type substances utilizing inexpensive achiral modifiers and the mechanism by which these separations take place and coupled to a mass spectrometer for unambiguous identification of the ATS compounds of interest.

This presentation will impact the forensic science community by describing a novel method for chiral separations offering advantages over the more time consuming and expensive methods currently in use. The high cost of chiral separation chromatography columns, the time needed, and the complexity is somewhat problematic for routine use. CIMS is a possible solution to these challenges by providing a high speed and low cost analytical technique not only for chiral separations but also as potential high throughput general drug analysis technique.

Chiral Separations have been a challenging aspect of analytical chemistry. There currently exists thousands of chiral separation phases predominantly used in liquid chromatography, gas chromatography and some capillary electrophoretic assays. The chiral phases themselves are expensive, while the time and resources required in selecting an appropriate phase for a particular enantiomer adds complexity. This presentation highlights a proposed separation mechanism when using achiral modifiers in the gas phase to separate enantiomers found in Amphetamine Type Substances (ATS). The presentation will show, for the first time, the use of straight chain achiral alcohols as drift gas modifiers and propose a mechanism for the gas phase chiral interactions responsible for the separations. Experimental results suggest that the interaction between the modifier and the analyte is ion-neutral based, with numerous modifiers adducting to a single analyte molecule. This interaction has been exploited to produce the desired separation of enantiomers of forensic interest using inexpensive achiral modifiers as opposed to the more expensive enantiomerically pure chiral modifiers such as S-2-Butanol. Common precursors used to make methamphetamine are ephedrine and pseudoephedrine, both chiral in nature. The ability to detect and separate these enantiomers as impurities in seized drugs has proven valuable as a forensic tool in many criminal investigations and has also been used for drug provenance studies. Achiral modifiers are used to separate RS and SR Ephedrine from SS and RR Pseudoephedrine. The resulting ion clusters formed have been characterized utilizing electrospray ionization to feed an ion mobility spectrometer coupled to a quadrupole mass spectrometer. The experimental data suggests that gas-phase chiral separations are possible and outlines a new, fast, and effective tool that may ultimately identify synthetic pathways and provide fast analysis and identification of seized amphetamine type substances.

This presentation will describe the capabilities of gas-phase chiral separations using commercially available electrospray ionization (ESI) ion mobility mass spectrometry (IMS-MS), to quickly separate and identify enantiomers introducing achiral modifiers into the drift gas. This approach provides an alternative technique to the more commonly used gas, liquid and capillary electrophoretic assays that are currently slower and more costly to perform. The proposed mechanisms of the separation will also be discussed.

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**Ion-Mobility, Chiral, Mechanism**

## **A144 Detection of Illicit Drugs by Linear Ion Trap LC/MS/MS**

*Yuriy Uvaydov, MS\*, 99 Tenth Avenue, Suite 721, New York, NY 10011*

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After attending this presentation, attendees will learn how linear-ion-trap liquid chromatography mass spectrometry (LCMS/MS) can be applied for the analysis of commonly encountered drugs of abuse. This presentation will highlight advantages and limitations of ion-trap detection of illicit drugs over traditional single quadrupole LC/MS and GC/MS techniques.

This presentation will impact the forensic science community by demonstrating how ion-trap LCMS/MS can be effectively applied for screening multi-unit submissions of evidence for the presence of controlled substances utilizing full-scan and tandem mass spectrometry (MS/MS).

Analysis of seized drug evidence consists of a series of presumptive and confirmatory tests. Traditional presumptive tests include color, microcrystal, thin-layer-chromatography (TLC), GC, and/or high

performance liquid chromatography (HPLC). Typically, mass spectral identification is required for confirmation of controlled substances in many forensic laboratories. The majority of cases encountered by forensic chemists are easily analyzed utilizing these traditional methodologies. However, with a continuing demand for faster, more efficient methodologies, the introduction of newer analytical instrumentation into the classical workflow is essential.

Advances in electrospray ionization (ESI) mass spectrometry have led to the increased popularity of direct analysis of drugs with minimal or no sample preparation allowing for faster analysis time. Additional advances in separation science have recently been attributed by the ion trap technology. For simple matrices, time-consuming chromatographic separations may not be required as multiple reaction monitoring (MRM) transitions can provide molecular confirmation. The purpose of this work has been to develop a method for rapid detection of drugs (target or unknown) utilizing a linear-ion-trap that will provide valid and reproducible data while maintaining cost-effectiveness.

In an effort to reduce case backlog and maintain quick analysis time, a non-chromatographic qualitative screening method was developed using ThermoFinnigan LXQ linear-ion-trap LCMS/MS. Analytes of interest were infused directly into the mass spectrometer using autosampler injections via a zero-dead-volume union. The mobile phase consisted of 50%/50% of (A) 0.1% formic acid in water, and (B) 0.1% formic acid in acetonitrile, delivered at 0.400mL/min. Mass spectral acquisition of data was performed with multiple scanning events using collision-induced-dissociation (CID) for full-scan and data-dependent auto MS/MS. Positive ionization mode was employed.

Applications presented will include the analysis of illicitly made tablets containing 3,4-methylenedioxyamphetamine (3,4 MDMA), benzylpiperazine (BZP), caffeine, and trifluoromethylphenylpiperazine (TFMPP) as well as pharmaceutical tablets containing oxycodone, hydrocodone, and codeine. In addition, the analysis of commonly encountered benzodiazepines such as diazepam, alprazolam, and clonazepam will be discussed. Preliminary results revealed sufficient MS resolving power for qualitative screening of analytes in multi-component mixtures without the need for chromatographic separation. The sensitivity of the linear-ion-trap LCMS/MS has been demonstrated in the nanogram range allowing for the detection of active ingredients in tablets containing less than one percent of the analyte of interest. For monoisotopic compounds such as methamphetamine and phentermine, detection utilizing chromatographic separation is required. The data showed that the linear-ion trap LCMS/MS can be used in compliment with GC/MS or LC/MS to provide principal means of identification. This approach, along with other limitations and challenges of different types of analyses, will also be discussed.

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#### **Linear-Ion-Trap, Seized Drugs, Mass Spectrometry**

### **A145 Evaluation of Fast Gas Chromatography Coupled With Hydrogen Mobile Phases in Drug Identification**

*Charles R. Cornett, PhD\*, and Rebecca L. Hansen, BS, University of Wisconsin - Platteville, Department of Chemistry, Platteville, WI 53818; and Leah J. Macans, BS, Wisconsin State Crime Laboratory - Milwaukee, 1578 South 11th Street, Milwaukee, WI 53204-2860*

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The goal of this presentation is to disseminate an assessment of both fast gas chromatography technology and the use of hydrogen as a replacement for helium mobile phase in drug identification.

This presentation will impact the forensic science community by presenting research which demonstrates a significant impact on the analysis time in drug identification. Fast gas chromatography significantly shortens the retention time of individual species and the replacement of helium mobile phase with hydrogen gas more than recoups lost resolution from the shorter columns while saving laboratories supply money.

The objective of this project is designed to assist crime laboratories' assessment of new separation techniques and gauge the feasibility of implementation. The first objective of this project is an assessment of the expected gain in resolution and sample throughput for a drug identification unit using a combination of Fast GC and hydrogen carrier gases. This area of application is prime ground for realizing the full potential of Fast GC – H<sub>2</sub>. In 2008, a joint project funded by the Midwest Forensics Resource Center between the University of Wisconsin – Platteville (UWP) and the Wisconsin State Crime Laboratory – Madison demonstrated incredible reductions of over 50% in retention times of ignitable liquids from arson debris using the Fast GC. This study also found that the use of hydrogen as a carrier gas more than compensated for resolution losses related to Fast GC. In fact, the more compressible hydrogen carrier produced improvements in resolution for the Fast GC analysis compared to a conventional GC technique using helium carrier gas ( $p < 0.01$ ). Fast GC-H<sub>2</sub> separation of drug identification samples could potentially decrease retention times of straightforward matrices to several tens to a few hundred seconds. This presentation addresses the limiting factors of this technique including the scanning rate of the quadrupole mass spectrometer and effects on detection limits of illicit drugs.

Experimental design involves the Fast GC and conventional analysis of one dozen Scheduled compounds including cocaine, tetrahydrocannabinol (THC), heroin, 3,4-Methylenedioxyamphetamine (MDMA), trifluoromethylphenylpiperazine (TFMPP), lysergic acid diethylamide (LSD), buprenorphine, synthetic cannabinoids, alprazolam, clonazepam, boldenone, and nandrolone. The figures of merit selected for ANOVA ( $p < 0.05$ ) comparisons center on retention times and chromatographic resolution of a master standard containing these twelve compounds. Given that many laboratories may hesitate to modify existing units for Fast GC, the first assessment examines the benefit in simply switching to the less expensive hydrogen mobile phases from the helium mobile phase. The control in this experiment is the conventional GC operating with a standard DB-5, 30 m column and helium mobile phase. The hydrogen is employed instead, and a data set is generated with resolutions compared to the helium gas. Subsequently, the heating ramp of the conventional GC is increased without altering other variables to assess just how aggressive of a heating ramp can be used to reproducibly equal the conventional use of helium. The second set of experiments uses solely helium while comparing Fast GC and conventional GC in the event that a laboratory is not ready or able to convert to hydrogen carrier but is interested in the Fast GC gains. In this particular case, the data demonstrate the resolution decrease for cost: benefit analysis. Finally, the two variables (Fast GC and hydrogen mobile phase) are combined to assess the maximum benefit.

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#### **Fast GC, Drug, Identification**

### **A146 2012 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)**

*Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081*

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After attending this presentation attendees will learn about the history and current development of the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). SWGDRUG was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance. This presentation will provide attendees with an update on SWGDRUG activities during the year 2011, including revisions to SWGDRUG recommendations and other work products in development.

This presentation will impact the forensic science community by providing current recommendations, new developments, and future projects and direction of SWGDRUG as it relates to the analysis of seized drugs.

During January 2011, version 5.1 of the SWGDRUG Recommendations was approved and made available to the general public via the group's website ([www.swgdrug.org](http://www.swgdrug.org)). This version of the recommendations included revisions to Part III A and Section 6 pertaining to "Reporting." Version 6.0 of the SWGDRUG Recommendations was approved in July 2011. This latest version includes a new section (Part III C) providing recommendations for the analysis of clandestine laboratory samples. Version 6.0 is also available through the SWGDRUG website.

In the first part of 2011, SWGDRUG also made available the first version of its mass spectral library. Laboratory analysts throughout the world can download this library from the SWGDRUG website and onto their laboratory instruments. The library contains more than 1400 spectra, including many of the recently encountered synthetic cannabinoids and their isomers. Initial feedback from analysts and library users has been highly positive. The library will be updated on a regular basis and contributions from the forensic community are strongly encouraged.

During 2010, the SWGDRUG core committee finalized and approved Supplemental Document SD-3. This document provides four examples of measurement uncertainty calculations for weight determinations. Since the document's release, comments were received from the forensic community and the document was revised to provide clarifications and useful information to future users. This presentation will include a general summary and discussion of the latest version of SD-3 dated July 2011.

During the first half of 2011, users of the SWGDRUG website were also encouraged to submit comments regarding the use and impact of SWGDRUG Recommendations within the forensic science community. The SWGDRUG core committee is constantly interested in obtaining direct feedback from laboratory analysts and managers as to the use of SWGDRUG Recommendations and other documents. A general overview of the community feedback received throughout 2011 will be presented.

Currently, members of the SWGDRUG core committee are working on the following projects:

- Examples of laboratory reporting
- Supplemental document containing examples of measurement uncertainty calculations for purity determinations
- Online resource center for drug analysis training

The SWGDRUG core committee is comprised of representatives from federal, state and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia, and Singapore. The following forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), the American Society for Testing and Materials (ASTM), and the National Institute of Standards and Technology (NIST).

#### **Criminalistics, SWGDRUG, Drug Analysis**

### **A147 Density Determination Via Magnetic Levitation**

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After attending this presentation, attendees will have learned of a new method of helping to characterize trace evidence items by determining their density.

This presentation will impact the forensic science community by introducing a method of determining the density of small, irregularly shaped objects and of liquids, that is non-destructive, quick, easy, and

inexpensive, is independent of instrument/operator/location, and provides values that may be entered into a searchable database.

Density is one parameter that may be used to characterize a trace object. Although the formula for density (or specific gravity) is simply mass divided by volume, this is not so easily done for tiny, irregularly-shaped objects. In the past the density of small objects has been compared/determined using the sink/float method. When the tested objects suspended in a liquid medium neither rose to the surface nor sank to the bottom, their density was the same as the liquid. This could be a slow process as the liquid medium was gradually made less or denser by the addition of drops of either a miscible heavier or lighter liquid followed by mixing. Once this equilibrium point had been reached, one could determine the density of the liquid and hence the density of the object by removing sufficient liquid to fill a previously weighed pycnometer and then weigh the now full pycnometer. One could then obtain an actual value of density that could be entered into a database.

This presentation will introduce an entirely different method of obtaining density that is quick (just a few minutes), requires no expensive instrumentation (not even a source of electrical power), does not require highly-trained operators, does not destroy the sample, is readily calibrated with a series of density standards, provides values that can be entered into a searchable database, and that can distinguish between samples whose density differs by as little as 0.0002 g/cm<sup>3</sup>. The method is based on magnetic levitation (MagLev) and involves placing diamagnetic samples into a container filled with a paramagnetic fluid, which is then placed between two permanent magnets. The vertical position of the sample, in the presence of the magnetic field, correlates with density. The vertical position depends on mass/volume rather than mass or volume separately and thus eliminates the need for standardized sample sizes.

Additionally, the MagLev concept may be used to separate similar items that vary in density. This will be illustrated by a photomicrograph showing how a mixture of two visually-similar types of glitter (both silver in appearance, both hexagonal and the same size) is clearly separated by vertical position in the cuvette containing a paramagnetic liquid. A disposable pipette may then be inserted to draw out those particles at a given level.

For heterogeneous samples, the distribution of densities may be a distinguishing characteristic. The MagLev method could replace the density gradient columns that in the past have been used to compare various size fractions of soil samples. In the past, the preparation of the density gradients was a slow, tedious process and involved liquids that are health hazards. Then once the soil fraction was added to the prepared density gradient column it might take a day or more for the sample to reach a stable distribution. With MagLev the entire process from start to finish would take only a few minutes.

For the determination of density, is there a limitation on particle size? Particles as small as 7µm in diameter are no problem, but for those 2µm or smaller Brownian motion prevent an accurate measurement. What if your sample is water soluble? Not a problem. There are paramagnetic ions that are chelated and are not water soluble, but are soluble in various non-aqueous solvents. Have a liquid sample? Not a problem; just add a drop to an immiscible paramagnetic liquid.

Does the MagLev process alter your sample? Results will show that smokeless gunpowder samples undergo no change. Also, the density of a glitter sample was measured and then some was added to a commercial nail polish. A portion of the liquid polish was diluted in hexane and then passed through filter paper, the glitter particles removed from the filter paper and their density again was measured and was unchanged.

Results will be shown from eleven different glitter samples, six different commercial smokeless gunpowder samples, and glitter particles extracted from a brand of commercial nail polish.

#### **Density, Magnetic Levitation, Trace Evidence**

## A148 Forensic Characterization of Surface-Modified Fibers Via X-Ray Photoelectron Spectroscopy

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After attending this presentation, attendees will understand how x-ray photoelectron spectroscopy may be used to characterize fibers that have received one or more surface modification treatments.

This presentation will impact the forensic science community by showing how single fibers (one of the most common and most important types of trace evidence) may be subcategorized by using x-ray photoelectron spectroscopy to detect and characterize any thin surface modification layers.

X-ray photoelectron spectroscopy (XPS) is virtually unknown to forensic science. X-ray photoelectron spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material. XPS requires ultra-high vacuum (UHV) conditions. XPS spectra are obtained by irradiating a material with a monochromatic beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1 to 10nm of the material being analyzed.

A proof of concept study shows that via XPS single fibers, that in all other respects are identical, may be characterized and distinguished if one fiber has no surface modification while the other does, or if one fiber has one manufacturer's proprietary surface modification while the other has some other manufacturer's (different) proprietary surface modification.

Today most fabrics have received one or more type of surface-modification treatment such as stain resistance, permanent press, or water proofing. For example, white cotton fibers are so common and have so few distinguishing features that today they are largely ignored by forensic scientists. XPS can distinguish, on a single fiber basis, between the following otherwise identical cotton fibers:

1. white cotton fiber with no surface modification,
2. white cotton fiber with some type of silicone surface modification,
3. white cotton fibers that have a surface modification (some type of fluorocarbon) applied by a plasma process,
4. white cotton fiber with some type of silicone surface modification plus the 3M Company's surface modification treatment (some type of fluorocarbon applied by a wet process),
5. white cotton fiber with some type of silicone surface modification plus some other company's (not specified) proprietary surface modification treatment (some type of fluorocarbon applied by a wet process but different than 3M's).

All elements except hydrogen and helium may be detected and measured by XPS. Low resolution survey spectra identify all the elements in the surface layer and their relative abundances, while separately for each element high resolution spectra show the different bonding states and the relative amounts for each state for a particular element. Because carbon can exist in so many different bonding states, XPS is particularly effective in characterizing different types of polymers that exist as thin surface layers.

Additionally, the thickness of a very thin surface layer, as well as the change in composition below it, may be determined by angle-resolved XPS. As with hammering in a nail at an acute angle compared to the normal, the penetration of the x-ray beam into (perpendicular) the sample is decreased as the angle become more acute.

Also, through XPS Imaging the coverage in the x,y plane of a given element may be mapped. It will be shown that by mapping for F the extent of coverage of a thin fluorocarbon surface layer may be determined on a single fiber.

This presentation will show how, from just a single fiber x-ray, photoelectron spectroscopy (XPS) can nondestructively distinguish these surface modifications.

## Fibers, Surface Modifications, X-Ray Photoelectron Spectroscopy

## A149 Raman Laser Polarization and Its Effect on Fiber Analysis

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After attending this presentation, attendees will have an understanding of the application of Polarized Raman spectroscopy for fiber identification and characterization. In addition, this presentation will introduce the phenomenon of how the polarization of a Raman laser affects the Raman spectrum of synthetic fibers.

This presentation will impact the forensic science community by providing an alternative approach to distinguish and discriminate between synthetic fibers since the Raman laser polarization effect on synthetic fibers has never been explored in the forensic community.

Fibers are a frequently encountered form of trace evidence. Many techniques and types of instrumentation have been developed and used to analyze fibers in the forensic community, for example, the comparison microscope, the fluorescent microscope, the polarized light microscope, the UV-Vis micro-spectrometer and the FTIR micro-spectrometer. Each of these techniques is capable of providing unique and useful information about a fiber.

Recently, Raman spectroscopy has started to establish its place in the forensic science community. Raman spectroscopy has been used to analyze paint chips, inks, drugs, condom lubricants, and dyes. The growth of Raman spectroscopy in the forensic science community is due to its short analysis time, minimum sample preparation, and non-destructiveness. Many articles published on fiber analysis have indicated that Raman spectroscopy is useful for identifying the dye(s) in the fiber. However, Raman spectroscopy should also be explored for fiber type identification and characterization.

Like Infrared spectroscopy, Raman spectroscopy yields vibrational spectra that indicate the chemical structure of a material. Raman spectrometers are often equipped with a polarized laser source. As in polarized light microscopy, the polarized laser interacts with different orientations of an anisotropic material. A drawn out fiber exhibits pseudo crystallinity that can be exploited by the polarized Raman laser source to yield additional information on the chemical structure of the fibers. This information may be useful in forensic fiber analysis. Depending on the orientation of the fiber, the polarized laser can interact differently with the functional groups of the fiber, producing a slightly different Raman spectrum for a given fiber orientation. Depending on the type of fiber, the difference can be seen in relative peak intensities, and additional peak(s).

Polyester, nylon, and acrylic fibers were analyzed with a WITec Confocal Raman Microscope with a 532 nm polarized laser source in this study. Five fibers for each fiber type were analyzed to ensure representativeness. Each fiber was measured in its perpendicular and parallel orientation with respect to the polarization of the laser source. For polyester fiber, dramatic differences were observed when comparing the perpendicular and parallel orientation at the 2800  $\text{cm}^{-1}$  to 3000  $\text{cm}^{-1}$  range. The peaks in the perpendicular orientation have relatively higher peak intensity. In addition, it has four distinguished peaks instead of three in the parallel orientation. The overall pattern of the peaks in that region is completely different. Minor changes were also observed in the 1300  $\text{cm}^{-1}$  to 1400  $\text{cm}^{-1}$  region, there are four peaks for the perpendicular orientation and two for the parallel orientation. For acrylic fiber, the only noticeable

change observed was at peak position 1220  $\text{cm}^{-1}$ , that peak was there in the parallel orientation and disappeared in the perpendicular orientation. For nylon fiber, the N-H stretching at 3305  $\text{cm}^{-1}$  is relatively more intense in perpendicular orientation. In addition, there is an extra peak at about 1550  $\text{cm}^{-1}$  in the parallel orientation. Also, the relative intensity of peaks at 1435  $\text{cm}^{-1}$  and 1630  $\text{cm}^{-1}$  were switched in the two different orientations. All the differences are due to the interaction between the polarized Raman laser and the different pseudo crystalline orientation of the fibers.

This preliminary study focuses on the most common synthetic fibers. Future studies can be conducted with different laser wavelengths and natural fibers.

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**Raman, Polarization, Fiber**

## **A150 Microwave-Assisted Extraction for Qualitative Analysis of Carpet Fibers**

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After attending this presentation, attendees will learn about how polymer additives can be employed to objectively compare carpet fibers.

This presentation will impact the forensic science community by providing a potential method for comparing carpet fibers that could one day be applied in crime labs.

Synthetic polymers are used in the manufacturing of many manmade products, including carpets. Synthetic polymers are long-chained molecules that are compounded with many, varied, and proprietary additives that chemically modify the polymer to improve its performance. In forensic science, carpet fibers can be used as evidence. Microscopy and spectroscopy are current methods used in the identification and comparison of carpet fibers. However, these techniques cannot determine the origin of a carpet fiber, thereby making these methods very subjective.

In this study, polymer additives were extracted from nine different carpets (made of nylon, polypropylene, or olefin) in 1:1 acetone:hexane through microwave-assisted extraction (MAE). Each carpet was tested four different times. The extracts were then analyzed through gas chromatography – mass spectrometry (GC/MS). Because each carpet yielded four chromatograms, the peak lists for these chromatograms were combined to make an averaged chromatogram for each of the carpets. These averaged chromatograms were then used to compare the carpets for base polymer and manufacturer differences. For comparison purposes, peaks were accepted at a 3:1 signal-to-noise (S/N) ratio. Moreover, it is hypothesized that similarities and differences can be seen between the various carpets.

The carpets were first compared in regards to their base polymer: nylon, polypropylene, or olefin. It was found that carpets made of nylon had a significant amount of differences when compared to polypropylene and olefin. Specific peaks and certain peak patterns were identified. Differentiating polypropylene and olefin carpets was more difficult considering olefin carpets are a mixture of polypropylene and polyethylene polymers. This in turn made it very hard to identify specific peaks and peak patterns between carpets made of polypropylene and olefin.

Because manufacturers chemically modify their carpets, it was determined if manufacturer type contributed to a carpet's profile. For example, the nylon carpets were compared among each other to establish whether any similarities or differences exist. With the nylon carpets, it was found that manufacturer type contributed to the observed differences. Moreover, a carpet made with a polymer (e.g., nylon) from the same company exhibited differences when different carpet manufacturers modified that polymer. Carpets made of polypropylene and olefin still exhibited some difficulties when comparing them in regards to manufacturer type. While significantly fewer peaks could be identified for polypropylene (and olefin) with at least a 3:1 S/N, carpets made of polypropylene (or olefin) could still be distinguished from one another.

In conclusion, this research successfully showed that carpet fibers can be analyzed and compared through a more objective method. Differences could be seen between carpets made of different base polymers. It could also be seen that carpets made of the same polymer can still be distinguished from each other due to the various chemical modifications that manufacturers apply to the polymers.

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**Carpet Fibers, GC/MS, MAE**

## **A151 Forensic Characterization and Identification of Dyes Extracted From Millimeter-Length Fibers Using Ultra-Performance Liquid Chromatography/Mass Spectrometry**

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The goal of this presentation is to communicate to the forensic community results from ongoing research on development and validation of methods for chemical characterization of dyes extracted from millimeter to sub-millimeter length trace evidence fibers.

This presentation will impact the forensic science community by using the working hypothesis that liquid chromatographic separation and detection of individual dye components by mass spectrometry (LC/MS) provides a qualitative and semi-quantitative fiber dye "fingerprint," with the prospect of enhancing discrimination for comparison of known and questioned casework fibers. Determining the number and relative amounts of dyes present, and characterizing those dyes at the molecular level by MS, offers an entirely new level of information that is not otherwise available from microscopic or spectroscopic methods. Such information may also open the possibility of tracing specific dye formulations to the textile manufacturer.

Determining the number and relative amounts of dyes present, and characterizing those dyes at the molecular level by MS, offers an entirely new level of information that is not otherwise available from microscopic or spectroscopic methods. Such information may also open the possibility of tracing specific dye formulations to the textile manufacturer. The target size for forensically relevant fibers derives in part from fiber examinations and population studies reporting that recovered fibers are often as small as 2mm in length, depending on the degree of dyeing. Because extraction of dyes from a fiber is destructive to the evidence, the ability to extract and identify dyes from trace fibers that are a millimeter or less in length is critical. The laboratory involved in this study has previously developed extraction/capillary electrophoresis (CE) methods for acid dyes on nylon, vat and reactive dyes on cotton, and basic dyes on acrylic, and demonstrated CE/MS analysis of basic dye extracts from two mm single acrylic fibers.

The current work explores the application of ultra-performance liquid chromatography (UPLC) for analysis of dyes extracted from fibers. UPLC uses high pressures (>10,000psi), smaller column particles (<2 $\mu\text{m}$ ), and short columns (~5cm) for high speed, resolution, and sensitivity. If sufficient fiber is available, UV/visible diode array detection may be suitable for rapid low cost screening of trace evidence samples by UPLC. With extractions from 10mm single fibers of nylon, acrylic, and polyester, the amounts of eight different corresponding acid, basic, and disperse dyes ranged from 5 to 20ng with standard deviations from 1 to 3ng. UV/visible detection limits are in the low ng range for dye extracts from 10mm fibers, although quality of absorbance spectra is compromised at lower lengths.

Liquid chromatography tandem mass spectrometry (LC/MS/MS) has been widely used in forensic toxicology due to its high sensitivity and selectivity. With tandem MS/MS with multiple reaction monitoring (MRM) of specific molecular ion-fragment ion transitions we have reliably detected extracted dye amounts as low as a few tenths of ng from commercial textile fibers as small as 0.5mm in length. For example, five

0.5mm length nylon fibers commercially dyed with three acid dyes resulted in the following mean extracted dye amounts and % relative standard deviations: C.I. Acid Yellow 49,  $0.15 \pm 0.02\text{ng}$  (13.22%); C.I. Acid Red 337,  $0.36 \pm 0.04\text{ng}$  (10.95%); and, C.I. Acid Blue 281,  $0.19 \pm 0.03\text{ng}$  (18.34%). Linear calibrations based on five concentration levels of dye standards produced coefficients of determination ( $R^2$  values) ranging from 0.983 to 0.999. A miniature guillotine for improving the reproducibility of cut fiber length was designed. However, the difficulty involved in cutting sub-mm fiber lengths reproducibly is the primary reason that percent relative standard deviations for extracted dye amounts from replicate 0.5 mm fibers is relatively high. Further LC/MS validation work and technology transfer is also in progress in collaboration with the Forensic Services Laboratory of the State Law Enforcement Division (SLED), Columbia, SC.

In summary, analysis of dye extracts from single acrylic, nylon, and polyester fibers of 0.5mm lengths has been achieved. In tandem MS, two or more stages of mass analysis are combined in one experiment. Each stage provides an added dimension in terms of specificity of structural information characteristic of the target analyte. Multiple reaction monitoring is a common LC/MS/MS detection mode in which pairs of target parent ions and unique fragment ions are used for quick and accurate identification of target analytes. MRM detection enables high discrimination between different dyes because transitions to a characteristic fragment ion are unique to the molecular structure of the parent ion. The specificity of analysis allows simultaneous identification and quantitation at exquisitely low detection limits (e.g., 0.1ng or less for acid dyes extracted from nylon). Using the collection of several hundred textile dyes in the laboratory, infusion of dye standards can establish an MRM transition library. Routine LC/MS/MS screening with an MRM library has the potential to provide unambiguous identification for those dyes. For dyes not in the library, MS/MS analysis can still provide discrimination based on molecular structure differences.

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**Trace Fiber Examination, Textile Dye Analysis, Liquid Chromatography/Mass Spectrometry**

## **A152 Use of Computer Controlled Scanning Electron Microscopy (CCSEM) Methods for the Analysis of Small Particles Adhering to Carpet Fiber Surfaces**

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After attending this presentation, attendees will understand how fine particles, adhering to the surfaces of individual carpet fibers, can be recovered and quantitatively analyzed by SEM/EDS and, in its current state of development, how this capability can be applied and integrated into conventional forensic trace evidence analysis.

This presentation will impact the forensic science community by demonstrating the feasibility of the analysis of these “piggy back” particles, and how they can be used to remove fundamental class-level limitations on the probative value of trace evidence and provide an independent quantitative means to test hypotheses of common origin.

Very small particles are ubiquitous in our environment and are virtually ignored by forensic science (gunshot residue being a notable exception). These particles range in size from an order of magnitude smaller than conventional trace evidence, down to the molecular level (now routinely exploited through DNA analysis). We move around in a soup that is a combination of these very small particles and they represent an

extraordinary, largely untapped resource for forensic associations and source attribution. The combinations of these particles are so complex that until recently there was no practical method to identify and interpret these combinations. Particle combination analysis (PCA) is a new capability that focuses on these methods. This project involves the application of PCA as a means to objectively verify and improve traditional trace evidence analysis of fibers.

An innovative instrumental trace evidence analysis approach that applies the PCA concept to the recovery and quantitative SEM/EDS analysis of fine particles found adhering to the surfaces of larger trace evidence particles is described. Ultimately this approach could fundamentally change the probative value of trace evidence from one of class association to one of highly individual, testable associations (akin to those arising from multiple-transfers of uncorrelated traces, or the co-occurrence of independent, highly variable events).

Methods were developed to quantitatively remove fine particles from carpet fiber surfaces and to prepare the particles for SEM/EDS analysis.

To assess within-carpet variability, computer controlled SEM analyses (CCSEM) were conducted on fine particles removed from three different areas from each of nine carpets (three domestic, three automobile, and three commercial carpets). From each of the three areas on each of the nine carpets, a set of ten carpet fibers was used to define the “known” or target fine particle profile of the carpet itself. Three individual fibers from each of the 27 areas were then used as test fibers, each representing a single recovered transferred fiber. To explore between-item variation, a broader survey of an additional twelve carpet particle profiles was conducted.

Principal findings from these studies were:

- Fine particles are present on the surfaces of individual carpet fibers.
- These particles can be recovered nearly quantitatively for CCSEM analysis by extraction with reagent ethanol.
- Quantities of particles adhering to individual carpet fibers varied from a few hundred to greater than 4000 (the maximum number examined).
- Particle classification schemes currently in use for environmental CCSEM applications are broad compared to variations in elemental composition seen among the individual CCSEM particle spectra.
- Carpets vary widely in the types and quantities of small particles adhering to their fiber surfaces.
- Particle distributions from the individual test fibers could not be explained based on a hypothesis of unbiased statistical sampling from a population defined by the target particle profile.
- Highly characteristic, semi-quantitative patterns of particle types found in target particle profiles were consistently represented in the particle distributions from individual test fibers from the same carpet, and consistently absent among those from different carpets.
- Based on these findings, particle distributions found on carpet fibers can contribute substantially to the weight of evidence linking fibers to a specific carpet.
- Further studies are needed to better understand this type of evidence, including: (a) the sources of within-item variation, (b) the effects of alternative methods of particle classification, and (c) the extent of between-item variation.

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**Evidence Interpretation, Fiber Analysis, SEM/EDX**

## A153 Characterization of Vectran LCP Fibers

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After attending this presentation, attendees will have information for the characterization and identification of Vectran LCP fibers.

This presentation will impact the forensic science community by expanding the knowledge and data on forensic fiber identification.

Vectran liquid crystal polymer (LCP) fibers are a relatively new commercially produced aromatic polyester fiber that was first developed by Celanese Acetate LLC in the 1990s and is now manufactured by the Kuraray Co., Ltd. Liquid crystals (LCs) are a state of matter that have properties between those of a crystalline solid and those of a conventional liquid. Like LCs the LCPs in the liquid state, either dissolved in a solvent or melted, have highly oriented anisotropic-molecular domains like a crystal but they flow like a liquid. A para-substituted aromatic polyamide, is a LCP fiber in the solid state and is wet spun from dissolved polymer in a liquid (lyotropic liquid-crystal polymer). Unlike conventional polyester that is melt spun from randomly oriented and fixable molecules, Vectran fibers are LCPs in the solid state and are the only LCP fibers being produced today that are melt spun from a highly ordered liquid crystal phase (thermotropic liquid-crystal polymer). Since Vectran fibers, like Kevlar fibers, are spun from a LCP it locks in the oriented crystalline nature and provides the exceptional high performance characteristics of strength, rigidity, and chemical resistance.

Both Kevlar and Vectran LCP fibers, as well as several other fibers, fall into the class of "High Performance Fibers." High performance fibers, as compared to commodity fibers, are "fibers that fall into special technical functions that require special properties unique to these fibers." These special functions may include chemical resistance, tensile strength, operating temperature, limiting oxygen index, and a modulus value. It should be noted that all high performance fibers are not classified as "High Temperature Resistant Fibers." A good example of this is high density polyethylene Spectra® fiber that is gel extruded and classified as a high performance fiber but not high temperature resistant fiber due to its low melting point of 250° F (121° C). A general definition of high temperature resistant fiber is "a synthetic fiber with a continuous operating temperature ranging from 375° F to 600° F (190° C to 316° C)" but this definition may vary depending on the end use of the fiber. Three types of Vectran fibers are being commercially produced by Kuraray Co, Ltd.: Vectran HT; Vectran NT; and, Vectran UM. Airbags made with Vectran woven fabric were used by NASA on the Mars Pathfinder spacecraft. A more down to earth use of Vectran fibers in everyday products include rope/cordage made of Vectran HT fiber or Vectran NT fiber; protective clothing made from Vectran NT fiber which is sometimes blended with another type of fiber; and fiber optic cables reinforced with Vectran UM fibers.

Therefore, Vectran fibers, especially HT and NT, may be encountered in forensic fiber evidence in casework. A review of literature commonly relied on by forensic fiber analysts failed to find identification characteristics for Vectran fibers. To fill this void, the microscopic optical properties, physical characteristics, as well as infrared and Raman data will be presented for the characterization and identification of Vectran fibers. Various equipment was used to determine this data.

**Vectran LCP Fibers, Liquid Crystal Polymers, High Performance Fibers**

## A154 Raman Spectroscopy Offers a Great Potential for Non-Destructive Confirmatory Identification of Body Fluid Traces

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After attending this presentation, attendees will have a better understanding of body fluid identification range of problems and advantages of Raman spectroscopic method as compared to conventional forensic methods employed for the identification.

This presentation will impact the forensic science community by providing information about a novel method of the fast nondestructive confirmatory identification of body fluids found at a crime scene.

The identification of traces of body fluids discovered at a crime scene is a major part of forensic investigation today.<sup>1</sup> The three most common fluids found are blood, semen, and saliva. Several methods are currently used to distinguish one from another. Blood can be presumptively tested using different color spot tests; these tests are destructive to the sample and can also yield false positives. Semen can be presumptively tested using destructive presumptive and confirmatory tests. However, saliva, has no confirmatory tests. Most presumptive tests can be performed in the field, but some sample preparation, such as extraction, is often necessary. Most confirmatory tests must be done in the laboratory. The main problem with these tests is the destruction of the sample. The forensic community is in great need of a reliable, non-destructive, on-field method for identification of all common body fluids.

Raman spectroscopy is a technique increasing in popularity among the different disciplines of forensic science. Some examples involve the identification of drugs, lipsticks, fibers, paint, and ink. The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, nondestructive laser light by a solid, liquid or gas sample. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, nonresonance Raman spectroscopic measurements do not damage the sample. The stain could be tested on the field and still be available for further use in the laboratory for DNA analysis. A portable Raman spectrometer is a reality now that should allow the identification of body fluids at the crime scene.

The development of a new method for identification of body fluid traces using Raman spectroscopy combined with advanced statistics is reported.<sup>2,3</sup> Dry traces of semen, vaginal fluid, sweat, saliva, and blood were analyzed using confocal Raman microscopy with a 785-nm excitation.<sup>4-6</sup> Dry samples of these body fluids are intrinsically heterogeneous. A library of multidimensional Raman spectroscopic signatures that allowed differentiating the traces of body fluids with high confidence was developed. In addition, traces of human and animal blood could be distinguished.<sup>7,8</sup> Overall, this preliminary study demonstrates the great potential of Raman spectroscopy for nondestructive, confirmatory identification of body fluids for forensic purposes.

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#### Raman Spectroscopy, Body Fluid, Biological stain Identification

### A155 Application of Surface Enhanced Raman Spectroscopy to the Forensic Analysis of Blood

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After attending this presentation, attendees will understand the current applications of surface enhanced Raman spectroscopy (SERS) to the forensic analysis of body fluids, specifically blood. This will include the dissemination of the results of the research conducted, as well as an explanation of the mechanisms behind SERS theory and relevant literature review concerning the utilization of SERS.

This presentation will impact the forensic science community by highlighting the benefits of surface enhanced Raman spectroscopy to the forensic analysis of blood through the comparison of techniques commonly used at crime scenes and in forensic laboratories.

Raman spectroscopy is in the defining stages of determination of application in the forensic arena for the identification of unknown materials. Already it has been used to identify various samples, including automotive paint, condom lubricants, gel pen ink, and contraband drugs. Its potential has been recently extended to the analysis of body fluids. Studies have shown that it is possible to achieve a Raman signature of blood that is characteristic of, and unique to blood, even between people. That, in addition to the advent of the handheld Raman spectrometer, makes this instrument an attractive tool for on-scene confirmatory analysis of physical evidence.

Previously, the dilution limitation detection of blood with the Raman spectrometer was investigated and found to be approximately 1:250, using an excitation power of 2mW at the sample plane. This was comparable to presumptive tests for blood performed on scene with swabs, but not comparable to detection limits of luminol and fluorescein. Analysis of blood samples using SERS and Raman Spectroscopy was investigated and was shown to greatly enhance signals coming from samples. The results of the research have demonstrated a SERS tip (nickel rods on a silicon plate, dusted with silver) allows for a new dilution limit of detection of approximately 1:100,000.

In addition to investigating the limit of blood detection with SERS, dried blood was also reconstituted from fabrics and tested using SERS.

Blood was swabbed from fabrics and then rubbed on the SERS tips. Reconstituted blood was found to show signal enhancement; however, no appreciable enhancement seen from the swabs rubbed against the SERS tips. Finally, various plant and chemical material known to give false positives during presumptive tests were examined using Raman spectroscopy. The Raman signatures produced were compared to the Raman signature of blood. Distinct spectra for all tested material were produced, showing Raman spectroscopy has the potential to be used as a confirmatory analysis tool.

In conclusion, SERS can be applied to the forensic analysis of blood, both from blood dilutions and reconstituted blood. Swabs of blood can be analyzed using a Raman spectrometer, but as of yet, no significant enhancement has been achieved using the swabs and SERS. Since Raman spectroscopy produces Raman signatures based on the chemical bonds present in the material tested, unique spectra are produced, leading to the possibility that this instrument may be used as a confirmatory analysis tool.

#### Raman Spectroscopy, Blood, SERS

### A156 Comparison of an Automated Image Analysis Software Versus Visual Examination to Search for Fluorescently-Stained Spermatozoa in Sexual Assault Cases

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After attending this presentation, attendees will have a better understanding of one image analysis software optimized and validated to automate the search for human spermatozoa stained using a fluorescently-based assay and how it compared to visual examination to count spermatozoa.

This presentation will impact the forensic science community by providing an alternative to visual counting of spermatozoa. A routine approach can be developed to automate the scoring of human spermatozoa in sexual assault exhibits. This can enhance case throughput, increase assay sensitivity, and standardize the search for spermatozoa.

The image analysis software was purchased in the hope of developing an automated method of counting fluorescently stained spermatozoa. The fluorescence-based staining assay was first optimized and validated as a replacement to the current human spermatozoa detection method based on phase contrast microscopy. In this assay, the mouse monoclonal antibody specific for human sperm heads is linked with Alexa 488, which fluoresces in green using a FITC filter. A second dye will appear blue when nucleated cells are present using a DAPI filter.

The development of appropriate classifiers within the software was challenging but essential to teach the system to specifically recognize human spermatozoa. As part of the optimization, different minimum and maximum integration times (exposure times) were tested in order to reduce the background without missing any human spermatozoa.

Optimized classifiers were tested/validated using a diverse sample of slides prepared from mock sexual assault samples containing a limited or a large number of human spermatozoa (fecal swabs, vaginal swabs, all mixed with different semen dilutions in addition to urine, blood, and yeasts for a subset of those swabs, contaminated with lubricants, spermicides, medicated creams, and non-human semen). Automated spermatozoa counts were compared to visual spermatozoa scoring. The performance of the image analysis software was recorded with respect to missed spermatozoa, false positives (rejected counts) and time required for the detection of human spermatozoa in each sample.

An excellent concordance was noted between automated and manual counts. Some human spermatozoa were missed by the image analysis software due to their location at the periphery of a classify field (area of search) or outside the predefined circle for searching. False positives or

rejected counts were caused by high DAPI or FITC background in that area and red dots (positive in FITC but negative in DAPI). Most false positives in the image analysis software were quickly rejected by visualizing the gallery (captured cells) on the computer screen. Ambiguous signals/cells were accepted or rejected by visual examination using both the FITC and DAPI filters. Manual scoring of human spermatozoa and the setting up of the image analysis software took the same amount of time. While the image analysis software carries out the automated scoring of human spermatozoa, other tasks can be performed. A major advantage when counting multiple slides is the elimination of eye strain as reviewing galleries shown on the large computer screen is an easy and quick step.

The results of this study indicate that automated scoring of fluorescently-stained human spermatozoa in mock sexual assault exhibits can be carried out reliably and reproducibly using well-developed classifiers for the image analysis software system. The automated scoring of spermatozoa combining the fluorescence-based staining assay and the image analysis software is currently being tested on a large number of sexual assault cases as part of a pilot project within an operational setting.

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**Automated, Spermatozoa, Image Analysis Software**

### **A157 Re-Evaluation of the Seratec® PSA Semiquant Test for Use at the United States Army Criminal Investigation Laboratory**

*James M. DiFrancesco, MFS\*, United States Army, Criminal Investigation Laboratory, 4930 North 31st Street, Building 925, Forest Park, GA 30297-5205; and Joel D. Sutton, MSFS, United States Army, Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297-5205*

After attending this presentation, attendees will be educated regarding the detection of PSA using the Seratec® PSA Semiquant test on samples and how the United States Army Criminal Investigation Laboratory (USACIL) has addressed low levels of PSA present in casework.

This presentation will impact the forensic science community by increasing awareness about false positive PSA results using the Seratec® PSA Semiquant test when performing the test on evidentiary samples. Knowing what substances can be attributed to false positive reactions to the Seratec® PSA Semiquant test, and how to dilute them from the sample without compromising the examiner's ability to identify semen, would help to improve confidence in the results that are presented in court.

It is known that semen contains a high concentration of PSA, making PSA a useful biological marker to identify semen. The Seratec® PSA Semiquant test has been determined to be a valid and reliable method for detecting semen in biological stains. The test works by detecting PSA using two monoclonal antibodies that combine with the PSA to form a complex which is visualized as a red line on a membrane. The sample is extracted in a buffered solution to maintain a constant pH and to help it travel through the test strip. It is also well documented that a small chance of false positive PSA results in the absence of semen. Sometimes these results are from elevated PSA due to a biological phenomenon in an individual. Other times this is a result of non-biological material mimicking a positive result on the test strip. In the literature it has been noted that a change in pH due to the addition of organic acids (citric acid, acetic acid, and oxalic acid) can cause a false positive band on the Seratec® PSA Semiquant card. Examiners at USACIL perform Acid Phosphatase (AP), PSA, and microscopic examinations on a sample to determine if semen is present. Sometimes all three tests are performed on the same cutting. If a sample is only

PSA positive an immunological indication of semen is reported. A review of cases at the USACIL has noted the presence of weak positive PSA results with no male DNA detected in multiple samples from different cases. In order to determine if a case sample is truly positive for semen, a study was performed to test various non-biological samples in order to isolate any that may show a false positive PSA result. Diet soda, alcoholic beverages, tooth paste, lubricants, douche, mouthwash, and various other substances were tested. During preliminary studies it was found that diet sodas gave false positive results when diluted one to one in PBS. The effects of AP reagent on the Seratec® PSA Semiquant test were also examined to determine if there was any interference occurring. Various dilutions were performed to determine if any changes to the current USACIL protocol can help eliminate these false positive results while still being able to detect low levels of PSA from semen.

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**Seratec®, PSA, False Positive**

### **A158 The Microscopic Characteristics of Antemortem and Postmortem Hairs at the Root End**

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After attending this presentation, attendees will see how the microscopic effects various environmental conditions may have on hairs that have been removed antemortem. This presentation will also demonstrate how to microscopically distinguish these hairs from hairs that have undergone postmortem changes.

This study will benefit the forensic science community by providing information on the microscopic characteristics that may be caused by environmental exposure and how these characteristics differ from postmortem banding. Postmortem banding and putrid roots are microscopic characteristics commonly observed in hairs that have undergone postmortem changes. Based on the experience of hair examiners, postmortem banding is generally accepted throughout the forensic hair community as a reliable indication of hair removal during the postmortem process. However, few research studies have been conducted to address the possibility that these characteristics may be observed in hairs removed antemortem.

Results from a study involving 600 hairs collected from fifteen living individuals will be presented. These hairs were exposed to various environments including indoors on a windowsill, submerged in water, buried in potting soil, outdoors on the ground surface, and inside vehicles. The hairs were subsequently microscopically examined at the root end to determine the type of changes that may have occurred as a result of storage in these conditions. Any changes at the root end were then compared to hairs removed from deceased individuals.

The majority of hairs studied (97%) contained roots in the actively growing anagen phase. Hairs in the anagen phase are not completely keratinized and thus more susceptible to changes due to environmental conditions. Two-hundred and fifty hairs were stored in vehicles or indoors on a windowsill for time periods ranging between nine days and 230 days. Two-hundred and fifty hairs were stored outdoors on the ground surface in shaded and non-shaded areas for time periods ranging between seven days and 106 days. One-hundred hairs were submerged in water or buried in potting soil for time periods ranging

between fifteen days and 100 days. No hairs stored indoors on the windowsill and no hairs stored in the vehicles exhibited characteristics of decomposition. Some of the hairs stored outdoors, most of the hairs submerged in water, and most of the hairs buried in potting soil exhibited characteristics of decomposition. Some of these characteristics are similar to characteristics observed in hairs removed postmortem. However, no hairs in this study exhibited characteristics of postmortem banding.

The results from a blind test on the identification of postmortem banding will be presented. The test consisted of over 200 hairs that included all hairs in the presented study with possible changes at the root end as well as hairs known to have been removed from deceased individuals. Initial analysis by the two examiners that completed the test resulted in greater than 99.5% accuracy in identifying postmortem banding. Following a confirmation process whereby each examiner reviewed the initial results, accuracy was increased to 100%.

Results from this study contribute towards the reliability that hairs exhibiting characteristics of postmortem banding are consistent with having been removed postmortem. This study also demonstrates the need for proper training and good quality assurance procedures when identifying postmortem banding.

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**Hair, Postmortem, Banding**

## **A159 The Evaluation of Possible False Positives With Detergents When Performing Amylase Serological Testing on Clothing**

*Nicole M. Novroski, HBSc\*, 58 Dufferin Avenue, Trenton, ON K8V 5C9, CANADA; and Andrea L. Feia, MA, 1430 Maryland Avenue East, Saint Paul, MN 55106*

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After attending this presentation, attendees will know that false positive detection of  $\alpha$ -amylases is not possible when testing clothing laundered in detergents containing  $\alpha$ -amylases to screen for the presumptive presence of saliva on evidentiary clothing.

This presentation will impact the forensic science community by increasing the understanding of presumptive saliva screening methods, and will clarify any misconceptions between the scientific and legal communities regarding the sensitivity and specificity limitations of the Phadebas<sup>®</sup> and RSID<sup>™</sup>-Saliva screening methods employed for the detection of  $\alpha$ -amylase on evidentiary samples.

Amylase detection has become a very useful tool in the screening process for possible saliva stains on forensic evidence. In particular,  $\alpha$ -amylase detection using commercially available tests such as RSID-Saliva or Phadebas<sup>®</sup> Amylase Test are two ways in which the forensic scientist is able to determine if saliva is presumptively present on an evidentiary sample.

The enzyme  $\alpha$ -amylase is naturally occurring in many species of bacteria, fungi, plants, and animals. As a result, the presence of two  $\alpha$ -amylase isoenzymes in humans has led to some difficulty in reporting the presence of salivary  $\alpha$ -amylase versus pancreatic  $\alpha$ -amylase in forensic casework. Moreover, there has been recent speculation from legal professionals that the  $\alpha$ -amylases present in common household laundry detergents may be contributing to positive detection of  $\alpha$ -amylase on evidentiary samples during forensic presumptive screening procedures.

For almost 40 years, detergent companies have been adding enzymes such as proteases, celluloses, and amylases to their products as a more effective method of breaking down tough stains created by polysaccharides and proteins. To determine whether or not  $\alpha$ -amylase detection is possible following routine clothing laundering, unworn, unwashed fabrics of different compositions were laundered in a variety of detergents and stain removing agents. Two assays, RSID<sup>™</sup>-Saliva (Independent Forensics, Hillside, IL) and Phadebas<sup>®</sup> Amylase Test (Magle Life Sciences, Lund, Sweden), that use different methods of detecting  $\alpha$ -amylase, were used to investigate whether detergent  $\alpha$ -amylases in laundered clothing are

detectable. RSID-Saliva detects human salivary  $\alpha$ -amylase via anti-salivary amylase monoclonal antibodies whereas the Phadebas<sup>®</sup> assay takes advantage of the enzymatic properties of  $\alpha$ -amylase present in a given sample. Thus, when using a screening method such as the Phadebas<sup>®</sup> Amylase Test, a positive amylase result is only suggestive, and not confirmatory, for the presence of human saliva.

Five fabric swatches were washed in a volume of laundry detergent pre-determined for a light load wash cycle, exceeding what would typically be required to launder five fabric swatches. This was employed in an attempt to maximize the retention of detergent and detergent additives in the laundered clothing. Nevertheless, all garments tested negative in response to alternate light source, Phadebas<sup>®</sup>, and RSID-Saliva. These findings suggest that at some point during the laundering cycle, the enzymes were damaged, degraded, or removed. The causes of  $\alpha$ -amylase loss during laundering were not examined; however, the data support that detergent enzymes should not contribute to a misidentification of a saliva stain using a presumptive screening method.

Additionally, unlike laundered clothing, undiluted detergents do contain detectable levels of  $\alpha$ -amylase, but these findings were only observed using the Phadebas<sup>®</sup> Amylase Test.

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**Amylase, Saliva Screening, Serology**

## **A160 Capturing the Moment: Photographing Low Level Signals From Serological Testing of Swabs and Cartridges**

*Francesca M. Daher, BA\*, Michael A. Donley, MS, and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

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After attending this presentation, attendees will have learned how to photograph, in a single image, multiple DNA swabs, or cartridge tests with varying levels of signal intensity from presumptive color tests or from cartridge tests for blood or semen.

This presentation will impact the forensic science community by providing an optimal way to photograph the results of time-sensitive Serological tests of varying signal intensities in order to document the findings of a Forensic Genetics analyst.

To photograph the results of presumptive color tests on swabs or the results of blood or semen cartridge tests, the photographs must be taken at specific time interval after the test is completed. The photograph must accurately capture what the analyst observes even when the results are faint. The Forensic Imaging Division and the Forensic Genetics Laboratory of the Harris County Institute of Forensic Sciences conducted a study to establish photographic standards and procedures that would accurately capture and corroborate the results observed by the analyst within the time constraints. This interdisciplinary effort was designed to identify and validate a photographic method so that the images could be used for verification of the analyst's work. These results are intended to provide guidance to other agencies in establishing and validating photographic methods in order that photographs may become a standard and useful part of the case record.

A Nikon D5000 camera with a Nikkor 85mm macro lens was used to perform the tests with an ISO of 200, a shutter speed of 1/125<sup>th</sup> of a second and an aperture setting of  $f/22$ . Due to the fact that several swabs or cartridges are often collected at the same time, these settings had to be able to accurately portray all levels of signal intensity in a single shot. For the swabs, a series of tests on various colored backgrounds, exposure settings, and lighting angles was conducted. Settings were validated by changing one variable at a time and holding the others variables constant. Successful results were observed at -0.3 exposure compensation on an 18% neutral gray background with direct lighting. This proved to yield an image that captured a wide range of signal levels on several different swabs within a single image.

For the cartridges, another series of images was photographed using more extensive exposure compensation settings and lighting angles. Since the cartridges completely fill the frame, there was no need to experiment with different colored backgrounds; however, due to the reflective nature of the cartridge material, additional exposure compensation settings were needed. These images were photographed by changing only one variable at a time in order to identify the best settings for the test. The most favorable results were achieved at an exposure compensation setting of -1.7 with direct lighting.

Presumptive color tests of Serological swabs as well as blood and semen testing on cartridges are integral parts of the Forensic Genetics Laboratory. It is the opinion that all results of visual tests should be photographically documented for verification. Photographs of faint results can be challenging, but a photograph taken under the right conditions can provide a permanent and accurate record of the test results. The tests done in this study will allow for time-sensitive test results to be successfully photographed and verified and will provide guidance for others to achieve the same results.

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#### **Photography, Presumptive Tests, Serology**

### **A161 To Freeze or Not to Freeze?: Science-Based Guidance for Preserving Biological Evidence**

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After attending this presentation, attendees will gain an understanding of research to date that provides guidance on how biological evidence can be best preserved. Further, this presentation will provide an overview of Technical Working Group on the Preservation of Biological Evidence activities to date and preliminary recommendations.

This presentation will impact the forensic science community by bringing awareness to issues in evidence storage and the solutions being proposed by the working group. The administrative burden of evidence management and storage has increased as advances in DNA technology enable better results with less material and more states enact post-conviction testing legislation. Recent DNA stability studies reveal conflicting justifications for biological evidence storage conditions. Crime labs and property and evidence rooms have different purposes, yet coordination is required among both in order to ensure that evidence is properly collected, analyzed, and preserved.

In August of 2010, the National Institute of Justice (NIJ) and the National Institute of Standards and Technology's Law Enforcement Standards Office (OLEs) convened the first meeting of the *Technical Working Group on the Preservation of Biological Evidence Preservation*. The primary objective of the working group is to establish best practices, based in science, to reduce the premature destruction and degradation of biological evidence, thus ensuring its availability for future analysis. After conducting an analysis of the state of biological evidence storage, the group determined that a key barrier to adequate management of biological evidence is the lack of communication and standardized protocols between property and evidence clerks and forensic scientists.

Recent headlines have highlighted significant problems with the storage of potentially exculpatory biological evidence in property and evidence storage units across the country. Court orders for the location of evidence have demonstrated inadequacies in the packaging, storage, and tracking process of some evidence. Investigations into these inadequacies reveal underlying factors such as: capacity of the storage facility, laboratory backlog, materials available for packaging, geographic distance between the collecting and storage facility, and the selected tracking system. The

disposition of evidence is a key consideration as the majority of evidence stored is of questionable probative value. Management of evidence in many long term storage locations is lacking, thus jurisdictions must develop standardized protocols to identify what evidence to keep, how to keep it, and for how long. This presentation will focus on describing ways that forensic scientists, in particular, can mitigate these issues.

The group's key deliverables will include a handbook outlining best practices and standardized protocols for property and evidence clerks, a report on legislative considerations, a report discussing current technological trends and possible applications, and a web-based clearinghouse for biological evidence handlers in the property rooms, courts, and law enforcement agencies.

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#### **DNA, Evidence, Storage**

### **A162 Mixed DNA Analysis**

*Matthew J. Bohn, PhD\*, Analytic Services, Incorporated, 1330 Inverness Drive, Colorado Springs, CO 80910; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

After attending this presentation, attendees will learn how statistically significant information can be extracted from mixed DNA samples. Some statistical techniques are relatively simple to implement such as making random match probabilities on a mixed DNA sample given an individual's DNA profile. Other more difficult techniques will be interesting to the warfighter attempting to capture a terrorist cell or a narcotics unit attempting to track a drug gang. These more sophisticated techniques extract minor commonalities in the DNA profiles using the techniques of Principal Component Analysis (PCA) and clustering algorithms.

This presentation will impact the forensic science community by demonstrating mathematical techniques that can be used to extract information from DNA samples containing more than one contributor.

Currently, mixed DNA samples face difficulties linking an individual to a crime scene or location. However, in an expeditionary environment faced by a military force, evidence linking a group of individuals could be of monumental importance. As a hypothetical example, a recently confiscated cell phone contains DNA from three individuals of al-Qaida and a mixture of four random, innocent individuals can be found to be statistically similar to a mixed DNA sample found on an IED. Cluster analysis can be used to separate the group of al-Qaida members from the random, innocent individuals. In addition, statistics can be used to ascertain the random match probability of an individual in the group. For example, the warfighter could ascertain with what probability the individual holding the cell phone is a member of the al-Qaida group that built the IED.

Analysis techniques will be discussed for mixed DNA from crime scenes, confiscated material, and opportunistic collections. A wealth of intelligence information hidden exists within mixed DNA samples. World-wide analysis and databasing of DNA mixtures will lead to increased awareness of the world-wide threat while simultaneously providing actionable intelligence to the commander in a local environment. Statistical analysis of mixed DNA samples using Principal Component Analysis (PCA) and cluster analysis enables tracking, group identification, and probability analysis of individuals in the group. Computer simulations will be presented demonstrating the exciting potential for the use of DNA mixtures. Based on a NIST database, a computer program was developed to generate random DNA profiles representative of the allelic frequencies in the database. These profiles could then be mixed and a random match probability of a random individual to the group calculated. The random match probability is then plotted versus the number of contributors. One example from the data is that with ten contributors to a mixed profile, the probability of a random individual's profile appearing in the mixture is one out of ten. With four contributors, the random match probability drops to one in 3,333. Obviously these numbers are far from the typical one in five billion random match probabilities for a single profile; however in an expeditionary setting, they may help to uncover a terrorist cell.

Given sufficient data, groups can also be identified and tracked in mixed DNA samples. A collection of 60 computer generated samples were analyzed. The 60 samples consisted of three groups: (1) completely random; (2) four random profiles plus three consistent profiles; and, (3) four random profiles plus three different, consistent profiles. The concept being modeled is that three members of a terrorist cell or drug gang might all touch a cell phone, which is then thrown away and touched by four innocent individuals. This leads to an aggregate DNA sample that contains a mixture of seven individuals. Can these groups be identified and separated? Yes, with reasonable probability, the method of PCA coupled with cluster analysis can separate the groups. In addition, the total number of alleles in these mixtures was analyzed using a Monte Carlo technique and depending on the total number of alleles, an estimate can be made on the total number of contributors.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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**Mixed DNA, Principal Component Analysis, Cluster Analysis**

## **A163 The Partial Match Process in New York City**

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After attending this presentation, attendees will be introduced to a functioning, large-scale partial match operation. The presentation will illustrate how the SWGDAM recommendations for partial matches have been put into practice within the State and City of New York.

This program will impact the forensic science community by addressing the uses of DNA to generate investigative leads when there have been no direct matches to a profile.

In early 2008, the FBI hosted a conference for CODIS laboratories and other interested parties about partial matches, familial searching, and the various ramifications thereof. The SWGDAM Interim Plan for Partial Matches was introduced (and subsequently adopted), which makes recommendations to the community but leaves policy decisions to each state. Shortly thereafter, the New York State Commission on Forensic Sciences, DNA Subcommittee also took up the discussion. In anticipation of a statewide policy, the New York City Office of Chief Medical Examiner (OCME) Department of Forensic Biology began to review potential partial matches to be pursued when the policy took effect.

Partial matches are inadvertently discovered—for instance, moderate stringency matches in CODIS during a routine search, or an evaluation of profiles within a case. These exclude the person brought forward in CODIS as a candidate match as being the actual source of the forensic profile, but there is a chance that a close relative could be the source.

Partial matches are NOT familial searches. Familial searches are deliberate searches seeking similarities between a forensic sample in a database and an offender. Searches of this sort require specific non-CODIS software and are only conducted in a few jurisdictions at this time. In order for a profile to be considered for partial match evaluation in New York City, there are some basic criteria that must be met before even performing any calculations. First, it must be a previously unmatched forensic profile of  $\geq 10$  loci. Second, it must be a clean (or fully-deconvoluted) profile—there can be no partially-determined loci, even though such profiles are eligible to be entered into CODIS. At the local level there is a distinction between partial matches obtained through a database search or within a case. At the state and national search levels, only forensic-to-offender partial matches are considered.

The next screening step is to determine how many alleles are shared between the two profiles. For a parent-offspring relationship, the threshold is 50%, because there only needs to be one allele in common at every locus. At any given locus, siblings are not required to share any alleles at all; even when they are the offspring of the same two parents (most siblings have around half in common). Conversely, some siblings share a high percentage of alleles, and these are the siblings for which partial matches may be useful. Based on results from the literature, it was determined that having  $\geq 70\%$  shared alleles maximizes the probability of a sibling relationship between two profiles, while minimizing the probability of more distant relatives, or of unrelated persons. Any pair of profiles fulfilling one or both of these scenarios is evaluated using the CODIS PopStats tool. Further testing (Y-STR or mitochondrial DNA testing) could be undertaken to confirm or refute the possible partial match between two local cases or samples if necessary.

For potential matches involving offender samples from SDIS or NDIS, the New York City Police Department (NYPD) and the appropriate District Attorney's Office (DAO) are queried regarding their interest in the case. Both entities must commit to pursue further investigation of the case if the name of the offender (potential relative) is released and to provide follow-up information to the New York State Division of Criminal Justice Services regarding the outcome. Only after affirmative responses are received from both NYPD and DAO will the OCME formally request the name of the partially-matched offender from the offender database. For non-New York requests, the process is also dependent on the CODIS unit (FBI) deeming it valid to pass along to the offender laboratory and whether that state will release offender names for partial matches.

For those requests from local labs within New York State, the SDIS staff at the New York State Police performs additional kinship calculations for the expected match ratio (EMR) and expected kinship ratio (EKR) as defined in the SWGDAM Interim Plan for Partial Matches. If the results meet their threshold, they advise the databank coordinators to release the name to the local lab. The name is then reported to the NYPD and DAO.

The partial match process began in the spring of 2011. Results regarding the outcomes for the partial match process are expected by early 2012.

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**Partial Match, DNA Database, Kinship Calculations**

## **A164 Combining DNA Evidence for Greater Match Information**

*Mark W. Perlin, PhD, MD\*, Cybergenetics, 160 North Craig Street, Suite 210, Pittsburgh, PA 15213*

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Most fields of scientific enquiry routinely combine data from multiple experiments. These experiments can be repetitions drawn from one item, or involve different items entirely. The motivation is to elicit maximal information from an experimental design. The statistical mechanism is the joint likelihood function.

DNA evidence is often challenging to interpret. When a DNA sample has low quantities, is degraded, or contains several individuals mixed together, no single allele pair may be able to explain the STR data. Multiple allele pair possibilities then become feasible, with an associated loss of identification information. It can then become important to use all the available experiment data in order to restore information.

In forensic DNA science, human data interpretation is usually performed on data derived from just one item. This practice is a natural consequence of "thresholds" – applying a preset peak height level to quantitative peak height data in order to simplify human data interpretation, which creates artificial all-or-none qualitative allele possibilities. However, the resulting genetic profiles cannot be mathematically combined. Some groups may heuristically combine profiles after data interpretation to form a "consensus" profile, but this practice has little statistical justification.

Quantitative computer interpretation of continuous electrophoretic STR data signals, however, has no such artificial limitation. It is therefore

natural to mathematically preserve identification information by inferring a genotype using a joint likelihood function that examines all the independent data simultaneously.<sup>1</sup>

A likelihood function mathematically quantifies how well alternative hypotheses explain a fixed data result. A joint likelihood function assesses these hypotheses on multiple data items simultaneously. Typically, the data are drawn from independent experiments. Therefore, the joint likelihood simply multiplies together the likelihood numbers from separate experiments, jointly conditioned on a common explanatory hypothesis.

This talk describes the joint interpretation of DNA evidence. It is shown how likelihood functions can be used to rigorously explain DNA evidence, and how joint likelihood functions can combine evidence. Results that show how the number of assumed contributors affects the inferred result, and why appropriately constructed likelihood ratios (LR) do not overstate the inferred DNA match information will be presented. These concepts on representative DNA mixture criminal cases and experiments will be illustrated.

In particular, using a joint likelihood function on DNA mixtures, we show:

- How a joint examination of the data signals from 10% and 50% mixtures can infer a highly informative genotype, with a combined LR match statistic a million times greater than either separate analysis;
- A criminal case where none of the three data signals from a low-template three person mixture was informative in isolation, but when jointly examined they together produced a DNA match statistic LR over a million; and
- Strategies for effectively combining the data signals of different mixture evidence (from one or more items) in a joint interpretation that retains more DNA identification information.

Forensic DNA is an information science that centers on inferring genotypes. More informative genotypes can lead to greater match information. By combining DNA evidence, as taught in this presentation, a practitioner can extract more information from their existing data, and make more accurate DNA identifications.

#### Reference:

- <sup>1</sup> Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. *Journal of Forensic Sciences*. 2011;56(November): in press.

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#### Combining Evidence, DNA Mixture, Likelihood Ratio

### A165 A Study of the Effects of Database Size and Loci Number on the Potential for Successful Familial Searches in Databases of STR Profiles

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After attending this presentation, attendees will understand the logic used to perform familial searches and how results are influenced by methodologies, database size, and genetic loci utilized.

This presentation will impact the forensic science community by clarifying potential problems involved with familial searches and how to best overcome them through the use of optimal scientific methods.

Over the past several years, the utilization of familial searching tools by law enforcement to locate relatives of the perpetrators of violent crimes

has received a good deal of publicity in the media. This has initiated a good deal of public debate over potential violation of civil rights of those individuals in DNA databases who are identified by familial searches as “persons of interest.” There is a legitimate potential for several individuals completely unrelated to perpetrators of violent crimes to arise as persons of interest when database searches are performed, particularly if the searching technique used does not have a strong discrimination power for locating relatives. Even when optimal methodologies are used to carry out familial searches, the number of false positive and false negative results in familial searches will be affected by the number of loci used and the number of profiles in searched databases.

A generally accepted methodology for assessing putative kinship is via the utilization of a Kinship Index (KI), which is a likelihood ratio comparing the likelihood of obtaining genetic results from individuals that are biologically related to the likelihood of obtaining the same genetic results from unrelated individuals. A less robust method of assessing kinship is via allele sharing (identical by descent in biological relatives vs. identical by chance in non-related individuals). In this study we compute KIs and number of shared alleles in comparisons of STR profiles from simulated evidence items to databases of varying size that also contain profiles of known relatives of the donor of the simulated evidence profile. In making these comparisons, the number of loci in the evidence profile is also varied to obtain data on the likelihood of successful searches based on loci used in the search. These data provide the practitioner of familial searches with a reasonable framework within which to establish guidelines to optimize the chances for carrying out successful searches in the future. Additionally, they illuminate the impact of database size on the frequency of false positive hits when performing familial searches. This presentation will examine possible means to reduce false negative and false positive hits that arise, such as the comparison of Y-STR haplotypes for male samples in databases, or comparison of most likely race of evidence and database profiles.

All databases used in these studies were generated from simulated profiles based on an algorithm that utilizes input STR allele frequencies from published data. Profiles of biologically related individuals for search purposes were either obtained from families of known racial/ethnic origin or generated from profiles in databases using rules of Mendelian inheritance.

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#### Familial Search, DNA Profile Database, Y-STR Haplotype

### A166 Alternative Sexual Assault Kit Processing

*Michael A. Donley, MS, and Roger Kahn, PhD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054; Christy Smejkal, MS\*, Harris County Medical Examiner's Office, Forensic Biology Section, 1885 Old Spanish Trail, Houston, TX 77054; and Michal L. Pierce, MS, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will understand the pros and cons of an alternative approach to processing sexual assault kits. This presentation will aid attendees in deciding the method to use when processing sexual assault kits. Presented here are the results after implementing the alternative processing method and its comparison to the traditional method.

This presentation will impact the forensic science community by demonstrating an alternative method of processing sexual assault kits and the impact it will have on the evidence being processed.

In 2010, the Harris County Institute of Forensic Sciences Forensic Genetics Laboratory (HCIFS) initiated the use of an alternate processing scheme for sexual assault kits. A portion of each available swab is cut and sent directly to differential extraction without presumptive or confirmatory testing for semen or saliva. This is done regardless of the information in the complainant's narrative. Vaginal, anal, oral, debris swabs (i.e., breast, neck), and swabs of fingernail scrapings are cut and sent directly to

differential DNA extraction. The new method simplifies the activities in Serology while greatly increasing the number of samples needing differential extraction. The HCIFS DNA Lab utilizes high capacity robots for DNA extraction, quantitation reaction set-up, sample normalization, and STR reaction set-up, making the large increase in the number of differential extractions possible.

After eight months of processing kits with this method, the results were evaluated for oral swabs, neck swabs, breast swabs, and swabs of fingernail scrapings – locations where semen is not typically present. Vaginal and anal swabs were not evaluated because semen, if present, is typically found on these swabs. Oral swabs from 153 cases were examined. In 131 cases, the samples were terminated (i.e., testing was halted after quantitation) due to a lack of male DNA. Of 22 cases which were amplified, 18 cases were from a male complainant and in all but one of the cases from a female complainant, only the complainant's profile was observed. In the one instance of a male profile in the male fraction of an oral swab, the narrative indicated that an oral assault had occurred. Differential extraction of these swabs does not appear to be necessary.

Differential extraction from neck and breast swabs is not necessary to observe male profiles. In the traditional method (without differential extraction), 50-70% of these samples yielded foreign male profiles. With the new method where debris swabs were tested only by differential extraction, all of the sperm fractions were terminated after quantitation due to a lack of male DNA. For neck swabs, 80% of the non-sperm fractions were amplified and all revealed mixtures that included male profiles. Similar results were seen in swabs from fingernail scrapings. Again, this indicates that differential extraction of these samples is not helpful since semen is not typically present on these swabs.

These results suggest a combination approach. Vaginal and anal swabs should continue to be tested without screening in Serology - testing for PSA and for sperm will continue to be conducted during DNA extraction. Oral, neck and fingernail swabs should be subjected to regular, not differential extraction unless the female complainant states in the narrative that the suspect ejaculated on her breast, neck, thigh, etc. If the female complainant reports the suspect licked her breast, neck, thigh, etc. then the swabs should be test by regular extraction. As an alternative, all such swabs could be screened for PSA. Those that are positive could be differentially extracted.

In any case, the handling will still be more efficient than conducting a full screen in Serology, although a change will increase the work on the Serology lab. Each laboratory can decide which method (traditional, alternative, or a combination) is best suited for their laboratory.

**Sexual Assault Kit, DNA Extraction, Processing Time**

## **A167 Update on DNA-PROKIDS: Fighting Human Trafficking With DNA Analysis**

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After attending this presentation, attendees will know about the impact and success of the DNA-PROKIDS program that has generated databases

in more than ten countries and has solved over 350 cases of child trafficking and illegal adoptions.

This presentation will impact the forensic science community by showing how human trafficking and illegal child adoptions are closely related, and how DNA analysis has shown to be an invaluable tool to fight these crimes.

Human trafficking has become one of the main criminal problems throughout the world, and it is rapidly becoming the primary crime in economic terms. One forensic science-based approach that can assist in the effort to combat human trafficking is through the use of the tools for human identity testing. DNA analysis can play an important role in verifying alleged kinship relationships. Forensic DNA testing for identification of human remains and routine casework is used throughout the world and is a technology that is readily accessible. Nevertheless, the use of DNA analysis is not used extensively in the efforts to address the epidemic of missing children. More than one million children are reported as actively "missing," and likely this number is grossly underestimated. Many of these missing children have been separated from their families and are being trafficked for various exploitations. DNA typing of these children and alleged relatives can verify or refute biological relationships that could be extremely useful in securing trafficked and exploited children and in assisting law enforcement in identifying traffickers and their networks. Moreover, database of DNA profiles from parents who report missing children and children who are offered for adoption or abandoned will facilitate the identification process on national and international scales. In 2006, the University of Granada, Spain, initiated the DNA-PROKIDS Program, and in 2009 the Center for Human Identification, University of North Texas Health Sciences Center, joined in the program as a primary partner. In addition to providing a DNA analysis service, DNA-PROKIDS is a program that develops DNA collection kits, protocols, methodologies, software, and educational materials for the use of DNA typing of these trafficked and abandoned children, and to assist in combating trafficking. This program has been able to function by support from the Spanish Government, several large Spanish banks including BBVA, Santander and CajaGranada-BMN, and donations from the Life Technologies Foundation.

Two databases are needed to form a functional search capability for child identification. One is a Reference Database (RD) of DNA profiles (composed primarily of autosomal STRs, and may include Y-STRs and mtDNA markers) obtained from biological samples (buccal swabs) voluntarily provided by mothers and other family members of missing children. The other database is a Questioned Database (QD) of DNA profiles (composed of the same genetic markers used for profile generation for RD) obtained from children who have been found without their families, are being exploited, or are known to be victims of human trafficking. Each country that has agreed to be part of the DNA-PROKIDS network maintains its own RD and QD. The DNA profiles are searched within and between a country's databases in an effort to identify missing children within the country. International sharing is performed on a case-by-case basis and currently is a manual process. DNA-PROKIDS has partnered with and has supported the development of such DNA databases in six of the countries within Latin America (Mexico, Guatemala, El Salvador, Paraguay, Peru, and the Dominican Republic) and within four of the countries in Asia (the Philippines, Thailand, Sri-Lanka, and Indonesia).

To date, over 2,500 samples have been collected by the different participating countries, and over 330 positive identifications have been made. Some experiences and notable cases will be described. In September 2010, Guatemala passed the Alba-Keneth Law that requires mandatory collection and analysis of biological samples from all children found without relatives and offers, by law, all relatives of missing children the opportunity to donate a biological sample for the DNA-PROKIDS Guatemalan database. It is believed that this is the first law of its kind throughout the world. The database is coordinated in Guatemala through its National Institute of Forensic Sciences (INACIF).

A greater global coordinated effort is needed to continue to identify trafficked and exploited children and to dramatically increase the success

that these DNA-PROKID pilots have experienced. The initial results and experiences obtained by DNA-PROKIDS clearly demonstrate the application of forensic DNA analyses and its supporting technical infrastructure can play an important role as a deterrent of this terrible crime.

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**DNA-PROKIDS, Human Rights, DNA Databases**

## **A168 Evaluating the Probative Value of Sexual Assault Evidence Collected From Suspects**

*Chani Sentiwany, MS\*, Oakland Police Department, 455 7th Street, 6th Floor, Oakland, CA 94607; William M. Green, MD, University of California Davis, Medical Center, 2315 Stockton Boulevard, Sacramento, CA 95817; Jennifer S. Mihalovich, MPH, Oakland Police Department, Criminalistics Division, 455 7th Street, Room 608, Oakland, CA 94607; Edward A. Panacek, MD, University of California Davis, Medical Center, 2315 Stockton Boulevard, Sacramento, CA 95817; and George Sensabaugh, DCrim, University of California - Berkeley, School of Public Health, 50 University Hall, MC 7360, Berkeley, CA 94720*

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After attending this presentation, attendees will appreciate the value of collecting suspect sexual assault kits on a regular basis and of the use of a standard sexual assault kit format.

This presentation will impact the forensic science community by introducing how victim DNA can be obtained from sexual assault suspects. Demonstrations on how victim DNA can be detected on penile swabs and finger swabs collected from sexual assault suspects up to and at least 28 hours after the alleged assault.

Over 80,000 forcible rapes are reported in the U.S. annually. Many states have established protocols for the medical forensic examination of sexual assault victims and a national protocol was published by the President's DNA Initiative in 2004. It is believed that only California has a standardized protocol with instructional guidelines, along with a forensic medical report form, for the collection of evidence from suspects. When a sexual assault suspect is known and available, collection of evidence from this suspect in a timely fashion may aid in corroborating the victim's report.

The objective of this study was to evaluate the probative nature of evidence obtained during forensic exams of suspects in sexual assault cases. This retrospective study reviewed findings from 106 suspect sexual assault kits (encompassing 102 cases) collected in the City of Oakland, California from 2000 through 2009. This included review of 49 suspect sexual assault kits collected at a county designated hospital prior to February 2006 and 57 kits collected by a contract service at the county jail after February 2006. Data from the California suspect forensic medical report (CalEMA 2-950 form) was collated with the appropriate data from the corresponding victim kit forensic medical report (CalEMA 2-923/930 forms). The data and relevant information were translated into a database structure. Information from the Oakland Police Department Criminalistics Laboratory analyst case notes including the victim and suspect kit inventory, examination notes, microscopic examination form, and genotyping data table(s) was integrated into the database.

The sample collection protocols differed between the two collection agencies. Prior to 2006, during collection at the county hospital, a single swab was used to collect from the suspect's penile glans and shaft. Scrotal swabs, separate glans and shaft penile swabs, and finger swabs were not collected on a consistent basis. After 2006, a procedural change occurred as collection was shifted to the county jail: finger swabs, separate swabs for penile glans and shafts, and scrotal swabs were collected on a regular basis. For this study, of the 106 kits examined, 104 kits contained genital swabs and 54 contained finger swabs. In total, DNA profiling was performed on 130 genital swabs and 94 finger swabs. No DNA typing was done on 18 of the kits based on analyst discretion, failure to detect epithelial cells, or redundancy of samples.

A standard protocol was used for the examination of the swabs. The swabs were extracted and the extracts were examined microscopically for cellular material. Typically, samples with epithelial cells or sperm were

taken to DNA typing. In some cases, based on analyst discretion, samples without epithelial cells were also taken to DNA typing. Prior to September 2007, amplification was performed with the AmpFISTR Profiler Plus® typing kit. For cases after September 2007, samples were amplified with the AmpFISTR Identifier® typing kit. DNA profiles containing DNA from someone other than the suspect were classified as having "Foreign DNA." Profiles with foreign DNA were categorized as "Probative" if the victim could not be eliminated as a possible source. The post assault interval (PAI) time was determined for each kit using date and time of the alleged sexual assault stated on the victim forensic medical report or police report and the date and time of penile swab collection noted in the suspect forensic medical report.

Probative DNA typing results were obtained from at least one genital swab in 42% of cases (44 of 104) or finger swab in 30% of cases (16 of 54). Half or more of the possible victim's DNA profile was detected on at least one genital swab in 24% (25 of 104) and on at least one finger swab in 17% (9 of 54) of cases. Full victim DNA profiles were obtained on at least one genital swab in 17% (17 of 104) or finger swab in 11% (6 of 54). A full victim DNA profile was obtained from a genital and a finger swab out to 18 hours and a 60% DNA profile on a genital swab was obtained at 28 hours.

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**Suspects, Sexual Assault, DNA Profiling**

## **A169 Retrospective Study of 150 Sexual Assault Cases**

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After attending this presentation, attendees will have an understanding of the importance of blind collection of potential oral contact site swabs, the possible discrepancy between vaginal smear slide results obtained by forensic medical examiners versus the forensic laboratory scientist and the importance of proper collection of rectal swabs. Additionally, overall DNA typing results of these cases will be reported.

This presentation will impact the forensic science community by adding to the practical knowledge of the laboratory scientist and forensic medical examiner and potentially affecting policy change with respect to sample collection post sexual assault.

According to the Bureau of Justice Statistics, approximately one in five women is sexually assaulted during their lives. DNA technology in combination with CODIS databases is a powerful tool for the identification of sexual assailants. However, without proper evidence collection, DNA typing from sexual assault cases could not be conducted. Thus the individuals responsible for the crimes would not be identified. In that vein, it is absolutely imperative to discern the best methods of evidence collection, preservation, and analysis.

The evaluation of 150 sexual assault cases collected by medical practitioners at Highland Hospital in Oakland California and examined by Criminalists at the Oakland Police Department (OPD) Criminalistics Laboratory was conducted. Cases assigned the California penal code: 261 and analyzed by the Criminalistics Laboratory between August 2003 and October 2007 were examined. No other penal code cases were utilized. These cases include only sexual assault examination of female victims aged 14 years and older and were selected from approximately 300 forcible rapes per annum from the same time period.

Microscopic examination of the vaginal smear slides by the forensic medical examiner resulted in observation of sperm 13% of the time. When the examiner observed sperm, the crime laboratory scientist also observed sperm on the corresponding vaginal swab 100% of the time. Of the 119

cases which were sperm negative when examined by the forensic medical examiner and the nine cases in which no wet mounts were prepared, subsequent examination of x-mas stained slides in the forensic laboratory detected sperm in 57 cases, a yield of 48%. Of these 57 cases, complete foreign DNA profiles were obtained in 37 cases (65%), partial profiles with sufficient data for upload to CODIS were obtained in 10 cases, and few alleles were obtained for six cases. The evaluation of these 150 cases resulted in probative DNA profiles being obtained from 100 cases. Oral contact samples yielded the only probative DNA profiles in 10% of cases. Twenty victims reported a lapse of consciousness. Potential oral contact samples from these victims were not ubiquitously collected. Policy should be implemented such that neck, face and breast swabs are routinely collected from victims.

There are 21 kits with reported penile anal penetration. One case had no anal or rectal swabs collected even though there was a report of anal penetration. Eleven (52%) cases had anal swabs collected and 10 (48%) cases had rectal swabs collected (one of these cases had anal and rectal swabs collected with an anoscope used for the rectal swab collection). Seven (33%) cases had rectal swabs collected using an anoscope. An additional case may have had an anoscope used for the collection of the rectal swab; however, the writing on the sexual assault medical report was illegible. This means, including the report with the illegible writing, rectal swabs were collected improperly (without the use of an anoscope) for four cases and no rectal swabs were collected for 10 cases. Rectal swabs need to be collected via anoscopy for all reports of anal penetration.

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#### **Sexual Assault, Forensic Evidence, Oral Contact**

### **A170 The Analysis of Sexual Assault Kit Backlogs: Scientific Results and Criminal Case Dispositions**

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After attending this presentation, attendees will be familiar with the scientific results from the testing of backlogged sexual assault kits, the success in uploading DNA profiles into CODIS, and the criminal justice dispositions of a sample of backlogged cases vs. a sample of sexual assaults that were tested currently.

This presentation will impact the forensic science community by increasing awareness of a growing national problem and how the role and impact of forensic science in backlogged sexual assault kit cases is mediated by the relationship between the assailant and victim and associated demographic and investigative variables.

Backlogged and untested sexual assault kits (SAKs) are a major problem facing forensic crime laboratories and law enforcement agencies throughout the United States. The combined untested SAKs from the Los Angeles Sheriff's Department (LASD) and Los Angeles Police Department (LAPD) reached 10,895 cases in the fall of 2008. The chief executives of both agencies announced that all backlogged kits would be tested, using outside private DNA testing laboratories. The Sexual Assault Kit Backlog Project was funded by the National Institute of Justice (NIJ) to: (1) evaluate the results of scientific tests performed on outsourced backlogged sexual assault kits; (2) review the sexual assault case processing literature; (3) determine the criminal justice dispositions of backlogged and nonbacklogged cases before and after kit testing; and, (4) identify case and evidence characteristics useful in prioritizing sexual assault evidence submitted in the future.

Researchers from California State University, Los Angeles collected a 20% random sample (1,948 cases) of backlogged cases from the more than

10,000 tested cases in order to evaluate the scientific results. The study design also included a smaller subsample of 742 backlogged and nonbacklogged cases to determine final criminal justice dispositions, before and after testing. The research design also included a review of the sexual assault case processing literature and focus groups with sexual assault investigators, prosecuting attorneys, and criminalists to discuss the role SAK evidence plays in resolving both stranger and nonstranger sexual assaults.

Data from tested SAKs were drawn from crime laboratory files and revealed 93% of victims were female, two-thirds knew their assailants, almost half reported they were intoxicated with alcohol or drugs at the time of the assault, and more than three-quarters (77.3%) sustained one or more injuries. The average post coital interval between the time of the assault and victim exam was 23.3 hours, three-quarters reported vaginal penetration, and more than one-quarter thought the assailant had ejaculated.

Laboratory results included screening tests to find different biological markers (sperm, P30, Y chromosome, acid phosphatase, amylase, and epithelial cells) from various orifices and in dried secretions on the body. Semen screening tests and Y chromosome tests were successful 40% to 50% of the time on samples from the vaginal and external genitalia areas and varied widely on samples from other areas. Y chromosome tests were also successful about 50% of the time for dried secretions. STR analyses found male DNA in about 80% of attempts with samples from the vagina and two-thirds of samples from external genitalia and dried secretions. Full DNA profiles were determined in two-thirds of DNA samples taken from the vaginal area, but in a lesser percent from other body regions. Success in finding foreign DNA and achieving CODIS Uploads decreased as post coital interval (PCI) increased.

The crime labs were successful in uploading profiles to CODIS almost 60% of the time and in achieving offender "hits" in about 45% of inquiries and case to case hits in less than 3%. For the SAK backlog sample (n=371), no new arrests occurred after SAK testing occurred, but one filing and two convictions did. Almost 40% of these sampled cases had previously resulted in arrests and 18% convictions without the benefit of a SAK analysis. For the matched sample of 371 nonbacklogged cases, almost the same percentage of cases resulted in arrest, filing, and conviction prior to SAK testing. After the tests, however, a modest percent of new cases resulted in arrest (2%), filing (5%), and conviction (12%). Focus group participants felt that testing of kits should not be mandatory and that future kits testing must reflect investigator evaluation. Many cases in the backlog were those where identity was not an issue and the alleged crime was a question of consent. A system of priorities needs to be established to determine which cases (and which evidence within the kits) need to be tested and recognize that forensic resources are limited. DNA can contribute to both stranger and nonstranger cases, but the SAK and the case at hand require evaluation before testing. Also, although uploading DNA profiles into CODIS may also have value in the long term, many of the backlog "hits" that occur are those where the assailant's DNA profile has already been entered (upon his arrest or conviction) on the same case and is redundant. A priority scheme with scientific, investigator and prosecutor input should be devised and implemented.

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#### **Sexual Assault Kits, Case Backlogs, Criminal Dispositions**

## **A171 Assessment of Acidified Hydrogen Peroxide vs. Cyanoacrylate Ester/Rhodamine 6G Processing for Developing Latent Fingerprints on Brass Cartridge Cases**

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After attending this presentation, attendees will have an understanding of the comparative performance of Acidified Hydrogen Peroxide vs. Cyanoacrylate Ester/Rhodamine 6G for developing latent fingerprints on brass cartridge cases.

This presentation will impact the forensic science community by discussing which processing method develops latent fingerprints with higher quality (Acidified Hydrogen Peroxide vs. Cyanoacrylate Ester/Rhodamine 6G).

Cyanoacrylate Ester (CA) fuming followed by Rhodamine 6G (R6G) fluorescent dye stain is a common processing technique for latent prints on non-porous evidence. The latent print residue will initiate the polymerization of the CA monomers forming a white, fibrous material thus developing the latent print. R6G fluorescent dye stain can then be applied which will absorb into the fibrous matrix of the CA polymer. The latent print can then be visualized at 532nm using a LASER or alternate light source. Another common method for developing latent prints, specifically on brass cartridge cases, is Acidified Hydrogen Peroxide (AHP). Fired cartridge cases typically appear dark in color due to the firing process. AHP will oxidize the brass cartridge case in all areas except where the latent print residue protects the brass resulting in a light colored appearance except in those areas below the latent print residue. The latent print can then be visualized under ambient lighting conditions. These two processes may also be used in sequence when processing cartridge cases (CA/R6G followed by AHP). Because AHP is an irreversible reaction, there may be a potential for the process to corrode the brass and interfere with forensic firearms examinations. Because of this, consideration should be given to determine the success of this technique over other non-destructive processing methods available. This research, therefore, seeks to understand the severity of potential corrosion to the brass and which of these processing methods (CA/R6G vs. AHP) develop latent prints with higher quality.

A total of 225 brass cartridge cases were used in this evaluation which was comprised of three different processing methods and using two types of latent print matrix standards (sebaceous and eccrine). One latent print was placed on each of the sixty 9mm cartridge cases, sixty .556 cartridge cases, and forty-five M16 cartridge cases using the sebaceous latent print matrix standard. Additionally, one latent print was placed on each of the sixty .556 cartridge cases using the eccrine latent print matrix standard. For each set of cartridge cases, one-third was processed with CA/R6G, one-third was processed with AHP, and one-third was processed with CA/R6G followed by AHP. All CA processing lasted approximately fifteen minutes followed by a ten minute vent sequence then rinsed with R6G. All AHP processing lasted until a latent print developed (range 29 – 75 seconds). All latent prints were photographed and the development times for all cartridge cases developed with AHP were recorded. Fifteen of each set of latent prints were digitally presented to a certified Latent Print Examiner for evaluation. The Latent Print Examiner rated the quality of development according to a numeric rating scale (0 to 5) corresponding to the quality of the friction ridges developed. Data was evaluated according to which processing method developed latent prints with higher quality, whether the results varied according to the type of cartridge case used, and whether the results varied according to the latent print matrix standard used.

Results indicate CA/R6G and CA/R6G followed by AHP consistently developed higher quality latent prints than AHP alone for all types of cartridge cases and latent print matrix standards ( $p < 0.001$ ). Statistical differences were observed between types of cartridge cases and the processing methods with CA/R6G developing higher quality latent prints on the M16 cartridge cases and AHP developing higher quality latent prints on both the .556 and M16 cartridge cases compared to the 9mm ( $p < 0.01$ ). The quality of latent print development for each processing method appears to be dependent on the type of latent print matrix with AHP developing higher quality latent prints using the sebaceous matrix and CA/R6G and CA/R6G followed by AHP developing higher quality latent prints using the eccrine matrix ( $p < 0.001$ ). Results also indicate no difference in the time of latent print development using AHP only vs. AHP following CA/R6G. Lastly, results indicate CA/R6G develops at a much quicker rate when using the eccrine matrix, but further research is warranted to better understand whether this time difference is significant. These results warrant further research to better understand how time and normal environmental degradation will impact the quality of the latent prints developed using these methods and using other types of cartridge cases.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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**Forensic Science, Cartridge Cases, Fingerprint Development**

## **A172 Latent Print Development Using Low Pressure Sublimation Vapor Deposition: Evaluation of a Prototype System**

*Henry Swofford, BS\**, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; *Calvin Knaggs, BS\**, Linde Canada Limited, 5860 Chedworth Way, Mississauga, ON L5R0A2, CANADA; and *Josh Ballard, BS*, Chipper Beegle, BS, and *Sarah Harbin, BS*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297

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After attending this presentation, attendees will be introduced to novel technology designed to improve the process of developing latent fingerprints using many of the traditional chemical and physical development methods while eliminating the need for the use of any hazardous and destructive chemical solvents.

This presentation will impact the forensic science community by discussing the mechanical operation of the system; the results of a performance evaluation comparing the quality of latent fingerprints developed using this system to traditional processing methods, and other improvements this system may provide over traditional processing methods for the development of latent fingerprints.

Numerous and various processing methods for the development of latent fingerprints have been introduced over the years, but many require costly hazardous and destructive chemical solvents to yield successful results. Chemical solvents are the most significant contributor to the destructive nature of latent print examinations. Because of this, laboratory examinations of evidence must adhere to specific processing sequences through the various forensic science disciplines in order to minimize or eliminate the potential loss of evidence as a result of a particular examination method. Novel technology involving a sublimation gas injection delivery system into a low pressure chamber environment has been developed in a prototype form to eliminate the use of chemical solvents for many of the most common processing methods for the development of latent prints. Each of the current processing methods can be achieved using this technology.

The performance of this prototype system was evaluated by comparing the quality of latent fingerprints developed using seven common traditional processes: (1) Cyanoacrylate Ester; (2) Cyanoacrylate Ester/Rhodamine 6G; (3) Cyanoacrylate Ester/Fluorescent Powder; (4)

Fluorescent Powder; (5) Iodine; (6) 1,2-Indanedione; and, (7) Ninhydrin. Eleven different substrates were used in this evaluation consisting of porous, non-porous and semi-porous substrates (including some with thermosensitive layers). Three latent fingerprints were deposited on each substrate sample using the appropriate latent print standard matrix. Each latent fingerprint was cut in half and processed separately using the comparable processing method (i.e. Cyanoacrylate ester, Ninhydrin, etc.). One half was processed according to established procedures for each traditional processing method and the other half was processed with the comparable processing method using the prototype system. The halves of each latent fingerprint were re-joined and photographed. Each image was digitally processed to convert the images to gray-scale to maintain anonymity of the processing method and minimize potential interpretation bias during the evaluation of each latent fingerprint. Each latent fingerprint was digitally presented to six Latent Print Examiners to evaluate whether one half of the latent print contained friction ridges that developed with higher quality and clarity or whether there was no distinguishable difference in the quality and clarity of development. Further examinations were carried out to determine if the processing regime using this low pressure system had any negative effect on other forensic examinations (Drug Chemistry, Forensic Document, and DNA). The Drug Chemistry evaluation consisted of placing a known sample of cocaine on sheets of multi-purpose office paper (20lb), cutting the samples in half and processing one half through each latent print processing method while retaining the other half as a standard control. Cocaine was used as a representative drug sample since it approximates the analytical behavior of most other drugs. Following the latent print processing, all samples and controls were analyzed using a GC-MSD. The Forensic Document evaluation consisted of an analytical comparison of six different ink marks and one pencil mark placed on a standard sheet of multi-purpose office paper (20lb). One set of samples were processed using each of the latent print processing methods in the low pressure system. One set of samples remained unprocessed as a control. Following the latent print processing, six different analytical methods were employed to detect chemical differences in the ink and pencil samples as a result of the latent print processing method. The DNA evaluation consisted of placing a sample of saliva on sheets of multi-purpose office paper (20lb), cutting the samples in half and processing one half through each latent print processing method while retaining the other half as a standard control. Following the latent print processing, saliva samples were evaluated to determine if DNA inhibition and/or degradation was present.

The overall results of the quality of developed latent prints were comparable to traditional processing methods. The Drug Chemistry results were analyzed and revealed conclusive results for cocaine with no apparent interference from any of the latent print processing methods using the low pressure system. The Forensic Document results were analyzed with variable results. For all latent print processes using the low pressure system, except for 1,2-Indanedione, one or more of the Forensic Document analytical methods indicated the latent print processing method negatively affected one or more of the ink and pencil samples. The DNA results were analyzed and revealed full DNA profiles from all samples with no apparent inhibition or degradation.

The results of this study indicate this method of developing latent prints using a low pressure sublimation vapor deposition system may provide improvements over traditional processing methods. In addition to the overall quality of developed latent prints being comparable to traditional processing methods, other improvements are related to the process, cost, and safety of developing latent fingerprints on multiple forms of evidence (porous, non-porous, semi-porous), no known interference with drug chemistry and DNA examinations, elimination of hazardous and destructive chemical solvents due to the dry process, standardized processing regimens programmed into the system computer, and the ability to rely on the single system for many of the most common processing methods for the development of latent prints.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

**Forensic Science, Low Pressure Deposition, Fingerprint Development**

### **A173 Assessing the Performance of Silver as a Post-Gold/Zinc Process for Vacuum Metal Deposition**

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After attending this presentation, attendees will understand the performance of silver as an effective post-gold/zinc process for vacuum metal deposition.

This presentation will impact the forensic science community by demonstrating the ability of silver to develop more and higher quality latent prints when used as a post-gold/zinc vacuum metal deposition process than the traditional gold/zinc process alone.

Vacuum Metal Deposition (VMD) using gold/zinc is a process which may be used to develop latent prints on many types of non-porous and semi-porous substrates. VMD has been found to be successful when other traditional processing methods have failed, such as with older latent prints and those previously exposed to water and other environmental conditions. The effectiveness of the gold/zinc VMD process relies heavily on the composition and surface contamination of the substrate and latent print residue. Variations in the composition and surface contamination of substrates may prevent successful development of latent prints using the gold/zinc process producing no or poor quality latent prints. Further research has suggested silver may be used as a post-gold/zinc VMD process having the potential to improve poorly developed latent prints and develop additional latent prints which were not developed using the gold/zinc VMD process alone. However, the amount of research demonstrating the performance of silver as an effective post-gold/zinc VMD process is limited. This research, therefore, seeks to better understand the performance of gold/zinc VMD process alone compared to the performance of silver as a post-gold/zinc VMD process on various types of substrates.

Nine different types of non-porous and semi-porous substrates were used in this evaluation, which included: Duct tape, packaging tape, pressure sensitive tape, adhesive tape, masking tape, cardboard, photo-paper, plastic sandwich bag, and thick plastic wrap. The ability of the gold/zinc VMD process to develop latent prints on the non-adhesive side of many types of tapes has been demonstrated in the literature, but research is limited on the success of developing latent prints on the adhesive side. Because of this limited research, this evaluation focuses on the ability to successfully develop latent fingerprints on the adhesive side of tapes. Each substrate contained a depletion series of five fingerprints using a sebaceous standard fingerprint matrix and a depletion series of five fingerprints using natural sebaceous fingerprint matrix obtained from the study coordinator's forehead. All fingerprints were deposited under controlled conditions on each substrate surface. Four sets of each substrate were used to evaluate the gold/zinc and silver VMD processes with fresh, one week, two week, and three week old fingerprints yielding a total of 360 fingerprints processed by each VMD process. All substrates were stored in controlled laboratory conditions until being processed. Following the VMD processing, each fingerprint was photographed and digitally presented to a certified Latent Print Examiner for evaluation. The Latent Print Examiner rated the quality of development according to a numeric rating scale (zero to five) corresponding to the quality of friction ridges which developed. Data were

evaluated according to the quality of fingerprints developed with the gold/zinc VMD process, the quality of additional fingerprints developed and those further enhanced with the silver VMD process, and whether the type of fingerprint matrix used, the age of the fingerprints, and depletion series impacted the ability of the gold/zinc and silver VMD processes to develop quality fingerprint impressions.

The gold/zinc VMD process consistently yielded poor quality results on the adhesive side of the tapes and plastic substrates; however, successful results were obtained on both the cardboard and photo-paper substrates. The silver VMD process yielded positive results on many substrates in which the gold/zinc VMD process failed or developed poor quality fingerprints. The quality of fingerprints developed using silver as a post-gold/zinc VMD process were greatly improved compared to the quality of fingerprints developed using the gold/zinc VMD process alone ( $p < 0.001$ ). Further analysis revealed no statistical differences in the quality of the fingerprints developed using the different fingerprint matrices and the fingerprint ages; however, those fingerprints having more matrix (deposition #1 in the depletion series of five fingerprints) yielded higher quality results when compared to the fingerprints having less matrix (deposition #5 in the depletion series of five fingerprints). These results suggest silver is an effective post-gold/zinc VMD process having the ability to develop additional fingerprints and further enhance those previously developed using the gold/zinc VMD process alone. Further research is warranted, however, to better understand the performance of the various VMD processes on other substrates, fingerprint matrices, and ages.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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**Forensic Science, Vacuum Metal Deposition, Silver**

## **A174 Shape Measurement Tools in Footwear Evidence: An Investigation to Determine Size Without Scale**

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The goal of this presentation is to examine the ability to use shape measurement tools to determine the class characteristic of shoe size without a scale in place on an image.

This presentation will impact the forensic science community by providing a method to allow for sizing of a shoe in circumstances when a scale is not included in the image, aiding the area of footwear impression evidence. This method also has potential applications in impression evidence in cases where key images lack a scale.

When footwear evidence is collected at a crime scene, inclusion of a scale in photographed images is necessary to determine the size of the shoe. If no scale is used, the evidentiary value can be lowered. The goal of this project was to explore the use of shape measurement methods with footwear impression evidence to determine if shape change alone, in element differences in the soles of different brands of shoes, can predict shoe size. These methods provide robust statistical approaches to shape comparison that do not require a scale.

One method that may be used to determine size without scale is geometric morphometric analysis (GM). This technique is used widely in other fields and is often used to describe change in the fossil record in evolutionary biology, in fisheries research and in addressing a range of

ecological questions. GM methods involve the placement of a series of landmarks on digital images, positioned on the image as to capture shape information of a structure of interest. These landmarks are then extracted and analyzed statistically as a unit. This allows for quantitative evaluation of large datasets.

The landmarks can be plotted in Procrustes superimposition, a method of optimally matching one shape to another. This allows for visualization of shape information and is a measure of the closeness in shape of superimposed specimens. Procrustes distances can be used to express the degree of similarity of individual specimens, means of populations, to summarize variations in populations, or to search for matches between specimens. Procrustes distances provide a general metric of shape distance, and allow for a wide range of statistical and data processing tasks to be readily carried out, including Principal Component Analysis (PCA). This allows for determination of which shape aspect is responsible for the most variation.

A size series of different brands of shoes were utilized from a local shoe store. The shoes were scanned on a flatbed scanner at 300dpi with an ABFO scale in place. Landmarks were placed on the repeatable locations on elements seen on the soles, specifically at the outer edges. It was noted that there were detectable and predictable shape differences between the sizes in several models of shoes examined, but not all. Results indicated that as the element altered shape and/or position, size could be determined even with no scale in place. Distances from a suspect shoe to each of the shoes in a measured size series of shoes could be used to assign the suspect shoe to a specific size, without use of the scale information. The error level was roughly one half shoe size as could be expected due to variation in manufacturing processes.

The ability to use this method will be brand dependent as not all brands may have repeatable elements relative to size. The method does require a reference set of shoes of varying sizes by which a suspect shoe may be matched. Landmark selection and placement was highly critical in success of this method.

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**Forensic Science, Footwear Impressions, Geometric Morphometric Analysis**

## **A175 Defining a Reliable Human Decomposition Odor Profile for Forensic Canine Investigations**

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After attending this presentation, attendees will discuss on-going research in determining the distinct volatile organic compounds and their relative ratios as they relate to the discovery of clandestine human burials.

This presentation will impact the forensic science community by providing insights into the difficulty of adequately and accurately

measuring the distinct components that will be used in canine training aids to assist in field investigations.

This presentation will provide a better understanding of the challenges facing forensic canine specialists in both locating clandestine burials as well as the challenges facing forensic research chemists in defining a reliable volatile organic chemical (VOC) composition profile from which to train their canines. There are several key issues that need to be addressed, most importantly determining and optimizing the set of volatiles that accurately represent human decomposition and reduce or eliminate associations to other odors resulting from background and/or non-human sources. Surface and sub-surface VOC comparative analyses could be important to adequately delineate any differences in odor profiles for investigations. Determination of this set of volatiles will allow for more appropriate training of victim recovery canines across the forensics community. If canines can be trained using a common training aid, the overall location accuracy of clandestine burials should improve.

This presentation will impact the forensic community by enlightening a wider audience as to these issues, which in turn may lead to advances in research and development to address these key issues. As a result, a greater number of well trained, efficient canines would be available to consistently locate clandestine burials (i.e., buried or concealed human remains).

The use of canines in law enforcement and military applications is well-known. Among their many uses, canines are used to screen for drugs and explosives, to locate missing persons, to associate crime scene evidence with a suspect, and to locate victims of violent crimes. Qualitative and quantitative characterization of the VOCs resulting from human decomposition (i.e., odor) has been elusive. Determining the relevant VOCs amidst a wide array of potential markers for human evidence, both living and deceased, has not been adequately achieved. For example, locating a clandestine burial efficiently and independent of the time since death has long been a significant challenge for canines due to the wide range of chemicals measured, as well as the number of chemicals that are similar from a variety of confounding decomposing matrices or sources. Traditional air sampling techniques, new sampling devices, human surrogate testing, chemical mimics, and new analysis tools have been developed over the years to assist in addressing these questions. The overall goal of this research is to improve the detection and characterization of the most relevant VOCs for forensic investigative reasons, as well as to improve training models for cases such as these.

Air sampling via solid phase microextraction (SPME) and 400-mL canisters has been performed in two biotopes: Knoxville, Tennessee (Anthropological Research Facility, (ARF)) and Boston, Massachusetts. A GC×GC TOFMS system with a preconcentrator was used for measurements of human materials (e.g., tissue or clothing) both fresh and decomposing. A GC/MS system was used for SPME sample analysis and also whole air samples when this system was interfaced to a dual preconcentrator thermal desorption system. An existing and a new hand-held preconcentrator have been computer modeled with flow simulations based on recent experiments with a baseline prototype device under high sampling air flow conditions. Chemical mimic development and testing was performed with a variety of mixtures and actual field trials were performed using canines. Bloodhounds and Springer Spaniels were used to test mimic performance and to test human decomposition and suspect scent hypotheses.

Prior research in human decomposition has shown that there could be over 450 different VOCs emanating from human subjects buried at the ARF in TN. Subsequent research supports focusing on a manageable 50 based on a variety of factors including a systematic statistical analysis. Further narrowing of this list is currently underway with careful consideration of biotope controls and the analytical results therein. Comparison of these reduced lists to a variety of currently available research studies and those performed in this laboratory using human surrogates such as pigs, has indicated that commercially available mimics may not be acceptable for efficient canine human decomposition training based on the GC/MS and GC×GC TOFMS data. Research into understanding canine olfactory capabilities has also influenced the development of improved VOC

collection devices and computational fluid dynamic simulations of a baseline system prototype, which indicates that a newly designed hand-held device once fabricated should be up to 40 times more sensitive for VOCs, once fabricated. This presentation will provide an overview of the many avenues of investigation involved in human (living/deceased) odor profile studies that are on-going in the research unit and in conjunction with the evidence response team unit's canine program.

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#### **Human, Decomposition, Volatiles**

### **A176 Imaging Using Synchrotron Radiation: A New Tool for Forensic Science**

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After attending this presentation, attendees will learn a different approach to latent fingerprint development which allows determining complete friction ridge details and important additional information regarding origin, chemical composition, and potential contaminants of the finger deposit, facilitating subsequent DNA analysis.

This presentation will impact the forensic science community by providing a new powerful tool for personal identification in high profile cases by means of Synchrotron Radiation facilities, particularly useful in the analysis of IED fragments.

Forensic science has experienced an increasing interest in research activities: all paths that allow the investigators to obtain more information about major crimes are tracked to solve offenses related to national security like terrorists attacks. The goal of the research, established in the framework of a project funded by the Italian Interior Ministry, by "Fondo Trieste" fund, is to adopt a multi-technique approach based on conventional and Synchrotron Radiation (SR) techniques, and to study latent fingerprints from the morphological and chemical point of view. The main goal of the research is to perform fingerprint analysis by its visualization with a SR source. The ultimate objective is to develop methods of image reconstruction that merge all the information coming from different SR techniques to produce valuable evidence gathered with additional information regarding the chemical nature and the contaminants of the enhanced fingerprint.

The analysis is performed using infrared micro spectroscopy (IRMS) on sweat fingerprints, by X-ray phase contrast (XPC), and by X-ray absorption fine structure analysis (XAFS) on contaminated ones.

Multiple donors, both male and female, were chosen to deposit the fingerprints. Prior to the deposition, each donor behaved normally and hands were not cleaned. Moreover, latent prints were not charged by inducing artificially sweat in any way. Each deposition was performed as a depletion series of eight fingerprints by the same finger, to take into account components abundance and to model, if possible, the real situation. The substrates chosen were silicon wafers and PET sheets. Contaminated fingerprints were realized shooting with a gun, prior to deposit, with the depletion technique.

With the intent to characterize the chemical nature of the fingerprint deposit and to make a chemical imaging of it, IRMS was used. It resembles

hyper spectral imaging: the fingerprint sample of interest is analyzed (either in parallel or serially) with a broad spectrum IR light source, ranging from 4000  $\text{cm}^{-1}$  to 500  $\text{cm}^{-1}$  (Mid IR region). Then, spectra are collected by a single-element detector. Each picture element is not characterized by a unique intensity value, but by a full spectrum in the same band mentioned above.

The broad methyl and methylene stretch bands (from 3000  $\text{cm}^{-1}$  to 2800  $\text{cm}^{-1}$ ) can be found in all donors spectra. In the 1800  $\text{cm}^{-1}$  to 1400  $\text{cm}^{-1}$  range some donors show an intense carboxylate band signal around 1590  $\text{cm}^{-1}$ , while others are characterized by the amide I (around 1650  $\text{cm}^{-1}$ ) and amide II (around 1540  $\text{cm}^{-1}$ ) stretching bands. Each donor shows different spectral shapes, with different relative intensities in the same bands, due to the different proportions of the fingerprint components. The ability to solve the amide I and amide II contributions in the 1800–1400  $\text{cm}^{-1}$  band, decoupling those from the intense carboxylate signal around 1590  $\text{cm}^{-1}$ , will allow the analysis of the fingerprint.

With the goal to characterize the contaminated fingerprints on silicon, XPC technique was used. This technique exploits the phase difference caused by the sample in the X-ray's path from the source to the acquiring sensor. The energy values chosen ranged from 8 keV to 35 keV. The analysis made on contaminated fingerprints with the XAFS technique showed the presence of metals, thus several images were taken with XPC just above or below the absorption of these elements. In practice, suitable preprocessing algorithm will have to be used to improve the signal-to-noise ratio which is very low due to the small quantities of contaminants and, consequently, to their low absorptions.

While being in its early stage, the research seems promising in visualizing latent fingerprints. Moreover, the added value of giving a complete morphological and chemical characterization of the fingerprint and its contaminants could enrich the classical approach with key additional information, facilitating also subsequent DNA analysis so as to identify the perpetrator.

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#### Synchrotron Radiation, Trace Analysis, Latent Fingerprints

### A177 Analytical Profile of 4-Methyl GHB, 4-Phenyl GHB, GVL, and Gamma-Phenyl GBL

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After attending this presentation, attendees will better understand the impact of the internet on current trends concerning the synthesis of “designer” drugs. Additionally, analytical data for several analogs and precursors of GHB will be provided for use/reference in the proper identification of this class of compounds.

This presentation will impact the forensic science community as it will discuss analogs of GHB that are extremely easy to synthesize and are currently being discussed on internet message boards by illicit drug users.

Illicit drug users are continuously searching for alternative ways to reach the biological effects of drugs which are federally controlled in an attempt to circumvent federal and state drug regulation laws. When  $\gamma$ -hydroxybutyric acid (GHB) was regulated as a Schedule I controlled substance in 2000, illicit drug users began to use 1,4-butanediol and  $\gamma$ -butyrolactone (GBL) as these drugs were shown to have similar sedative effects. As a result, the government made it increasingly difficult to obtain these compounds, classifying GBL and 1,4-butanediol as List I chemicals. Federal and state laws also made it illegal for stores and online websites to sell products marketed as health supplements, sleep aids, and cleaning products that contain the precursors of GHB.

Recent literature and internet searches have revealed additional analogs of GHB that drug users are synthesizing. Several internet message boards and websites contain concise instructions for synthesizing analogs, chemicals needed, narratives describing the biological effects and users'

experiences, and published scientific research regarding the efficacy of these analogs in mice. Scientific research suggests not only are certain analogs more potent than their original drugs, but that they are also more toxic. These websites provide a forum for users who are trying to stay one step ahead of the law by producing analogs that forensic laboratories have not yet seen. Two GHB analogs,  $\gamma$ -hydroxyvaleric acid (4-methyl GHB) and 4-hydroxy-4-phenylbutanoic acid (4-phenyl GHB), are among the newer GHB analogs that have caught the attention of illicit drug users. There has been little work done on these compounds regarding their identification, making it difficult for forensic laboratories to determine whether samples coming in for analysis are newer analogs or precursors to these analogs.

The analysis of GHB and its analogs is problematic due to their chemical properties. Conventional methods of analysis utilizing gas chromatography-mass spectrometry (GC/MS) have shown interconversion between analogs and their precursors, making the differentiation challenging. Due to their small molecular weights and polarity, GHB and its analogs are also difficult to analyze utilizing liquid chromatography-mass spectrometry (LC/MS) as they are not easily retained on column for analysis.

In this study, 4-methyl GHB and 4-phenyl GHB were synthesized using  $\gamma$ -valerolactone (GVL) and  $\gamma$ -phenyl- $\gamma$ -butyrolactone ( $\gamma$ -phenyl GBL), respectively. Preliminary results have indicated that the compounds can be separated by high performance liquid chromatography (HPLC) using (A) 10 mM sodium phosphate buffer ( $\text{NaH}_2\text{PO}_4/\text{NaHPO}_4$ , pH~7.3) and (B) acetonitrile, at 2% (B) isocratic for two minutes followed by a linear gradient to 40% (B) over eight minutes. The components were separated using HPLC columns 4.6 x 50mm, 1.8  $\mu\text{m}$  column. UV detection was performed at 215nm. Confirmation of the GHB analogs was also achieved using GC/MS derivatization via trifluoroacetic anhydride (TFA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA, 99% with 1% TMCS). LCMS/MS linear ion trap results indicated that optimal detection of 4-phenyl GHB can be achieved in negative mode requiring chromatographic separation from its precursor,  $\gamma$ -phenyl GBL. In addition, the presentation will discuss results using standard color tests and Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR).

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#### GHB Analogs, Precursors, Mass Spectrometry

### A178 Spectral Analysis of the of Gamma-Hydroxybutyrate (GHB) and Gamma-Butyrolactone (GBL) Using Near Infrared Spectroscopy in Varying Beverage Matrices

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After attending this presentation, attendees will have a better understanding of the use of Near Infrared Spectroscopy (NIRS) to study the chemistry and equilibrium of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) in different beverage matrices.

This presentation will impact the forensic science community by serving to provide a critical understanding how GHB and GBL behave in typical sample matrices of common beverages that may be involved in a drug-facilitated sexual assault.

GHB is a central nervous system depressant classified as a sedative-hypnotic and abused for its euphoric, sedative, and anabolic effects. GHB can be studied using the spectroscopic technique of NIRS because it is a quick non-destructive technique that allows for the measurement of organic compounds with vibrational overtones between 700nm and 2500nm, similar to mid-IR. GHB is classified as a federal Schedule I drug as an illicit substance, but can also be obtained in prescription form. GHB commonly cyclizes into a lactone, GBL, with the equilibrium dependent on

the matrix conditions. The equilibrium ratio between GHB and GBL can vary with the pH of the medium as well as the temperature of the matrix. The equilibrium reactions can be measured accurately using NIRS in the transmittance mode. Due to the solutions being essentially clear and colorless, the transmittance mode is most appropriate for this beverage study. The unique changes in the carbon-hydrogen groups can be used as the main parameter of comparison in shape, size, and intensity of each spectrum resulting in an understanding of how GHB and GBL behave in beverage matrices. All beverage samples were spiked with GHB or GBL at a concentration of 3.00g per serving. Either GHB or GBL was dissolved directly in the beverage, and measured in a cuvette (2mm) using NIRS. Thirty two scans for each sample were taken and the intensity was measured as it occurred at a given concentration. To standardize the spectral data all spectra were analyzed using multivariable mathematical software provided with the instrument.

The results show that GHB and GBL can be correctly identified in spiked samples versus unadulterated samples. When GHB or GBL is added to the beverage matrix, temperature controlled, there is a clear distinction between the two. Calibration curves of each of the eight beverages yield a straight line with  $R^2 > 0.98$  for each beverage. A calibration curve made from similar beverages using spectral correction has been shown to accurately yield concentration information. A similar beverage composition can be used to quantitate the GHB in solution; the exact beverage is not needed. The GHB and GBL in a beverage matrix has also been shown to be stable in concentration and composition over a period of greater than ten days, even after freezing the sample and defrosting it. Confirmation of concentration of GHB in the beverage matrices was achieved using LC/MS. Results from an equilibrium study was also completed for GHB and GBL coupled with the beverage study yielding data consistent with Ciolino.<sup>1</sup> All relative percentages and equilibrium times using GHB and GBL in buffered solutions agree with previous reports.<sup>1</sup>

#### Reference:

1. Ciolino L.A., Mesmer M.Z., Satzger R.D., Machal A.C., McCauley H.A., Mohrhaus A.S. The chemical interconversion of GHB and GBL: forensic issues and implications. *J Forensic Sci* 2001 **46**(6):1315–1323.

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Forensic Science, GHB, GBL

## A179 Objective Discrimination of *Salvia divinorum* From Related *Salvia* Species Using Chromatographic Techniques and Multivariate Statistical Procedures

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The goal of this presentation is to demonstrate statistical differentiation of the hallucinogenic plant, *Salvia divinorum*, from related *Salvia* species based on chemical profiles obtained using both gas chromatography-mass spectrometry (GC/MS), and liquid chromatography-mass spectrometry (LC/MS).

This presentation will impact the forensic science community by providing an objective methodology for the differentiation of *S. divinorum* from four related *Salvia* species using a combination of chromatographic techniques and multivariate statistical procedures. The application of such statistical procedures directly addresses one of the recommendations highlighted in the 2009 *National Academy of Sciences Report*; that is, the need for statistical evaluation of forensic comparisons.

*Salvia divinorum* is a hallucinogenic herb that is currently regulated in 25 states in the United States. Although there are over 900 *Salvia* species, *S. divinorum* is the only one known to contain the hallucinogen, salvinorin

A. As such, forensic identification of *S. divinorum* currently relies on the extraction and identification of salvinorin A in the plant, typically using GC/MS. Despite being more widely available in forensic laboratories, GC/MS is limited to the analysis of sufficiently volatile compounds that do not thermally degrade at the high operating temperatures used during analysis. In contrast, LC/MS is more suited to the analysis of non-volatile compounds and hence, may offer additional discriminatory information.

In this research, five different *Salvia* species were investigated: *S. divinorum*, *S. guaranitica*, *S. nemorosa*, *S. splendens*, and *S. officinalis*. Dried leaves from each species were extracted in triplicate for 16 hours, using a rotary agitation procedure. For GC/MS, the extraction solvent was dichloromethane while, for LC/MS, acetonitrile was used. All extracts were analyzed in triplicate using the appropriate chromatographic technique and, following analysis, two separate data sets were formed: one containing total ion chromatograms (TICs) obtained from GC/MS and the second containing TICs from LC/MS. Compounds present in extracts of each species were identified through mass spectral interpretation.

Prior to data analysis, TICs in each data set were subjected to various preprocessing procedures to remove instrumental sources of variance. This is necessary to ensure that subsequent data analysis procedures do not identify these non-chemical sources of variance as differences within the data set. Various preprocessing procedures were investigated for each data set, including background correction, smoothing, retention time alignment, and normalization. Background correction was used to minimize differences in background signal, while smoothing was used to minimize random variations in noise. Chromatograms were then retention time aligned, to account for retention time drift during analyses, and normalized, to account for variation in injection volume.

The pretreated data sets were then separately subjected to principal components analysis (PCA) to investigate differentiation of *S. divinorum* from the other four *Salvia* species based on the chemical profiles. Principal components analysis is an unsupervised statistical procedure that identifies groupings of samples within a data set. The procedure is termed “unsupervised” since no prior knowledge of groupings within the data set is required. The two outputs from PCA are a scores plot and loadings plots. The former is a scatter plot in which samples that are chemically similar are clustered closely, while chemically different samples are distinct. For both GC/MS and LC/MS analysis, *S. divinorum* was differentiated from the other four *Salvia* species in the corresponding scores plots. Student’s t-tests were then used to statistically assess the positioning of the samples in the scores plots. Additionally, the PCA loadings plots were used to identify those compounds contributing most to the variance among the *Salvia* species. These compounds have potential use as chemical markers for the discrimination of *Salvia* species.

Using a combination of statistical procedures, discrimination of *S. divinorum* from four related *Salvia* species was demonstrated based on profiles of both the volatile compounds, obtained by GC/MS, and the non-volatile compounds, obtained by LC/MS.

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***Salvia Divinorum*, Chromatographic Techniques, Multivariate Statistical Procedures**

## A180 Assessment of Methods for the Chemical Identification of the Psychoactive Plant Kanna (*Sceletium tortuosum*)

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After attending this presentation, attendees will be able to describe analytical techniques for the chemical identification of the alkaloids present in the Kanna plant (*Sceletium tortuosum*).

This presentation will impact the forensic science community by providing information to allow the identification of the plant material using forensic testing techniques that follow SWGDRUG recommendations and standard testing protocols.

Kanna is a ground cover plant that grows in the arid climate of South Africa. Kanna contains the primary alkaloids mesembrine, mesembranol, mesembrenone, hordenine, sceletenone, sceletium A4 among many others. Some of these alkaloids have pharmacological properties reported to elevate mood and decrease stress in addition to calming anxiety and producing euphoric effects. These effects, commonly sought after by drug users, add to the overall appeal of the plant as a substance to abuse. The drugs are not controlled by the federal government so it is important to be able to distinguish these alkaloids from other vegetable material submitted for forensic examination and analysis. Kanna is featured as a natural alternative to traditional anti-depressants on many online sites that describe psychoactive experiences with the drug, and as a result of its uncontrolled status it is easy to purchase online as capsules, powders, tinctures, and seeds.

This presentation describes the evaluation of the content and characteristics of commercial Kanna preparations using common chemical color tests and thin layer chromatography (TLC), with confirmation by gas chromatography/mass spectrometry (GC/MS). The following materials were purchased from an online supplier: *Sceletium tortuosum* (Kanna) finely shredded plant material; Herb Spirits Kanna 5:1 liquid extract; Kanna whole white leaf; and Kanna 5:1 powdered extract.

A basic extraction optimized for the recovery of mesembrine along with a methanolic extraction was performed on each of the four commercially available products. Color tests including Cobalt Thiocyanate, Dille-Kopanyi, Dragondorff, Froehde, Janovsky, Liebermann, Mandelins, Marquis, Mecke, and Simons were applied to the plant material and the above extracts. A Duquenois-Levine test was performed on the Kanna whole white leaf. Color tests showed positive reactions with the optimized extraction method, but produced non-specific reactions with the methanolic extracts. The color tests that yielded the most distinguishing results included Cobalt Thiocyanate, Janovsky, Dille-Kopanyi, Dragondorff, Mecke, and Liebermann.

Extracts of all products were compared using TLC. The solvent system was a mixture of ethyl acetate, methylene chloride, methanol, and concentrated ammonium hydroxide (74:72:12:4). TLC bands were visualized using short and long ultraviolet radiation and with both acidified iodoplatinate and Dragondorff reagents. This technique produced distinctive and characteristic results from the extracts of the Kanna products. Mesembrine, mesembranol, and mesembrenone were confirmed in three of the four Kanna products analyzed; hordenine was identified in two of the products, while sceletenone and sceletium A4 were each identified in one of the products. One of the products contained no Sceletium alkaloids.

Together this battery of forensic techniques proved effective in the characterization of Kanna and used SWGDRUG compliant testing options. Analysis of commercial Kanna products showed that their content was highly variable and counterfeit products containing no alkaloids are being sold as Kanna.

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**Forensic Science, Kanna, *Sceletium Tortuosum***

## **A181 Chemical Characterization of Kratom and Associated Alkaloids**

*Theresa Scott, BS\*, Fredric Rieders Family Renaissance Foundation, 2300 Stratford Avenue, Willow Grove, PA 19090; Jillian Yeakel, MSFS, Fredric Rieders Family Renaissance Foundation, 2300 Stratford Avenue, Willow Grove, PA 19090; and Barry K. Logan, PhD, and G. John DiGregorio, MD, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*

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After attending this presentation, attendees will be able to identify the most prevalent and unique alkaloid, mitragynine, present in the plant Kratom and various commercial preparations, using standard forensic chemical testing procedures that meet SWDRUG recommendations.

This presentation will impact the forensic science community by raising awareness of the existence of mitragynine containing products when they are submitted for analysis.

Kratom (*Mitragyna speciosa*) is a tree found mainly in Asian countries whose leaves, when consumed in low doses, provides both a stimulant and analgesic effect. When consumed in high doses, Kratom has a depressant effect and its major alkaloid, mitragynine, has been found in combination with other CNS depressant drugs in a number of fatalities. Although use of Kratom and mitragynine products is not yet widespread in the United States, the alkaloid has been detected in "legal high" products. Increasing numbers of internet sites market a number of different Kratom products including liquid extracts, dried leaves, powdered leaves, and the plants themselves.

An analytical standard of the alkaloid mitragynine was obtained commercially and characterized using various chemical color tests. The tests did not work well when applied directly to the plant material consequently a simple methanolic extraction was required. The botanical material caused the methanol to be tinted green and after addition of the reagents, the colors seen were natural botanical colors (such as greens and browns) making a color change caused by the reaction difficult to visualize. Therefore, the color tests tested thus far are not ideal presumptive tests to utilize for mitragynine.

Following methanolic extraction, Kratom related products were also analyzed by thin layer chromatography (TLC) using a 9:1 chloroform to methanol solvent system which yielded good separation of the components of the botanical material. Viewing under short wave ultra violet light allowed visualization of the mitragynine and final development with iodoplatinate spray produced a mitragynine band initially purple in color, then turning yellow shortly after, and finally turning orange overnight. Retention factors of the bands produced from the methanol extraction of the purchased products were compared to the bands from the standard mitragynine and found to be similar.

Following a mitragynine optimized extraction, confirmatory analysis was performed by gas chromatography/mass spectrometry (GC/MS). Mitragynine has a base peak of 214 and distinctive and prominent fragments of 398, 397, and 383 m/z, and was consistent with the mass spectrum in various mass spectral databases.

Analysis of several commercial products, six products labeled as "Kratom," as well as various products that were believed to be Kratom through appearance, confirmed the presence of mitragynine. Of the thirteen samples for this study, six were purchased over the internet by our laboratory and seven were obtained as evidence submitted to the lab for analysis. The six purchased were obtained from reputable sites from which samples for other research projects have been purchased and all were labeled as Kratom products. Of the seven samples acquired through the lab, one was a bag of capsules each of which contained a green powder, another was a K2 product, and the rest were either silver bags with a zip top or small, plastic containers all appearing to contain a green, botanical powder.

This approach of TLC followed by GCMS meets SWGDRUG requirements for forensic testing and proved suitable for the identification of mitragynine in these products.

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**Forensic Science, Kratom, Mitragynine**

## A182 Identification of Methamphetamine and Select Regioisomers

Harry F. Skinner, MS\*, Southwest Laboratory Drug Enforcement Administration, 2815 Scott Street, Vista, CA 92081; and Sandra Sachs, PhD, Oakland Police Department, Crime Laboratory, 455 7th Street, Room 608, Oakland, CA 94607

After attending this presentation, attendees will be able to identify methamphetamine and select regioisomers unequivocally from one another by chromatography and mass spectrometry.

This presentation will impact the forensic science community by allowing the forensic scientist to identify methamphetamine from the similar regioisomers.

Regioisomers are positional isomers which vary only in the position of a functional group or other substituent. Positional changes will not only alter a compound's physical, chemical, and physiological properties, but in the case of methamphetamine, variations will also affect the legal status as a controlled substance. Methamphetamine has four regioisomers: N, $\alpha$ -dimethylphenethylamine,  $\alpha,\alpha$ -dimethylphenethylamine (phentermine), N,N-dimethylphenethylamine, N-ethylphenethylamine, and  $\alpha$ -ethylphenethylamine.

Mass spectral data from these five compounds has been subjected to close scrutiny, since misidentification by using only a library search is a possibility. All four regioisomers have a molecular weight of 149 a.m.u. and have the same  $m/z$  58 base peak seen in methamphetamine. The four regioisomers also display similar fragmentation patterns and ratios seen in methamphetamine. Despite these similarities, it has been shown that differentiation of methamphetamine from its various regioisomers is entirely possible, through the use of routine laboratory equipment. Various studies have been performed with methamphetamine and its regioisomers, and it has been shown that methamphetamine can be both chromatographically separated from its regioisomers and accurately characterized by confirmatory analysis.

The gas chromatography separation of the regioisomer mixture on both the non-polar HP-5 and mid-polar DB-17 columns will be discussed. Amphetamine and N,N-dimethylamphetamine was included with the methamphetamine regioisomer mixture as reference. Methamphetamine with a retention time of 1.575 minutes with the HP-5 column and 2.640 minutes with the DB-17 column did not co-elute with any of the four regioisomers.

The LC chromatography separation of the regioisomer mixture is comparable to other work. Methamphetamine had a retention time of 5.101 minutes and did not co-elute with any of the regioisomers. Complete baseline separation of all compounds in the regioisomer mixture was achieved.

The mass spectral data collected from the regioisomers closely match published spectral data. In the analysis of fragmentation patterns of similar compounds such as the regioisomers, it is necessary to expand the spectra in order to show the specific fragments and their abundance ratios. All four regioisomers and methamphetamine have the characteristic  $m/z$  58 base peak. Furthermore, the fragmentation patterns are very similar except for a few distinguishing key ions. Sachs and Woo focus on the low mass region ( $m/z$  39 – 56) as an area of distinction. In addition to the low mass region, a few unique/key ions are found in some of the compounds that allow for differentiation from methamphetamine and each other. For example, methamphetamine has a significant  $m/z$  119:115 ratio not found in any of the regioisomers, which is a unique identifier for methamphetamine in addition to the low mass analysis. Both the nitrogen substituted compounds, N-ethylphenethylamine and N,N-dimethylphenethylamine have a significant  $m/z$  105 fragment which would allow exclusion from methamphetamine. The regioisomer,  $\alpha$ -ethylphenethylamine contains a uniquely identifying peak at  $m/z$  120. Lastly, phentermine can be identified by its strong  $m/z$  134 peak and lack of an  $m/z$  148/ 149 (contains no alpha hydrogen's). In conclusion, methamphetamine can be distinguished from its regioisomers using chromatographic retention time *or* a thorough evaluation of the mass spectroscopy fragmentation pattern.

### **Methamphetamine Identification, Regioisomers, Mass Spectrometry**

## A183 Herbal Incense and Bath Salts Cases in Harris County, Texas

Warren C. Samms, PhD\*, and Ashraf Mozayani, PhD, PharmD, Harris County Institute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054

After attending this presentation, attendees will learn about the timeline of appearance, case overview, analysis, and reporting of "herbal incense" (cannabinoid receptor agonist) and "bath salts" (methcathinone or pyrovalerone derivative) at the Harris County Institute of Forensic Sciences, located in Houston, TX. Unlike other portions of the Texas Controlled Substances Act (CSA), the subsections referencing these drugs (effective September 1, 2011) are written with broad language. While examples are given in the law for the different types of "herbal incense" and "bath salt" compounds, the list is not all-inclusive. As attorneys have no direct manner of correlating some of these chemical names with the Texas CSA, Harris County Institute of Forensic Sciences (HCIFS) Drug Chemistry Laboratory (DCL) provides additional information on the laboratory reports for these types of results.

This presentation will impact the forensic science community by communicating the analytical experience the DCL has had with regards to these two classes of designer drugs. It is hoped that the case history, compounds encountered, types of exhibits, and methods of reporting may be beneficial to other laboratories and agencies, especially in the Northeastern and Western United States, areas identified by the National Forensic Laboratory Information System (NFLIS) as having very low occurrences of these substances compared to the rest of the country.

The HCIFS DCL received its first encounter with "herbal incense" type substances in May 2010 – a foil pouch of "Space Blend" herbal incense which was found to contain the substance JWH-018. Since then, exhibits have been submitted on a near-exponential frequency in both opened and unopened commercial pouches, unlabeled plastic bags, in hand-rolled cigarettes, and mixed in with marijuana. Around that same time, the DCL also received an exhibit consisting of approximately 25 grams of JWH-018 powder, having an appearance similar to cocaine. Approximately three months later in August 2010, a suspected ecstasy tablet was analyzed by GC-FID, GC-MS, and DART-TOF, and found to contain 3,4-methylenedioxypyrovalerone (MDPV), m-trifluoromethylphenylpiperazine (m-TFMPP), and caffeine, marking the first appearance of a "bath salt" compound at the HCIFS. As of August 2011, the DCL is currently capable of confirming 22 "herbal incense" and six "bath salt" compounds, with others being added as analytical standards become available. The DCL is also able to distinguish between 3- and 4-fluoromethcathinone isomers.

During the 2011 session, the Texas Legislature passed two separate bills relating to these designer drugs, using general language as opposed to an all-inclusive list of substances. Senate Bill 331 authored by Senator Florence Shapiro of Plano, TX, controls herbal incense as "any quantity of a synthetic chemical compound that is a cannabinoid receptor agonist and mimics the pharmacological effect of naturally occurring cannabinoids." Seven major structural classes are listed, and examples are given for each, listed by systematic name (ex. JWH-018, AM-2201). Penalties for possession are analogous to those of marijuana. House Bill 2118, authored by Rep. Garnet Coleman of Houston, TX, controls bath salts as "any compound structurally derived from 2-aminopropanal by substitution at the 1-position with any monocyclic or fused-polycyclic ring system." including subsequent modifications. This bill also gives examples of some applicable compounds, listed by chemical name. The language of both bills was based on the general language added to the United Kingdom's Misuse of Drugs Act in 2010, regarding these substances.

As substances, therefore, may be controlled without being listed by name, when reporting a result containing a cannabinoid receptor agonist, the DCL uses the systematic name and adds the following result statement: "This compound is also known as [chemical name]. It is a cannabinoid receptor agonist classified as a [structural class]." In order to refer to a substance as a "cannabinoid receptor agonist," the DCL requires peer-

reviewed scientific literature on file discussing the compound's cannabinomimetic properties. Upon reporting a methcathinone or pyrovalerone derivatives, the following statement is added, as appropriate: "This compound is also known as [common name]. It is a structural derivative of 2-aminopropanal."

**Designer Drug, Herbal Incense, Bath Salts**

## **A184 Analysis of Synthetic Cannabinoid (AM2201) by LC/MS/MS and GC/MS: A SPE Approach**

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After attending this presentation, attendees will learn about the analysis of a new synthetic cannabinoid (AM2201) from seized material using readily available solid phase extraction (SPE) cartridges and gas chromatography/mass spectrometry (GC/MS) liquid chromatography/mass spectrometry (LC/MS/MS). Use of this SPE method will permit analysts to provide data on this compound in samples

This presentation will impact the forensic science community by offering analysts in forensic facilities a method that permits samples of synthetic cannabinoids to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of SPE and GC/MS/ LC/MS/MS.

**Method:** Extraction (SPE) was performed on a mixed mode column (C8/WAX) conditioned with methanol, deionized water, and 0.1 M phosphate buffer (pH 6 (3mL, 3mL and 1mL, respectively)) prior to sample loading. Methanolic extracts of seized material (1mL) were adjusted to pH 6 with 0.1 M phosphate buffer (5mL) and an internal standard added (THC-d3). After loading the sample, the sorbent was washed with deionized water and a solution of the phosphate buffer containing 20% by volume of acetonitrile (3mL). After drying each SPE column was eluted with 3 mL of a solvent consisting of ethyl acetate containing 10% methanol (two x 3mL). The individual eluates were collected, evaporated to dryness and dissolved in mobile phase. These solutions were combined for analysis by LC/MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of AM2201 and THC-d3 respectively. For GC/MS analysis, after evaporation, the eluates were dissolved in 50µL of ethyl acetate/ BSTFA (containing 1% TCMS) and heated prior to injection. Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm C18 analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes. In terms of GC/MS, a temperature program starting at 100°C for one minute rising to 310°C at 40 °C/ minute was used employing a 30m x 0.25mm (250 µm) capillary column. Mass spectrometry was performed in selected ion monitoring/full scan mode (50- 500 m/z). In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis.

**Results:** The limits of detection/quantification for this method were determined to be 50ng/g and 100ng/g, respectively for both. The method was found to be linear from 100 ng/ g to 2000 ng/ g ( $r^2 > 0.999$ ). Data is presented to show that recoveries of AM2201 were found to be greater than 85%. Interday and Intraday analysis of AM2201 were found to < 5% and < 8%, respectively. Matrix effects were determined to be < 6%. Details of genuine samples are given at the presentation.

**Conclusion:** The use of this new procedure for the analysis of a synthetic cannabinoid (AM2201) will be of great use to analysts in the field

of forensic drugs analysis as the concentrations of this can now to be reported rather by either GC/MS or LC/MS/MS techniques.

**Spice, LC/MS/MS, SPE**

## **A185 Synthetic Cannabinoid Colorimetric Detection**

*Danielle Green\*, Albany State University, 504 College Drive, Albany, GA 31705; Candice Bridge, PhD, and Sue Lenhard, MS, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

After attending this presentation, attendees will learn what a synthetic cannabinoid is, how they were originally used, and the detection of marijuana and synthetic cannabinoids.

This presentation will impact the forensic science community by providing a field test specific to detecting synthetic cannabinoids.

Marijuana is illegal and highly abused used due to the psychoactive effects that the user would experience. The active component in marijuana is tetrahydrocannabinol (THC) which is what gives the psychoactive properties.<sup>1</sup> In the last 40 years compounds known as synthetic cannabinoids (SC) have been developed in an effort to research the possible medical uses of marijuana without having the consequences of the negative unwanted side effects.<sup>2</sup> Synthetic cannabinoids are able to produce similar effects to the body as THC because they bind to the same receptor sites however they do not have a similar structure.<sup>3</sup>

In 2004 an herbal mixture of plant material called "Spice" came into the drug community.<sup>4</sup> The plant material was believed to give a similar or a more potent "high" as THC. In truth, "Spice" is a combination of plant material that has been sprayed with one or more SCs and then passed as an herbal drug. Synthetic cannabinoids were not detected in "Spice" until late 2008 by a German pharmaceutical company called THC Pharm. The first reported death due to "Spice" occurred in 2010 in Iowa and since the identification in 2008 many hospital cases across the globe have been reported. Currently there is no ban placed on all SCs, only a few specific cannabinoids have been banned in numerous countries. Little research and literature is available on the detection of SCs in the field and this study will lead to exponential progress in that area.

Recently, a colorimetric field test has been developed that positively identifies the THC compound and claims to positively identify SCs. There are two tests marketed: a general screening test and a THC specific test. To conduct this study test samples were broken up into two different sections: known positives and known negatives. These groups would determine the test's specificity toward SCs. The positive samples were comprised of SC standards, THC standards, and "Spice" samples that had tested positive for SCs. The known negatives had samples of "Spice" that had tested negative for known SCs and household items that would not contain SCs like sugar, flour, oregano, and tobacco paper. "Spice" samples and known negative samples were tested as a solid and in solution for the presence of SCs. A 1mg/mL solution was prepared and tested to determine if there was a positive reaction. A positive reaction for identification occurs when the test solution in contact with the sample turns red.

The study yielded a range of results. In regards to the known positive samples only THC; CP-47, 497; and HU-210 had positive results for both tests when analyzed in the solid form and in a solution form. The other SCs and most of the true negatives did not have a color change for both the general screening test and the cannabis specific test. A few of the "Spice" samples had faintly positive results for both the tests. The study did yield some ambiguities including a lack of consistency in tests results depending on the physical state of the sample when tested. Some samples had a positive result in the solid test but did not have the same result while in liquid form. The test shows signs of movement in a positive path in regards to field detection. It is able to detect classic cannabinoids as well as THC which is a necessity in field detection.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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#### Synthetic Cannabinoids, Spice, THC

### A186 Differentiation of Isomers of Synthetic Cannabinoids

*Christina England\**, United States Air Force Academy, 2304 Cadet Drive, USAF Academy, CO 80841; *Sara Roper, BS\**, and *Lee Fadness, BS*, United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297; and *Michael J. Salyards, PhD*, 45 High Street, Sharpsburg, GA 30277

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After attending this presentation, attendees will be familiar with the combination of analytical procedures that are necessary and sufficient to conclusively identify synthetic cannabinoids and preclude the possibility of false positives. This will be accomplished by outlining, for each of fourteen commonly encountered synthetic cannabinoids, the specific combinations of tests that can differentiate the particular cannabinoid from a series of its structural isomers.

This presentation will impact the forensic science community by describing a rational approach to the analysis and positive identification of an important and growing class of abused compounds.

The first herbal smoking blends laced with synthetic cannabinoids are believed to have been produced about six years ago. Sold in foil packets with a distinctive glinting eye logo, "Spice" was the forerunner of hundreds of similar products that have been sold in head shops and on the Internet under various names. In late 2008 and early 2009, JWH-018 and cannabicyclohexanol were identified as the active ingredients in Spice. Since then, a bewildering array of synthetic cannabinoids has been encountered by forensic laboratories. The purpose of this presentation is to introduce data and analytical schemes that will allow forensic laboratories to confidently identify many of these compounds.

In this two-part presentation, attendees will be introduced to the commonly encountered synthetic cannabinoids and will be presented with direct instrumental data comparisons of isomers of these compounds. All of the analyzed compounds were synthesized by Cayman Chemical Company.

Part one of the presentation will discuss the following compounds: the 3- and 4-methoxy isomers of JWH-250, the 3- and 4-chloro isomers of JWH-203, the 2- and 3-methoxy isomers of RCS-4, the 3- and 4-methoxy isomers of RCS-8, the 3- and 4-iodo isomers of AM-694, and the 2-naphthyl isomer of JWH-200.

Part two of the presentation will discuss the following compounds: fifteen structural isomers of JWH-018 formed by pentyl chain rearrangements and 1- and 2-naphthyl linkages, seven structural isomers of JWH-073 formed by pentyl chain rearrangements and 1- and 2-naphthyl linkages, the six isomers of JWH-081 as determined by the six possible positions of the methoxy group on the naphthyl ring, the six isomers of

JWH-122 as determined by the six possible positions of the methyl group on the naphthyl ring, the six isomers of JWH-210 as determined by the six possible positions of the ethyl group on the naphthyl ring, the six isomers of JWH-398 as determined by the six possible positions of the chloro group on the naphthyl ring, the 3- and 4-methyl isomers of JWH-251, and the 2-, 3-, and 4-fluoropentyl isomers of AM-2201.

The compounds listed were analyzed by GC/MS, GC/IRD, LC/UV/MS, and NMR. The chromatographic and spectral data revealed adequate separation and/or distinguishable spectral features among all the samples. The necessary and sufficient tests to discriminate between the parent compounds and their respective isomers will be discussed.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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#### Cannabinoids, Isomers, Spice

### A187 Differentiation of MDMA Exhibits Using Liquid-Liquid Extraction, Headspace Solid Phase Microextraction, and Gas Chromatography-Mass Spectrometry

*Karlie M. McManaman, BA\**, 89 South Dietz Road, Webberville, MI 48892; and *Ruth Waddell Smith, PhD*, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824

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After attending this presentation, attendees will have learned about impurity profiling procedures for 3,4-methylenedioxymethamphetamine (MDMA). This research compares liquid-liquid extraction and headspace solid phase microextraction procedures, as well as different GC temperature programs.

This presentation will impact the forensic science community by investigating the effect of extraction procedure and GC temperature program on the association and discrimination of MDMA tablets based on organic impurities. Determining the more effective extraction procedure and GC temperature program would be a step towards standardizing the procedures used in forensic laboratories for this application. This would allow better comparison of data collected in different laboratories which will be necessary for the successful identification of drug trafficking routes.

MDMA is a Schedule I controlled substance, which is often found as an active ingredient in ecstasy tablets. Surveys such as the 2010 Monitoring the Future Survey indicate that abuse of controlled substances, including MDMA, continues to be a growing trend in the United States, with a significant increase in MDMA use for all ages questioned since the previous study five years prior. This increase has created a greater need for quick and easy identification methods for illicit tablets, as well as improved methods for the identification and prevention of drug trafficking.

Impurity profiling can be used to determine the synthesis method of MDMA in illicit tablets, potentially linking tablets to a common production source, based on the impurities present. Profiling is conventionally done using liquid-liquid extraction (LLE) and gas chromatography-mass spectrometry (GC/MS), but recent literature has also shown the potential benefits of using headspace solid phase microextraction (HS-SPME) as an alternative extraction procedure. However, reports in the literature use different GC temperature programs to analyze extracts and different statistical methods are used to evaluate the impurity profiles.

The objective of this research is to investigate the effect of the extraction procedure and GC temperature program on the association and discrimination of MDMA tablets based on the organic impurities. Determining the more effective extraction procedure and GC temperature program would be a step towards standardizing the procedures used in forensic laboratories for this application. This would allow better comparison of data collected in different laboratories, which will be necessary for the successful identification of drug trafficking routes.

Three different MDMA exhibits were used for this preliminary study. Tablets from each exhibit were homogenized to minimize variation within each exhibit due to the clandestine manufacturing process. Samples from each exhibit were extracted using both LLE and HS-SPME. Extracts were then analyzed in replicate (n=5) using four different GC temperature programs that ranged in the number of temperature ramps (one-, two-, and three-step ramps), as well as the total analysis time (36-53 minutes).

The resulting total ion chromatograms from each extraction/GC temperature program combination were treated as separate data sets and subjected to principal components analysis (PCA). This multivariate statistical procedure is used to identify sources of variance within a data set, while reducing dimensionality to allow simpler visualization. In this case, the PCA scores plots were used to assess association of replicates of the same exhibit and discrimination among exhibits. In addition, the PCA loadings plots were also used to identify those impurities contributing most to the variance in the data set. Such impurities are potentially useful chemical markers for discrimination of MDMA exhibits. Additional statistical procedures, such as Pearson product moment correlation (PPMC) coefficients and student t-tests, were used to evaluate the association and discrimination of exhibits observed in the PCA scores plots. Association of replicates from the same exhibit and discrimination among exhibits was possible irrespective of GC temperature program and extraction procedure.

#### **MDMA, Impurity Profiling, Multivariate Statistics**

### **A188 Investigation Into the Influence of Precursor Chemicals on the Chemical Profiling of Methylamphetamine**

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After attending this presentation, attendees will understand the basic principles of gas chromatography Mass spectroscopy analysis and how it is used in the chemical profiling of methylamphetamine. The paper will discuss the eight routes employed in the clandestine synthesis of methylamphetamine and the precursors used in the manufacture (e.g., ephedrine, pseudoephedrine, and phenyl-2-propanone (P2P)).

This presentation will impact the forensic science community by providing useful insight in the identification key route specific impurities of two popular clandestine synthetic routes of methylamphetamine: (1) Moscow; and, (2) Hypophosphorous routes. This research explores differences in impurity profiles extracted from samples prepared from pure grade materials and batches of methylamphetamine synthesized from precursors extracted from cold medication using different solvents and other pharmaceutical grade essential chemicals, i.e. iodine tinctures, hydrogen peroxide.

The goal of this research is to synthesize methylamphetamine using *pseudoephedrine* hydrochloride extracted from decongestant tablets which are available in pharmacies in Glasgow, United Kingdom. This precursor was extracted from the decongestant tablets with three different solvents. The three different solvents used in the extraction were ethanol, laboratory prepared methylated spirits, and commercial methylated spirits. Two synthetic routes were investigated in this research: (a) Moscow route; and, (b) Hypophosphorous route. The mentioned routes are popular in clandestine synthesis of methylamphetamine due to its simple process in a large scale production. The mentioned routes are variations of the Nagai route. In both of these routes, hydroiodic acid is made *in situ* during the reaction process. These modifications are most likely to have occurred as

a result of the difficulty of obtaining the hydroiodic acid in the market. In the Moscow Route, hydroiodic acid is made "*in situ*" by adding red phosphorous, iodine and water. For the hypophosphorous route or "Hypo route," hydroiodic acid is made "*in situ*" by adding hypophosphorous acid and iodine. This route does not require any red phosphorous in the reaction because hypophosphorous acid itself acts as the reducing agent and can be used as an alternative to red phosphorous. The two routes were chosen due to their popularity in clandestine synthesis of methylamphetamine.

Other key catalysts such as red phosphorous were extracted from matchbooks and iodine crystals derived from iodine tinctures using hydrogen peroxide. Most of the methodology of the extraction of the precursor and extracting the essential chemicals were obtained from the clandestine literature. The methylamphetamine synthesized from the two routes was analyzed by gas chromatography mass spectrometry (GC/MS) and the route specific impurities elucidated. The determination of an optimized impurity extraction method and gas chromatography mass spectrometry is essential in the investigation related to the profiling of route specific impurities of methylamphetamine. This presentation will demonstrate an ideal extraction method that will efficiently extract the maximum number of route specific impurities from two mentioned routes and optimum gas chromatography mass spectrometry conditions that produced chromatograms with well resolved peaks.

This work forms part of a wider investigation into the ability of other analytical technique to discriminate the inter and intra batch variation of methylamphetamine synthesized from the two popular clandestine routes, focusing in particular on stable isotope analysis at natural abundance level of the elements C, N, and H by Isotope Ratio Mass Spectrometry (IRMS) for this purpose.

#### **Methylamphetamine, Illicit Synthesis, GCMS**

### **A189 The Effect of Season and Soil Type on the Microbial Degradation of Gasoline**

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After attending this presentation, attendees will understand the concept of microbial degradation of gasoline and the effects of soil type and season on this process.

This presentation will impact the forensic science community and the justice system by highlighting seasonal and soil type differences in the microbial degradation of gasoline.

Substrates rich in organic matter such as soil provide an excellent source of carbon and therefore contain a high bacterial load. Since ignitable liquids are comprised of various hydrocarbons, the bacteria can utilize these fuels as a carbon source. In particular, common soil bacteria have been shown to selectively metabolize lower substituted alkylbenzenes (e.g., toluene, ethylbenzene, and propylbenzene) and normal alkanes (e.g., n-octane, n-nonane and n-decane). Previous work completed on various ignitable liquids (e.g., petroleum distillates, gasoline, isoparaffinic, and naphthenic-paraffinic products) has shown a significant loss of normal alkanes in the range of C<sub>9</sub> to C<sub>16</sub>. Branched alkanes appear to be more resistant to degradation than normal alkanes. In addition the degree of degradation was positively correlated to the length of the alkyl chain on the mono-substituted alkylbenzenes. Also, the position of the alkyl branches plays a significant role in the ability for the bacteria to metabolize the alkylbenzenes. This is problematic for fire debris analysis as samples often sit for many weeks before they are analyzed due to case backlog. As a result, selective loss of key components due to bacterial metabolism can make identifying and classifying ignitable liquid residues by their chemical composition and boiling point range very difficult.

Of interest to this study are the effects of soil type (e.g., residential, industrial or agricultural) as well as the physical and chemical characteristics of soil on bacterial populations. These characteristics

include the pH, nitrogen and phosphorus content, total organic carbon (TOC), and the soil composition (e.g., silt, clay, or sand). In addition, the season (e.g., fall, winter, spring, or summer) can strongly influence the nature and activity of some bacteria. Overall, these variables (soil type and season) could impact the degree of microbial degradation observed in fire debris samples containing soil substrates. Therefore, the microbial degradation of gasoline in agricultural, industrial, and residential soils was monitored for up to 30 days over four different seasons. The top two inches of these soils were collected from specific areas in Northern Indiana each season. Degradation studies were carried out by spiking these soils in quart-sized paint cans with 20 $\mu$ L gasoline and stored for analysis after 0, 2, 4, 7, 11, 15, 22, and 30 days. These samples were then subjected to passive headspace concentration and GC-MS analysis.

The chromatographic profiles showed that residential soil was the most active and the industrial soil was the least active in terms of the microbial degradation of gasoline. Furthermore, a positive correlation was noted between the outdoor temperature when the soil was collected and the degree of degradation. It was noted that storage at ambient conditions upon collection prior to degradation studies had no apparent effect on the rate of degradation of the gasoline.

Principal Components Analysis was also utilized to elucidate trends of microbial degradation among the different soil types and seasons. These trends showed clear differences in the overall rate of degradation of the ignitable liquid in the different types of soil as well as over the various seasons. In particular, the industrial soil lagged significantly behind the agricultural and residential soils. Even though gasoline was degraded more slowly, by 30 days the chromatographic profile was significantly altered. The aromatic profile became very important for PCA, particularly for the C<sub>3</sub>-alkylbenzenes. It was noted that as degradation progressed, the chromatographic resolution was lost between 3-ethyltoluene and 4-ethyltoluene. This was due to benzaldehyde (a possible hydrocarbon metabolite) becoming more abundant relative to the other components being degraded. Benzaldehyde has major ions of 77 and 105 in its mass spectrum. Therefore, the previously used aromatic profile which included m/z 91, 105, and 119 was adjusted to include only m/z 91 and 120. This allowed for the exclusion of benzaldehyde while still allowing toluene, the C<sub>7</sub>- and the C<sub>3</sub>-alkylbenzenes to be captured.

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#### Fire Debris, Ignitable Liquid, Principal Components Analysis

### A190 The Association and Discrimination of Gasoline and Lighter Fluid Using Multivariate Statistical Procedures in the Presence of Evaporation, Thermal Degradation, and Matrix Interferences From Surface-Treated Wood

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After attending this presentation, attendees will have an understanding of an objective method that may be used to identify the presence of an ignitable liquid extracted from wood in simulated fire debris. Principal components analysis (PCA) and Pearson product moment correlation (PPMC) coefficients are used to associate an ignitable liquid residue to the corresponding standard, in the presence of interferences from untreated and surface-treated wood, despite evaporation and thermal degradation effects.

This presentation will impact the forensic science community in two ways. First, the compounds inherent to combinations of treated or untreated and burned or unburned wood will be characterized. This may allow analysts to determine if compounds in a chromatogram of fire debris

originate from the wood matrix and/or its surface treatment. Secondly, an objective method for identifying the presence of an ignitable liquid will be demonstrated. It has become increasingly important to establish methods in which objectivity replaces subjectivity, especially in light of the National Academy of Sciences report from 2009.

In order to characterize the inherent interference compounds, samples of untreated/unburned and surface-treated/unburned wood were sealed in nylon bags and extracted at 80°C using a passive headspace extraction with activated charcoal strips. Each extract was then analyzed using gas chromatography-mass spectrometry (GC-MS). Separate untreated and surface-treated wood samples were also burned for different periods of time to determine the optimal burn time that generated the maximum amount of matrix interferences. These samples were analyzed in an identical manner to identify the compounds introduced during the burning process.

Next, two ignitable liquids (gasoline and lighter fluid) at four different evaporation levels were individually spiked onto unburned wood samples that were either untreated or surface treated. This allowed the data collected to take into account the effect of evaporation of the liquid. The liquids were also spiked onto burned wood samples that were either untreated or surface treated, which allowed the data to reflect the effects of the matrix interferences as well as the evaporation of the ignitable liquid. Lastly, fire debris was simulated by spiking the liquids onto the unburned wood (untreated and surface treated), which was then burned. This last data set took into account thermal degradation of the sample in addition to evaporation and matrix interferences.

The association of the ignitable liquid residues extracted from the wood to the ignitable liquid standards was assessed using PCA in combination with PPMC coefficients. Principal components analysis identifies the variance among samples and will cluster chemically similar samples accordingly in the scores plot. The PPMC coefficients provide a pairwise comparison between chromatograms and produce a value, which describes the similarity between the chromatograms. Multivariate statistical procedures were used to objectively associate the ignitable liquid residue extracted from the wood to the corresponding standard in spite of evaporation, matrix interferences, and thermal degradation.

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#### Ignitable Liquids, Wood Fire Debris, Multivariate Statistical Procedures

### A191 The Persistence of Gasoline on Fabrics Constructed of Natural and Manufactured Fibers

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After attending this presentation, attendees will understand that it is imperative to collect and secure fabric samples in vapor-proof packaging within 24-hours of the gasoline deposition to ensure the highest potential of detection and identification. Furthermore, the fabric fiber type and the environmental conditions to which the fabric was exposed must be known in order to predict the elapsed time since deposition of the gasoline.

This presentation will impact the forensic science community by describing some of the challenges associated with the detection and identification of gasoline on fabrics and demonstrating the impact of environmental factors on the prediction of the elapsed time between the deposition of an ignitable liquid and the detection of the residue on fabrics.

This study examined the persistence of gasoline on single-component fabrics constructed of the natural fibers cotton and silk and the manufactured fibers acetate, acrylic, nylon, polyester, olefin, and rayon. The study was designed to determine if the type of fabric affected the detection of evaporated gasoline and if that information could be used to predict the length of time between the deposition of the gasoline to the collection and analysis of the material. The persistence was evaluated with constant and variable airflow, humidity, and temperatures ranging from 34°F to 87°F. All samples were placed in vapor-proof nylon bags and

extracted using the passive headspace concentration method (ASTM E1412). One-half of an activated charcoal strip (ACS) was placed in the bag with each fabric. The bag was resealed and heated at 80°C for 16 hours. Each half strip was eluted with carbon disulfide and analyzed by gas chromatography-mass spectrometry (ASTM E1618). The extracted ion chromatograms (EIC) for the molecular ion 105 of the gasoline-spiked substrates were compared to the C3 alkylbenzenes profile of gasoline which consists of m-ethyltoluene (1-methyl-3-ethylbenzene), p-ethyltoluene (1-methyl-4-ethylbenzene), o-ethyltoluene (1-methyl-2-ethylbenzene), and 1, 2, 4-trimethylbenzene (pseudocumene). The plots of the selected ion total peak areas versus time for these compounds demonstrated the subjectivity of gasoline evaporation from the different types of fabric substrates when exposed to various environmental conditions. Generally, there was an inverse relationship between the exposure temperature and the time interval that gasoline remained detectable on the fabrics. The results for the constant and variable environment conditions were not consistent among the fabrics tested. For example, cotton retained gasoline for up to five days in the EIC at a temperature of 76°F and constant airflow. However, with variable conditions, gasoline was only detectable in the EIC for up to 24 hours. Gasoline could be detected in the EIC for nylon, olefin, and polyester for up to five days after deposition depending upon the conditions. In contrast, gasoline was identifiable in the total ion chromatogram for acetate for up to five days after deposition in both constant and variable conditions. The fabrics that were least retentive of gasoline were acrylic, rayon, and silk. Given the results of this study, it is imperative to collect and secure fabric samples in vapor-proof packaging within 24 hours of the gasoline deposition to ensure the highest potential of detection and identification. Furthermore, this study shows that the environmental conditions to which the fabric was exposed must be known in order to predict the elapsed time since deposition of the gasoline.

**Gasoline, Fire Debris, Fibers**

## A192 NIST Trace Explosives Test Bed

*Marcela Najarro, MFS\*, and Greg Gillen, PhD, National Institute of Standards and Technology, 100 Bureau Drive, Mailstop 8371, Gaithersburg, MD 20899*

After attending this presentation, attendees will learn about the laboratory research being conducted at NIST in the area of trace explosives detection and the efforts in transitioning research findings into real-world applications.

This presentation will impact the forensic science community by reporting test bed evaluations to critical stakeholders for the potential modification of explosive trace detection protocols.

There is a critical need to protect government infrastructure from potential terrorist threats. Government agencies have expanded security measures by increasing surveillance, manpower, and threat detection capabilities. The National Institute of Standards and Technology (NIST), Surface and Microanalysis Science Division, focuses on the development of measurements and standards that facilitate improvements in the reliability and effectiveness of currently deployed explosives trace detectors (ETDs) and next-generation detection technologies. Ion mobility spectrometers (IMS) - based ETDs are widely used for the rapid screening of trace explosives and narcotics residues collected by physical swiping of a suspect surface. In this technique, residues collected on a sampling swipe are thermally desorbed by rapid heating to produce neutral vapor molecules that are subsequently ionized with a <sup>63</sup>Ni source at atmospheric pressure. Although extensive research is focused on the development of next-generation technology, long-term evaluation of instrument field performance is a need in the area of trace explosives detection.

New efforts have focused in the development of a NIST Trace Explosives Test Bed. The goal of this field test bed is to test NIST laboratory findings in real-world field conditions to determine end-user

utility and provide stakeholders with operation improvement recommendations (OIRs). The development of the test bed has involved the deployment of trace detection systems throughout the NIST campus. In addition, a training program has been developed and implemented for NIST Physical Security. Laboratory research findings have been integrated into the training program including standard operating procedures for proper instrument operation and best practices for sample collection and alarm resolution. To date, over 40 NIST security clerks/police officers have been trained in the daily operation and maintenance of explosives trace detectors. Field experiments with our trained officers have yielded data supporting the improvement of collection media (swabs) as well as hand-held wands used to harvest a sample. In addition, researchers provide field screeners with well-characterized explosives test materials used daily to validate instrument performance under operational environmental conditions. Inkjet printing technology capable of depositing a known mass of explosive with better than one percent precision is used to produce the quality assurance/quality control (QA/QC) test materials. The stability of the test materials as well as testing different storage methods to establish sample shelf-life is being evaluated. Analytical figures of merit such as measurement repeatability and instrument sensitivity are evaluated as part of the analysis. Preliminary QA/QC results of ETDs deployed at the NIST test bed show typical measurement repeatability of approximately 10% RSD, when n = five using these high-level test materials. In summary, the ability to compare field data versus laboratory data allows us to compare factors such instrument drift, environmental effects, test material stability in the field, monitor need for maintenance of the detectors, as well as compare instrument response and measurement repeatability. Operational improvement recommendations developed through the test bed are now being leveraged by stakeholders in a series of pilot studies to determine their value for airport security screening. In the future the test bed will be expanded to take advantage of other resources available such as an explosives canine team and a cargo-screening facility.

**Trace, Explosives, Test Bed**

## A193 Evaporation Rates of Unconfined Explosive Liquids

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After attending this presentation, attendees will understand the evaporative trends of various nitrated explosives and their relation compounds, under unconfined conditions. Attendees will also understand how evaporative theories (Langmuir, Deegan, Berry) hold up in experimental conditions; ultimately a mathematical model to be used to describe the evaporation of unconfined explosive liquids is suggested.

This presentation will impact the forensic science community by helping understand how explosives behave under different conditions and how this behavior affects a canine's ability to detect and alert to an explosive compound.

Although canines are regularly utilized by law enforcement agencies to detect explosives, the mechanism by which canines respond to explosive vapors is not well understood. In particular the factors that govern the amount of explosive vapor available for canine sampling are often confused, leading to difficulties in canine training and testing. For example, it is a common misconception that the amount of explosive itself is the chief contributor to the amount of odor available to a canine. In fact, the concept of odor availability is decidedly more complex in that it depends very little on the amount of explosive material present. More importantly, it is known that the amount of vapor generated from explosive compounds is dependent upon several factors including sample amount, vapor pressure, rate of transport, and the degree of confinement. Underlying these factors are the

basic processes of evaporation of unconfined explosives, which are crucial to understanding how their vapors behave in other, more confined, systems.

The concept of odor availability remains controversial in the explosive-detecting canine community because the quantity of explosive used for canine testing and/or training is easily measured while the degree of confinement and the amount of vapor available for sampling is not. It has also been shown that vapors emanating from certain nitrated explosives tend to adsorb onto surrounding surfaces. Ultimately, odor availability is dependent upon evaporation rates, or the rate at which the mass of explosive material is decreases over time. Because evaporation involves both heat and mass transfer, the unconfined evaporation of an explosive must be modeled in order to fully account for odor availability. In this study, evaporation rates were determined for several explosive liquids using an analytical balance. These rates were compared to one another as well as to theoretical models for the evaporation of small liquid pools.

In general and as expected, the mass of explosive liquid decreased linearly with time with evaporation rates ranging from  $-9.57 \times 10^{-6}$  mol/sec to  $-9.09 \times 10^{-8}$  mol/sec for the most and least volatile species, respectively. Furthermore, it was shown that sample amount (i.e. surface area) and vapor pressure (as reflected in the boiling point of the substance) were determining factors in the evaporation of unconfined, nitrated liquids. For example, the mass loss of nitromethane ranged from 0.024 mg/sec (for 10 $\mu$ L) to 0.16 mg/sec (for 10mL) and the evaporation of nitromethane (374°C) proceeded approximately three times faster than nitropropane (405°C). The overall trend for the nitroalkane and nitroaromatic species was an exponential decrease in evaporation rate with increasing boiling point.

Overall, it was determined that the evaporation of unconfined nitrated liquids, exposed to open air, can be described by Deegan's model for the evaporation of liquid drops on flat surfaces, where the determining variables are the surface area of the pool and the vapor pressure of the substance. While several examples of solvent "pinning" on a metal surface (Deegan) were observed, these phenomena may be attributed to surface abnormalities in the container (i.e., unflatten surface and/or surface irregularities) as Deegan's model specifies the need for a completely flat surface.

**Explosives, Evaporation Rate, Canine Detection**

## **A194 Further Studies Investigating Zeolites for the Recovery of Oxygenated Compounds From Fire Debris Samples**

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After attending this presentation, attendees will have gained knowledge of further studies into the use of zeolites for improved recovery of oxygenated compounds from fire debris samples which may supplement the activated carbon strip technique for recovering petroleum-based products.

This presentation will impact the forensic science community by demonstrating the feasibility for implementing zeolites into forensic casework as a viable technique in fire debris analysis for the recovery of oxygenated ignitable liquids.

Heated passive headspace concentration is presently the most commonly utilized technique for the extraction of ignitable liquid residues from fire debris evidence. This process, introduced by William Dietz in 1991, typically involves suspending an activated charcoal strip within an airtight container such as a metal can and incubating the sample for a period of time. ASTM Standard Practice E 1412-07 advises heating the sample for 2 to 24 hours at a temperature of 50 to 80° Celsius. Subsequently, the compounds are easily eluted from the adsorbent with a suitable solvent, often carbon disulfide, and analyzed using gas chromatography/mass

spectrometry (GC/MS) for the potential identification of any ignitable liquid residues. It is a simple, sensitive, and nondestructive method, and can often be performed within the original sample packaging. The activated charcoal strip, which does not interact with water or nitrogen, is advantageous in its affinity for hydrocarbons and resistance to oxidation. The technique is highly efficient for recovering petroleum-based ignitable liquids, however, it has had limited success with adsorbing and concentrating oxygenated species.

In an effort to improve the recovery of ignitable liquids containing oxygenated compounds, previous studies have suggested zeolites are a suitable adsorbent for the recovery of acetone through heated passive headspace concentration. Zeolites are inorganic, microcrystalline materials that have a well-defined internal structure and uniform pore size. Most frequently aluminosilicate with cations dispersed internally, zeolite beads attract small organic molecules including alcohols. Their high thermal and chemical stability make them ideal adsorbents for heated passive headspace applications. An additional advantage to utilizing zeolite beads involves selective adsorption of small organic molecules due to their pore size. Molecular modeling has shown that the 13X zeolites were effective for recovering analytes smaller than 10 Å, such as acetone (6.3 Å). Any compound with a molecular diameter greater than the pore size may not gain access to the internal zeolite channels, and thus may not be adsorbed.

The primary aim of this study was to further optimize the conditions for implementing zeolites as a viable extraction technique within fire debris casework, complementing the activated charcoal strip method. Extraction time and temperature, desorption solvent, and GC parameters were all examined to provide for the most efficient recovery of oxygenated volatile compounds, including (but not limited to) ethanol, 1-propanol, 1-butanol and isopropanol. For example, approximately a 900% increase in recovery was observed for 1-butanol by the use of zeolites in comparison to an activated charcoal strip. This is in accordance with previous studies that reported a 300% improvement in acetone recovery by utilizing zeolites. In an effort to evaluate the ability of zeolites to selectively adsorb oxygenated volatile compounds, comparative recoveries of mixtures of petroleum and alcohol-based ignitable liquids were studied utilizing both activated charcoal strips and zeolites. In the presence of both adsorption media within the same can, 100% of three major components of gasoline (toluene, 1,2,4-trimethylbenzene, and naphthalene) selectively adsorbed to an activated charcoal strip, while approximately 90% of isopropanol adsorbed to zeolites. This phenomenon may be attributed to the size exclusion properties and polarity of the zeolites. An ideal technique for the analysis of ignitable liquids would allow for the efficient recovery of both petroleum-based and oxygenated products in a single concentration procedure.

**Zeolites, Volatiles, Fire Debris Analysis**

## **A195 Pattern Recognition Based Library Searching Techniques for IR Spectra of Clear Coat Paint Smears**

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After attending this presentation, attendees will understand the principles of pattern recognition techniques, advantages of preprocessing and deconvolving vibrational spectra using the wavelet transform, and the importance of coupling search prefilters with library searching algorithms to improve the accuracy of IR spectral searches in the PDQ database.

This presentation will impact the forensic science community by being aware of modern automotive paints using thinner undercoat and color coat layers, but a thicker clear coat layer. All too often, a clear coat paint smear

is the only layer of paint left at the scene of a hit and run accident where damage to vehicles or injury or death, especially of the pedestrian, has occurred. In these cases, the text-based portion of the PDQ database will not be able to identify the motor vehicle as to the manufacturer and model. However, crucial investigative lead information from infrared spectra of clear coat paint smears can be extracted from wavelet-transformed spectra using pattern recognition techniques.

Applying a wavelet packet tree to de-noise and deconvolve infrared absorbance spectra of clear coats by decomposing each spectrum into wavelet coefficients which represent the sample's constituent frequency, a genetic algorithm for pattern recognition and feature selection has been used to identify wavelet coefficients characteristic of the manufacturer and model of the automobile from which the clear coat paint sample was obtained. Wavelet coefficients characteristic of the manufacturer and model of the automobile are formulated into search prefilters. Even in challenging trials where the samples evaluated were all the same model (Chrysler or General Motors) with a limited production year range, the respective manufacturing plants could be correctly identified using a search prefilter.

Utilizing search prefilters, many of the problems encountered in library searching can be addressed. Most spectral comparisons performed during a search are of little use because the spectra in question are very dissimilar. A prefilter can quickly spot dissimilar spectra, thereby avoiding a complete spectral comparison. Prefilters allow for more sophisticated and correspondingly more time-consuming algorithms to be used for spectral matching since the size of the library is culled down for a specific match.

Searches currently performed using the PDQ database often generate a large number of hits because the chemical information in the current PDQ database is only described in terms of generic chemical formulations. The major advantage of using the pattern recognition approach to identify paint samples is an increase in search accuracy because spectra from the entire database are searched. Improving discrimination capability between spectra in the database using the wavelet packet transform for spectral preprocessing and the pattern recognition GA to identify informative coefficients permits inter-comparison of original equipment material (OEM) automotive paint layer systems using the infrared spectra alone. This allows comparison of all possible pairs in the database, reducing dependence on the text-based portion of the database, resulting in improved ease of use and fewer errors. By coupling the proposed pattern recognition searches with a library search algorithm that utilizes the cross correlation function, it will be possible to perform similarity searches (i.e. identify spectra in the library that are similar but are not identical to the unknown). If a paint sample is not contained in the PDQ library, similarity searching is crucial for a tentative identification. Currently, there are no algorithms commercially available to perform similarity searching, so this feature is expected to have significant impact in the field of spectral library searching.

**Library Matching, Search Prefilters, Spectral Pattern Recognition**

## **A196 Determination of Unique Fracture Patterns in Glass and Glassy Polymers**

*Allison C. Baca, BS\*, Frederic A. Tulleners, MA, and John Thornton, DCrim, University of California-Davis, Forensic Science Graduate Program, 1333 Research Park Drive, Davis, CA 95618*

After attending this presentation, attendees will gain information on current research in the field of fracture pattern analysis. Particular attention will be given to the uniqueness of each fracture pattern. A statistical treatment of observed data will be presented, together with a discussion of potential error rate and measurement uncertainty.

This research will impact the forensic science community by providing information relevant to the reliability of physical match and fracture pattern interpretation. In particular, the issue of coincidental fracture duplication will be addressed.

Not infrequently, investigators ask if two glass fragments collected from different locations fit together in a manner demonstrating their former adjacency in a now fragmented glass object. Clearly, the evidentiary value of such a fit depends on the reliability of methods that demonstrate the uniqueness of fractured glass surfaces and the physical properties of glass itself. But fracture mechanisms, not uniqueness of fracture patterns, have been the focus of research in the forensic science community. The goal has been to understand such mechanisms and to reveal ways of identifying the fracture mechanism in any given situation. Thus one does not know how unique fracture patterns may be or how reproducible they are.

Also, within the forensic sciences, the uniqueness of fracture margins of glass and other brittle solids has been assumed. While there is empirical evidence of this, there has been little effort to develop a rigorous fundamental basis to support this assumption. Much of the forensic literature dealing with glass and glassy polymer evidence has spoken of the mechanisms of fracture propagation rather than fracture uniqueness. The present work has focused on the issue of uniqueness.

Fractures were initiated using two different methods, velocity impact and static pressure. For the velocity impact method, a drop weight was released at a predetermined velocity to initiate the fractures in glass window panes and glass bottles. Ten of each item was fractured for each of the three interchangeable tips used in the drop weight. The three tips used included a round tip, a sharp tip, and a blunt tip. These three tip types were chosen to determine if the type of surface that impacted the glass had any effect on the fracture pattern.

For the static pressure method, a hydraulic press was used to apply pressure to the glass window panes and glass bottles until fractures were obtained. Again, ten of each item was fractured for each of the three tips used in the hydraulic press. The round, sharp, and blunt tip types were again used to determine if the type of surface that impacted the glass had any effect on the fracture pattern. Once all fracture experiments were complete, the fracture patterns were converted to digital images and an inter-comparison analysis was completed to determine the uniqueness of each fracture pattern.

It was found that in an inter-comparison of the sixty glass window pane fractures, none of the 3,600 comparisons were determined to be duplicate fracture patterns. In an inter-comparison of the sixty glass bottle fractures, it was also determined that none of the 3,600 comparisons were found to be duplicate fracture patterns.

Study sponsored by NIJ Grant 2010-DN-BX-K219 "Determination of Unique Fracture Patterns in Glass and Glassy Polymers"

**Glass Fracture, Physical Match, Fracture Pattern**

## **A197 Glass Analysis by Laser Ablation Inductively Coupled Plasma Mass Spectrometry: Casework Experience**

*David Ruddell, PhD\*, Brenda Burton, MBA, Hendrik Dorn, PhD, and Alexander Heydon, BSc, Centre of Forensic Sciences, 25 Grosvenor Street, Toronto, ON M7A 2G8, CANADA*

After attending this presentation, attendees will have a better appreciation of how elemental analysis works in real cases. They will also be able to acquire software that will assist in reporting of results and in research.

This presentation will have an impact on the forensic science community by demonstrating the utility of Laser Ablation Inductively Coupled Plasma Mass Spectrometry in forensic casework.

The use of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) for the analysis of glass has been the subject of a great deal of research; however, reports of the use of this technique in casework are lacking. This presentation will examine the use of elemental analysis of glass from samples collected since 2009. In that time, of the cases where elemental analysis had potential to be useful (i.e., excluding

cases with no questioned glass recovered, or where the questioned glass was different in refractive index or thickness), 64% had sufficiently large questioned samples to perform LA-ICP-MS analysis.

A total of 82 glass samples from cases submitted to the CFS have been analyzed with LA-ICP-MS, with the following trace elements used for discrimination: 49Ti, 55Mn, 85Rb, 88Sr, 90Zr, 137Ba, 139La, 140Ce, 146Nd, and 208Pb. Using pairwise comparison analysis (3321 pairs) with a modified  $\pm$  four standard deviation match criterion, three Type II errors were found, giving a false inclusion rate of approximately one in 1000. Further analysis of the casework samples has shown little correlation between refractive index and the concentration of any of the ten trace elements that are used for comparison (the largest R2 value among the ten elements is for 49Ti, at 0.19). Given this, it is not unreasonable to assume that the trace element concentration and refractive index are independent. Using this assumption, along with the observation that in typical cases, the refractive index range for a known sample will match 1-4% of the samples in the CFS database, the coincidental match probability can be estimated. For two unrelated pieces of glass, when refractive index, thermal history, and elemental concentration have been determined, this is approximately one in 25,000-100,000.

In order to present the results of the elemental analysis in a more readily understandable form, a spreadsheet has been developed that not only determines if samples are indistinguishable from one another, it also presents the information in a clear manner, suitable for reviewers or presentation in court. A related spreadsheet has also been developed that can perform pairwise comparisons for up to 500 samples (124,750 comparisons). This has great utility in determining Type I and Type II error rates. Both spreadsheets are freely available to interested parties.

Two interesting cases will be discussed. The first had questioned and known sources that were indistinguishable in refractive index, but differed in elemental concentration. This is the first case seen at the CFS where samples indistinguishable in refractive index were distinguished by elemental analysis. The second case, a “smash and grab” at a jewelry store, had four known samples from one location. Questioned glass was indistinguishable from all four known samples in refractive index, and indistinguishable from three of the known samples in elemental concentration. One known sample was different in just one element.

#### Glass, Elemental Analysis, ICP-MS

## A198 Nonparametric Permutation Hypothesis Testing: Applications to Physical Evidence

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The goal of this presentation is to introduce the audience to nonparametric permutation testing which frees the analyst from distributional assumptions, and to demonstrate the application of the method to physical evidence evaluation.

This presentation will impact the forensic science community by demonstrating methods in which physical evidence is evaluated in the laboratory and presented in court.

This presentation will provide an overview of a nonparametric permutation hypothesis testing methodology and demonstrate the application of the method to laser-induced breakdown spectroscopy (LIBS) analysis of paint and glass samples, and analysis of fiber evidence by microspectrophotometry.<sup>1-3</sup> Research results will be presented and a method for reduction to practice will be described. The methodology described in this presentation directly addresses the need for improved statistical assessment of physical evidence.

Parametric statistical tests often rely on the assumption of normality which frequently does not hold for experimental data and the robust nature of the statistical test is relied upon to insure reliable results.

Nonparametric tests free the analyst from rigid distributional assumptions. The nonparametric permutation test exhibits excellent discriminating power for the physical evidence analyses mentioned above and rigorously holds the actual size of the Type I error at the nominal level.

The nonparametric permutation test has been applied to multivariate data sets comprised of spectral profile representations of the samples. Multiple spectra from each of two samples are compared through a similarity metric (i.e., Fisher transformations of Pearson product moment correlation coefficients). The difference between the sums of the similarity metric within spectral groups and between spectral groups,  $W_n$ , is calculated for all  $n$  permutations of the set of spectra. The fraction of  $W_n$  greater than or equal to the original spectral grouping,  $W_0$ , is a measure of the p-value for the test.

Results from laboratory tests of glass, paint and fiber analyses show that the test is sensitive enough to detect within-sample variations at a prescribed significance level (i.e.,  $\alpha = 0.05$ ). An example of LIBS analyses of glass samples is shown in Figure 1. Analyses were conducted in five different areas on each of ten different automobile side windows. Twenty comparisons each were made between all same window-same area (SWSA), same window – different area (SWDA) and different window (DW) samples, for a total of 4,100 comparisons. The distributions of the p-values for each set of comparisons are shown as box plots in Figure 1. The SWSA p-values are randomly drawn from a uniform distribution with 3.5% of the values less than or equal to 0.05 (i.e., 3.5% Type I error which is near the nominal  $\alpha = 0.05$ ). A total of 54.8% of the SWDA p-values fell below 0.05, indicating a significant discrimination between different areas of the same window. Substantial variation of elemental concentration within float glass panes has been reported based on LA-ICP-MS analyses.<sup>4</sup> A total of 98.7% of the DW comparisons gave p-values below 0.05 (i.e., a 1.3% Type II error rate), reflecting the excellent power of the test. A methodology will be described which combines the nonparametric permutation test with a Wilcoxon rank sum test to allow the nonparametric permutation method to be effectively applied to casework samples.

All calculations were performed using software written in-house.

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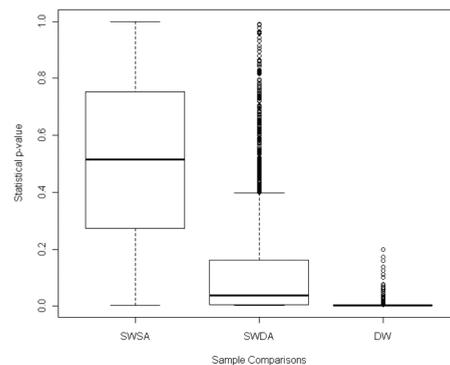


Figure 2. Statistical p-value distributions from the comparison of LIBS spectra taken from the same window-same area (SWSA), same window-different area (SWDA) and different windows of automotive side windows.

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**Hypothesis Testing, Nonparametric Permutation Methods, Physical Evidence**



ATLANTA 2012

# DIGITAL & MULTIMEDIA SCIENCES



ATLANTA 2012

## B1 Scientific Validation of Digital Forensics Tools: A Case Study

*Marcus Rogers, PhD, 401 North Grant Street, West Lafayette, IN 47907; and Philip Craiger, PhD\*, Daytona State College, Advanced Technology Campus, 1770 Technology Boulevard, Daytona Beach, FL 32117*

After attending this presentation, attendees will: (1) become familiar with the importance of digital forensics tool validation; (2) will be provided an overview of a large-scale study demonstrating digital forensics tool validation process; and, (3) will be presented lessons learned from a validation study.

This presentation will impact the forensic science community by showing how scientific validations of digital forensic tools are important. Anecdotal evidence; however, suggests that the tools do not appear to undergo the same scrutiny as forensic tools in other more established disciplines. Therefore, it is important that examiners (some of whom may not have a good background in computing) understand the tool validation process and how the lack thereof may affect the interpretation of the results.

As with all other forensic disciplines, the results obtained from digital forensics tools must meet basic evidentiary and scientific standards to be allowed as evidence in legal proceedings. In the United States, the requirements for the admissibility of scientific evidence and expert opinion were outlined in the precedent setting U.S. Supreme Court decision *Daubert vs. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579*. The U.S. Supreme Court found that evidence or opinion derived from scientific or technical activities must derive from methods that are proven to be “scientifically valid” to be admissible in a court of law. The term “scientifically valid” suggests that the tools and methods are capable of being proven correct through empirical testing. In the context of digital forensics, this means that the tools and techniques used in the collection and analysis of digital evidence must be validated and proven to meet scientific standards.

The recent case of *State of Florida vs. Casey Anthony* underscores the importance of tool validation. Examiners conducted an initial analysis of the family computer and found that someone had performed an internet search for the keyword “chloroform,” and that the same user (apparently) visited a web page — a single time — that contained information on “how to make chloroform.” A subsequent analysis with a different tool indicated the same web page had been visited **84 times** within a few minutes. Such a discrepancy between tool results clearly indicates a problem with the validity of one, if not both, tools.

This presentation presents an overview of a large-scale digital forensics tool validation study consisting of over 150 validation tests, as well as lessons learned. Funded by the National Institute of Justice, the purpose of the study was to validate several popular digital forensics tool suites with respect to the most commonly employed functions (e.g., keyword search, identifying deleted files, file recovery, hashing, internet history, etc.), across multiple versions of operating systems as well as across multiple file systems. The validation protocol described in the *Scientific Working Group on Digital Evidence’s Recommended Guidelines for Validation Testing (Version 1.1)* was used for each validation test. Testing protocols required the creation of multiple source evidence sets for a crossed design (e.g., XP-FAT32; Vista-FAT32, Vista-FAT32, XP-NTFS, Vista-NTFS, 7-NTFS, etc.). Evidence creation scripts were developed and used to create sample evidence for each experimental “cell” (combination of operating system and file system where appropriate).

Although a few anomalies were identified, in general, the results of validation testing suggested that the digital forensic tools were capable of accurately performing the functions tested. Operating system version did

not appear to affect accuracy of the tools, nor did file system type (e.g., FAT32 vs. NTFS). One interesting finding was that the user manuals for the tools occasionally did not precisely specify the limitations of a particular function, which could affect the results and consequently an examiner’s interpretation of the results. This may be of some consequence if examiners are unfamiliar with the tools, and have little knowledge or experience with validation testing.

**Digital Forensics, Tool Validation, Scientific Validation**

## B2 An Alternate Methodology for Validating Hardware Write Block Devices

*Benjamin R. Livelsberger, MS\*, National Institute of Standards and Technology, 100 Bureau Drive, Stop 8970, Gaithersburg, MD 20899-8970*

The goal of this presentation is to show how the Computer Forensics Tool Testing (CFTT) project at National Institute of Standards and Technology has developed an alternate methodology for validating Hardware Write Block (HWB) devices. After attending this presentation, attendees will be familiar with this methodology and the benefits it offers.

This presentation will impact the forensic science community by showing how the current methods used to validate HWBs have an inherent weakness. This presentation will have the impact of educating the community to the nature of this weakness and to introduce an alternate NIST-developed method that seeks to account for it.

Before being used in an investigation, the correct functioning of a forensic tool must first be established. For Hardware Write Block tools this involves testing: (1) that the HWB allows informational and read commands to be passed to the drive and their responses to be returned to a host computer; and, (2) that it blocks modifying commands from reaching the protected drive.

The current commonly used method for validating a HWB involves using forensics tools and/or common operating system utilities and operations to attempt to read and write to a protected drive. There is a weakness inherent in this approach, namely that it only tests a small subset of the commands that could be used to read from or write to a drive. For example, testing a HWB’s ability to block modifying commands by attempting a file copy operation to a protected ATA drive using an operating system that implements the WRITE DMA EXT command for ATA devices will only test the hardware write block’s ability to block the WRITE DMA EXT command; it will not test whether the HWB blocks the WRITE SECTORS EXT or WRITE MULTIPLE EXT commands. In this scenario, a malfunctioning HWB that incorrectly allows the WRITE MULTIPLE EXT command to be passed to protected drives will not be identified as faulty. A more thorough approach, one that tests the HWB’s behavior with a broader range of commands than those implemented by a given operating system is desirable.

The Computer Forensics Tool Testing (CFTT) project has developed an alternate methodology for validating HWBs. With this methodology, testing is not limited by the subset of commands implemented by the operating system being used. HWBs are instead tested with all read and write commands as defined in ATA specs 4-8 and SCSI Block Commands-2 and as implemented by an extended version of the ATArw Linux library written by Kyle Sanders and Simson Garfinkel of the Naval Post Graduate School. Three Linux programs were written to implement the CFTT methodology. These programs tie into the ATArw library to send ATA or SCSI commands to devices via the Linux SCSI Generic driver. The three programs are:

\* Presenting Author

**try\_read** sends all defined SCSI or ATA read commands to a drive;  
**try\_write** sends all defined SCSI or ATA write commands to a drive, and;  
**write\_verify** measures whether any hard drive sectors have been successfully written to.

Using these programs, a HWB tool may be validated in the following manner:

1. For each hard drive interface supported by the HWB, initialize a drive with known content.
2. Calculate a before reference hash for each drive.
3. For each permutation of host-to-blocker and blocker-to-drive interfaces execute the `try_read` and `try_write` programs.
4. Calculate an after reference hash for each drive.
5. Use `write_verify` and a comparison of the reference hashes to measure whether any sectors on the test drives have changed.

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### Digital Forensics, Hardware Write Block, Tool Validation

## B3 Creating Deleted File Recovery Tool Testing Images

*James R. Lyle, PhD\*, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8970, Gaithersburg, MD 20899*

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After attending the presentation, attendees will be made aware of some of the issues in the creation of test data for testing computer forensic tools used to recover deleted files from digital evidence and strategies used by the Computer Forensics Tool Testing (CFTT) project to address the issues.

The presentation will impact the forensic science community by increasing awareness of what impact tool test strategies have on the ability to reveal anomalies in tool behavior. The presentation will aid the forensic practitioner in the preparation of test data sets for testing forensic tool capabilities for the recovery of deleted files.

The CFTT project at the National Institute of Standards and Technology develops methodologies for testing computer forensic tools. This presentation covers creating test data images for testing digital forensics tools that recover deleted files using residual meta-data after a file is deleted to recover file name and file content.

A file system is used to store data for access by a computer. This data is normally stored within a tree-structured hierarchy of directories and files. When a file or directory is deleted from a file system, the associated *metadata* entry and the stored data are no longer directly accessible to the user and appear to be completely removed. However, in many file systems, e.g., FAT, neither the metadata associated with the file nor the actual content is completely removed. This creates a situation where there is *residual metadata* (metadata remaining after a delete has occurred) that is still accessible by direct access outside the usual operating system methods and can be used to reconstruct deleted files. Many forensic tools exploit the behavior exhibited by file systems of leaving metadata behind after a file is deleted to attempt to recover these deleted files. Metadata-based deleted file recovery should not be confused with *file carving*, i.e., scanning unallocated memory for the file signatures present within a file itself to identify a deleted file. The scope of this presentation is limited to metadata-based deleted file recovery tools that use file system metadata from file system structures such as directories or i-nodes to identify recoverable deleted files.

The basic approach to creating a test image is as follows:

- Create a file system on a secondary storage device.
- Create some files.
- Delete some of the created files.
- Image the storage device.
- Use the tool under test to attempt to recover the deleted files.

This basic approach requires refinement so that the tool testing can produce verifiable results; the following issues need to be addressed:

1. The content of the image file needs to be documented. In particular, the following should be noted:

- Active files and blocks allocated to each active file
- Deleted files and content history for blocks allocated to a deleted file and time of file deletion
- MAC (modify, access & create) times for each file
- File attributes
- Each operation (create, append or delete) on files.

2. Identification of conditions within a file system that are relevant to tool behavior and should be present in test images.

3. Development of techniques to bring about the relevant conditions.

Four programs were created to aid documentation of image file content as follows:

- **not-used** writes the text message “not used” to each sector of the storage device. This is run as a first step before the file system is created.
- **mk-file** is used to create files. Each block of the file is tagged with the file name and a sequence number. Blocks allocated to a file are easy to identify and track.
- **ap-file** is used to append to an existing file. This appends blocks to the file already created and continues the block numbering where it left off.
- **layout** scans an image file and classifies blocks as files, metadata or not used.

These four programs simplify classification of blocks from an image file as either *allocated to a file, never been used* or *file system metadata*.

This presentation gives an overview of the issues in creating deleted file recovery test images and techniques that can be used to address the issues.

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### Digital Evidence, Software Testing, Deleted File

## B4 The Role of Global Telecommunications Providers in Tracking and Investigating Information Security Incidents

*Christopher W. Day, BS\*, 2 South Biscayne Boulevard, Suite 2800, Miami, FL 33131*

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After attending this presentation, attendees will have a better understanding of how global telecommunications providers can provide assistance to digital forensic investigators investigating a host of information security breaches and incidents. Global providers have a vested interest in tracking and mitigating many forms of information threats such as so-called advanced persistent threats, organized crime activity, botnets, child pornographers, and so on. Many providers offer their customer base various services against these threats, while others are compelled by their national governments to maintain certain records for law enforcement use. All are interested in protecting their infrastructure from today’s increasingly sophisticated threats. The types of information potentially available to those investigating an information incident may include extensive flow records (including multi-hop, multi-jurisdictional data), access logs, reputational statistics for IP blocks and addresses, peering data, and many others. In certain limited circumstances, even captured packet data may be available.

This presentation will impact the forensic community by providing practitioners with awareness of the types of information that may be available from global telecommunications providers, how the information can provide evidence to support an investigation, and how to go about requesting and preserving the evidence. There are also a number of formats in use today for reporting incidents as well as requesting support from a provider for an investigation. A number of case examples will be discussed to better demonstrate how the various types of data have been used in this study to investigate a wide array of computer intrusions and incidents in the past. Finally, various legal frameworks and privacy issues that come into

play when requesting and utilizing this sort of information will be discussed. As well as a number of industry initiatives to capture, utilize, and normalize data submission. This useful data in an appropriate manner for investigation purposes while preserving and protecting privacy and civil liberties will be shared.

Many incidents today involve multiple systems in various international jurisdictions. Piecing together the evidence to not only understand the scope of the incident, but also determine attribution of the perpetrators, can be a daunting and time-consuming process. In many cases, investigators are not aware of what types of information may be available to them from a given provider. In some scenarios, access to a known-malicious host, in its entirety, has been made available for forensic analysis. In certain limited circumstances, even captured packet data may be available under the appropriate legal frameworks. Additionally, with the ever-growing movement of computing workloads into various cloud computing infrastructures, many owned and operated by telecommunications providers, investigators must be cognizant of what types of evidence and information cloud providers are maintaining to support their businesses and how that information can be useful to an investigation. The presentation will also discuss issues relating to the scale of the evidence acquisition problem brought about by increases in bandwidth, system memory, data storage, and the elasticity of today's cloud computing environments. Investigators must be prepared to receive and process potentially massive amounts of data (possibly on the order of terabytes) when requesting incident information from large providers.

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**Telecommunications, Global, Investigation**

## **B5 Hand Identification Based on Skin Characteristics**

*Meredith Dilley\*, United States Air Force Academy, 2304 Cadet Drive, USAF Academy, CO 80841; Christina A. Malone, MFS\*, United States Army Criminal Investigation Laboratory, Digital Evidence, 4930 North 31st Street, Building 925, Forest Park, GA 30297; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

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After attending this presentation, attendees will understand inter- and intra-observer reliability when counting commonly found skin features of the hand that may be used in photographic hand comparisons.

This presentation will impact the forensic science community by determining if different examiners see the same features on the same hands, and if the same examiner sees the same features in the same hands. A second goal of this study is to examine how observer reliability is correlated with examiner experience in pattern recognition.

The markers used in this project include moles, freckles, sunspots, and scars. These dermatological features vary in appearance and are a potentially valuable resource for establishing individuality. As these features may form throughout an individual's life, current data and relatively recent photographs are essential. In general, these markings are constant, and such observable skin features have been useful for facial identification; however, oftentimes a suspect's face is not present in a photograph. Hands are a useful alternative, as they may be depicted in images of crimes such as: kidnappings, trophy killings, sexual assault, and child pornography.

Previous research suggests the use of a method for conducting photographic comparisons of the dorsal surface of the hand using fourteen regions. The current research consisted of examining inter-/intra- rater reliability of observers assessing a series of images of the right hand and counting the number of markers in each of these fourteen regions of the hand. Three of the thirty-four images were repeated three times, creating a total of forty images. The sets of images were examined by individuals with various experience levels in pattern recognition and dermatology. It was hypothesized that examiners with more experience would have similar

results and that the three repeated hands would have closely correlated values.

Raw data was compared by examiner, region, hand, and repeated hands. The values were cross-correlated to determine the similarity between results for each examiner and for each hand. The results show that high correlation values were obtained for different examiners comparing the same hands, while slightly lower correlation values were obtained when comparing different hands. In general, those examiners with more experience had more correlated results than those with less experience. The repeated hands had comparable values to one another. Further, it seemed that some hands were particularly difficult to examine based on their high ranges and standard deviations across all examiners.

Using hands for identification of an individual will give investigators another tool to use in identifying suspects. Improvements are needed, but this method shows promise for future research and demonstrates the need for experience in image analysis and pattern recognition when conducting photographic comparisons of the hand.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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**Inter-/Intra- Observer, Identification, Photographic Comparison**

## **B6 Photographic Analyses Using Skin Detail of the Hand: A Methodology and Statistical Evaluation**

*Christina A. Malone, MFS\*, United States Army Criminal Investigation Laboratory, Digital Evidence, 4930 North 31st Street, Building 925, Forest Park, GA 30297; and Michael J. Salyards, PhD, 45 High Street, Sharpsburg, GA 30277*

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After attending this presentation, attendees will understand a method for examining micro-level skin detail of the dorsal surface of the hand in photographic comparisons. Such comparisons have been conducted worldwide by experts in image analysis and human variation, using skin features to demonstrate unique aspects of unknown individuals.

This presentation will impact the forensic science community by demonstrating a method which can be easily employed to assess the distribution and frequency of commonly found skin features on the dorsal surface of the hand.

Lesions, scars, marks, tattoos, and other imprints on the skin can aid in the identification of unknown individuals and have been employed by law enforcement agencies for suspect and victim identification. The importance of hand analysis has arisen in recent forensic image comparison casework, including sexual assault and child exploitation cases, where images have been submitted in which the individual's face was not present in the image.

This research utilizes a database of 128 hands collected from employees of the U.S. Army Criminal Investigation Laboratory to examine the frequency and distribution of micro-level skin detail on the dorsa of the hand. To assess the location of features, the hand was segmented into fourteen regions using readily discernable anatomical landmarks. Each hand was assessed for the number of features found in each region. Pigmented lesions were observed in 14/14 regions, and scars or injuries were observed in 13/14 of the regions. Overall, 2,618 pigmented lesions and ninety-two scars or injuries were documented.

Descriptive statistics (range, mean, and variance) were calculated to compare each of the regions to one another. Additional statistical techniques were employed to determine which regions of the hand demonstrated significant difference in the distribution of features. When the location of pigmented lesions was considered, Region Thirteen expressed the most pigmented lesions per hand, and Region One demonstrated the least pigmented lesions per hand. When evaluating the location of scars and injuries, Region Eleven exhibited the most

scars/injuries per hand, and Region One showed the least scars/injuries per hand. The quantity of lesions present and how they were distributed across regions provided information on how the hands could be differentiated from one another.

Through the assessment of skin detail in this study, an examiner has another tool that can be employed when performing photographic comparisons and when explaining findings in a courtroom. With the increased demand for photographic comparisons of skin and the expanded availability of digital cameras, the current study presents a set of data and calls for the implementation of further research.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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#### **Photographic Comparisons, Image Analysis, Forensic Identification**

### **B7 A New Technique for Photo Skull Superimposition Using CT and Presentation Software**

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After attending this presentation, attendees will learn about a new technique for rapid superimposition of a skull and facial photograph for postmortem identification with standard software, following simple steps, on almost any personal computer.

This presentation will impact the forensic science community by illustrating this new method which, using universal technology, quickly and simply replicates the results obtained by the widely accepted but complicated and time-consuming video sequencing process.

The technique of using complex video setups with film editing techniques can be greatly simplified with custom modern CT technology and PowerPoint® presentation software including custom animation.

The validity and usefulness of craniofacial superimposition in identification cases has been demonstrated for decades.<sup>1</sup> When the anatomical features of the skull do not align with the photograph, a match is eliminated. However, when there is alignment between the anatomical features or anomalies, it offers strong evidence of identity, even though the evidence is circumstantial. In cases with unusual cranial morphology, alignment between the skull and photograph is even more compelling evidence.

Craniofacial superimpositions were initially made by using tracings of skull and faces from photographs. Since these early attempts, other craniofacial superimposition techniques have been developed. Early photographic comparison of a skull and a facial photograph required scaling and orientation of the skull photograph to match the precise pose of the head when photographed.<sup>2</sup> Clyde Snow, in 1976, may have been the first to use video superimposition, which has become the method of choice since the early 1980s.<sup>3</sup> It has often appeared to be the best solution for correcting the many variables encountered: the most difficult being the scaling and orientation of the skull image since the photograph of the person in question is unalterable.

A modern video superimposition laboratory is described as requiring several people and substantial equipment.<sup>1</sup> Two video cameras are needed; one is directed at the photograph, the other at the skull, which must be supported in some fashion to permit altering its orientation in three planes. The two images thus acquired are superimposed and blended on a third monitor while correcting for scale.

Given the remarkable advances of 3D reconstruction of CT generated images, this process can be simplified. This paper defines the technique of using 3D rendering software and PowerPoint® presentation software to

produce quickly and simply the same image sequences rendered by video superimposition techniques. This can be accomplished using almost any personal computer and modern CT equipment with minimal manpower. Further, a great advantage of this new technique is that it does not require defleshing a skull, thus permitting examination of a live subject who is unrecognizable due to injury, or a partially decomposed decedent.

With this new technique, unlimited skull orientation is accommodated using 3D reconstruction software available with any modern diagnostic CT software or DICOM viewer software. Standard features built into presentation software permit easy scaling and superimposition of both images. Using these techniques, standard video sequences— fades, sweeps, and box sweeps — can be replicated quickly and with minimal effort.

A real-time sample case demonstrating this new technique will compare traditional concordant craniometric landmarks on a skull to cephalometric landmarks of two individuals of same sex and ethnicity, similar age and body stature.<sup>4,5</sup> Possible match or exclusion will derive from superimposition of such landmarks as: skull to face proportion; cranium width to forehead; eyebrows to superorbital margin; eye/pupil to orbit; external nose; and, nasal ala to nasal aperture.

In conclusion, this craniofacial superimposition technique offers an expedient method for comparing unidentified skulls to photographs for purposes of identification. This innovative technique was devised to meet the challenge of matching a skull to one of three robbers involved in the infamous 1876 Northfield Bank Robbery.

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#### **Identification, CT, Superimposition**

### **B8 Heart Beat Detection in Snuff Movies**

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After attending this presentation, attendees will have an insight into the forensic possibilities for the determination of the presence and rate of a heart beat from video.

This presentation will impact the forensic science community by investigating snuff movies and determining if a heartbeat is measurable from the veins in the face with ordinary webcams or video to help determine if a person is dead or alive.

Experiments are conducted to obtain the heart rate from a movie for forensic use in, for example, snuff movies. The approach is based on the principle of photoplethysmography. This means that volumetric changes are measured with the aid of a light source. Here, the changes of interest are the volumetric changes of the blood vessels and the light source used is ambient light.

The heart rate obtained from a movie is compared with the heart rate measured with a finger pulse oximeter and the two methods are used for the comparison. The first method is the root mean squared error and the second is based on the, so-called, Bland-Altman statistics.

Results are obtained from movies of the facial area (recorded with a digital camera and a webcam), an arm (inside and outside, digital camera), a hand (inside and outside, digital camera), and a leg (outside, digital camera).

The maximum obtained root mean squared error is 2.69 beats per minute (hand inside) and the maximum Bland-Altman standard deviation is 2.44 beats per minute (hand inside). Therefore, it can be concluded that the heart rate determined from movies as well as the heart rate measured with the finger pulse oximeter do not differ significantly.

In the experiments that examine the facial area, a face tracker is used. The region of interest is a centered rectangle. The agreement, between video and finger pulse oximeter, might be further improved by using a different region of interest, e.g., the forehead or cheek area. No tracker is used in the experiments with the arm, hand, or leg. Introducing a tracker in these experiments will likely reduce errors caused by motion artifacts.

The parameters used to analyze the photoplethysmographic signal extracted from a movie are determined with a genetic algorithm. Changing the parameters from their optimal values has a great impact on the determined heart rate. This aspect seemed not present in the results reported by Poh of MIT.<sup>1</sup> It is assumed that the difference is caused by the presence of an additional light source. Extra experiments have been performed to validate this assumption.

Additional experiments showed that decreasing the resolution of the movie as well as compression of the movie both increase the root mean squared error and the Bland-Altman standard deviation.

In current implementation, the algorithm always selects a frequency in the range of interest (0.75-4.00 Hz). Therefore, the implementation needs to be modified in order to apply it to snuff movies. A possible approach is to examine the photoplethysmographic signal obtained from the video) for typical photoplethysmographic features. A good feature could be the standard deviation and a standard deviation lower than normal could indicate a subject without a pulse. Validation of the method is necessary before it is applied in casework.

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#### Heart Beat, Measurement, Video

## B9 Implementation of the Likelihood Ratio Framework for Camera Identification Based on Sensor Noise Patterns

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After attending this presentation, attendees will understand some of the typical problems that may arise when applying the Bayesian framework of evidence evaluation to camera identification based on sensor noise patterns.

This presentation will impact the forensic science community by illustrating practical issues when the Bayesian framework of evidence evaluation is applied based on similarity scores between traces.

In digital forensics, the question of which particular camera was used to make a certain photograph may arise, e.g., in child pornography casework where an accused is suspected of producing photographs in addition to possessing them. Instead of looking at metadata, one may look at identifying characteristics present directly in the image due to small deviations in the image sensor itself. These small deviations in the image sensor mostly arise from the pixels in the image sensor having non-uniform

sizes, and hence capture more or less light, even when all pixels are under the same illumination. This phenomenon is called photo response non-uniformity, or PRNU, and can be used as an identifying characteristic. Therefore, camera identification comes down to verifying whether the PRNU pattern from a questioned image corresponds to the PRNU pattern from reference images from a camera.

Extraction of PRNU patterns can be done effectively and efficiently with state of the art methods. The similarity between patterns is calculated using Pearson's correlation coefficient, which will increase if the similarity increases. The goal of the presentation is the assessment of the strength of the evidence of eventual similarity of PRNU patterns. Assessing the similarity between two sources, i.e., individualizing the sources, is classically approached by using a verbal scale (e.g., "strong support" for a certain hypothesis). This scale may be based on estimations of probabilities or on thresholds set by the expert. It is clear that both approaches are to a certain extent subjective: it likely depends on the amount of experience of the investigator and may vary from investigator to investigator. In forensics, a framework gaining popularity to assess the value of the evidence is the likelihood ratio (LR) framework under a Bayesian reasoning approach; from here on: "LR framework." The goal of the LR framework is to accurately assess the strength of evidence in the light of clearly defined opposing hypotheses, and not to comment on the probability of traces being from a common source, which is considered principally impossible. Furthermore, it should harmonize the value of the evidence and ease the interpretation of the evidence in different disciplines.

In Nordgaard and Høglund, the LR framework is implemented for PRNU-based camera identification, where the focus is on the measurement uncertainty of the strength of evidence.<sup>1</sup> The current presentation focuses on general problems that are encountered when interpretation of results is performed in the LR framework. In two (fictive) case examples, namely for a mobile phone camera and a good quality Sony DSC-S500 camera, the results of the LR approach are described. It turns out to be very possible to obtain statistical distributions underlying the reference data for both "matching" and "non-matching" comparisons, for both types of cameras. Based on these, LRs can be calculated. For the mobile phone cameras, it turns out that in the tail of the distributions problems will emerge: the LR decreases as a function of the correlation between PRNU patterns, which is nonsensical. For the Sony camera this point is even clearer. Again the LR function is not increasing on the whole range of correlations encountered. Moreover, LRs under  $H_p$  are absurdly high. The reason for this is that the statistical fit of the distribution for "non-matches" is constantly evaluated in a range where there is no reference data. Clearly the numbers that are being returned cannot be trusted. The problem is that extrapolation takes place in the tail of the fit for correlation scores under  $H_d$ , which is a bad statistical procedure. All in all, under these circumstances it is not possible to come up with reliable LRs, and the reason for this is that the correlation scores under both hypotheses are separated too well.

The issue of widely separated distributions, and the resulting unreliable LRs is not a problem that is unique for PRNU-based comparison: if the informative value of any forensic comparison (be it fingerprints, speech, glass particles, etc.) is high, the problem emerges. Although this may be considered to be a problem of luxury, the question remains how to deal with it. The alternative of checking whether comparison scores are larger or smaller than some threshold value yields LRs that are smaller but more reliable.

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- <sup>1</sup> J Forensic Sci. 2011 Mar;56(2):390-402. doi: 10.1111/j.1556-4029.2010.01665.x. Epub 2011 Jan 25.

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#### Camera Identification, Bayesian Framework, Likelihood Ratios

## B10 Analysis of Corrupt Video Files With Open Source Initiative Defraser

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After attending this presentation attendees will explore the forensic possibilities to analyze and restore video from files that are broken.

This presentation will impact the forensic science community by demonstrating the possibilities to investigate broken and partial video files and restoring them properly with less time compared to other methods.

Cameras may be seen in many places, recording persons and events. Sometimes the recordings are helpful as forensic evidence in court, providing insight into what happened at a crime scene, or which scenarios are possible. Also people often record videos of crimes with the purpose of assisting the police, or to record what has happened for other reasons. Sometimes people try to erase the video before it is given to or seized by the police, or the data storage may get corrupted, and the video is not playable anymore. Also, in cases such as lawful internet interception only parts of a video file may be recoverable. Slack space and unallocated clusters on a hard drive may also contain partial video files.

In these cases, the data carriers or damaged files can be examined and fragments that are found may be repaired with a hex editor and the specifications of the video file format. This usually involves a lot of reverse engineering and comparison with reference files from the same recording device or storage device.

For this type of examination the Netherlands Forensic Institute developed the software Defraser (abbreviation for Digital Evidence Fragment Search & Rescue). It helps the digital forensic investigator by searching for fragments of video files and displaying metadata such as recording time and video resolution. In the current version the user can view any valid keyframes that are found. It also allows creating new files by combining syntactic elements (headers) of video file fragments by dragging and dropping. This way, a broken video file can be repaired using headers from valid reference video files that have the same settings. Ideally, such reference files are recorded with the same camera type as the one under investigation. Defraser also has a wizard to help with header replacement. The software can log the links between the output of the software and the source evidence files. In court, such information can be used to completely reconstruct any resulting video files from the source evidence material.

The knowledge about video file formats is stored in plug-ins. Currently there are plug-ins available for the AVI, MPEG-1, 2, & 4, 3GPP/QuickTime/MP4, and ASF/WMV video file formats. A plug-in for H.264 is currently being developed. The software is developed in .NET and C#; and on the software engineering side, the structure is such that new plug-ins can be developed easily without too much rework by software engineers, reducing development time. The software is open source, so it can be downloaded for free from <http://sourceforge.net/projects/defraser>.

The benefits to making it an open source program are to give reviewers and other experts the possibility to do a code review of the forensic software as well as writing new plug-ins for other video formats, and also give vendors the possibility to include it in their software for further development. In practice, the code review does not often happen, since in general codes of thousands of lines are not easy to follow, even if there are many comments included on what exactly happens.

In practice, Defraser has proven to be useful in an increasing number of forensic cases. The approach saves time compared to reverse engineering and working with hex editors. The most notable advantage of Defraser over other recovery software is that it recognizes fragments of video files, as opposed to just full video files.

In this presentation several examples of where it works will be shown, but also cases in which the approach could not be used, as well as guidelines for carving of video files. Examples include cameras that have been used in

skimming devices for banking cards and partially erased video files from mobile phones.

**Defraser, Video, Multimedia**

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## B11 Quantifying Phase Changes in Audio Authenticity Examinations

Kenneth Marr, MS\*, David J. Snyder, BS\*, and Andrew Galotti, BS\*, Federal Bureau of Investigation, Engineering Research Facility, Forensic Audio, Video and Image Analysis Unit, Building 27958A, Quantico, VA 22135

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After attending this presentation, attendees will be presented testing results of automatic methods for detecting phase changes in forensic audio recordings when conducting audio authenticity examinations. Additionally, attendees will learn to compare the results of different automatic phase detection programs used in this series of tests.

This presentation will impact the forensic science community by exploring how phase changes by themselves may not provide insight as to whether an event is an alteration or not, but show promise as a method to correlate multiple events. If the accuracy of detecting phase changes is unreliable, this will also impact the value of it being used as potential method for forensic audio authentication.

**Hypothesis:** To determine whether a phase change in a forensic audio recording can accurately be identified as an alteration or edit and develop criteria or parameters classifying the event as such.

**Synopsis:** A series of test recordings that include various digital formats containing both altered and unaltered recordings will be analyzed by two different automatic phase detection systems. The test recordings will consist of reference tones recorded at various amplitudes as well as background environments consistent with forensic recordings. Testing results will address the question: Is a phase change in an audio file synonymous with alterations; and, can they accurately be detected?

The use of changes in phase is not a new analysis method for forensic audio authenticity examinations. Interest in expanding its role to detecting edits in digital recordings has increased in the last several years. Several concerns accompany this increased interest. Automatic detection of phase shifts can help speed a cumbersome time-consuming process of manually locating events, but the accuracy and thresholds of such detection methods are not widely known. Even if events are detected accurately, there is no specific criteria to determine whether the phase shift is the result of an alteration or naturally occurring event during the time of recording.

Test recordings will be produced to include reference tones at various amplitudes to determine if changing the amplitude of the reference tone will directly impact the accuracy of automatic phase detection systems. The test recordings will also contain a range of naturally occurring events and files with various alterations. The use of several common digital formats that represent the type of audio being received for examination and the method in which they are recorded may show that some formats may or may not maintain phase. An automatic detection system may falsely identify phase changes that may be inherent to the recording process, format, or recording environment and not necessarily the result of an edit or alteration. It may also identify phase changes where they do not exist or not identify them at all. Being able to accurately account for the number of phase changes detected or not detected is important.

The second part of this presentation will attempt to clarify what a phase change means to the authenticity of an audio recording. Even if the detection of phase changes is accurate, what correlation does a phase change have to an alteration or edit? Examination of known events may help correlate phase changes caused by edits or naturally occurring ones. Criteria is lacking for establishing which phase changes are the result of an alteration and which ones are not. This problem is similar to identifying events as pauses or as voice-activated for analog recorders. Both of these events are caused by stopping the transport of the recorder without disengaging the record and erase heads. A pause event is generally

associated with a potential alteration, where as a voice-activated event is generally not. Often the events are identified through testing the operation of the analog recorder. This analog analysis method does not translate to digital analysis of record events.

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### **Digital Audio, Authenticity, Data Analysis**

## **B12 Strategies for the Forensic Collection of Digital Evidence From Shared Storage and Virtualized Environments**

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After attending this presentation, attendees will gain a basic understanding of proposed strategies to obtain digital evidence from shared storage technologies such as, iSCSI, Fiber Channel (FC), and Network Attached Storage (NAS), which are commonly used with virtualization.

This presentation will impact the forensic science community by empowering digital forensic examiners with strategies involved in acquiring, investigating, and preserving digital evidence from shared storage technologies and virtualized environments that have become increasingly prevalent.

With the increase of virtualization being adopted by the IT industry, coupled with the continued decrease in data storage costs per gigabyte, shared storage technologies have become more prevalent. The advantages of shared storage technologies are vast. They allow for extremely large amounts of data to be disseminated to both individual computers and servers. These storage areas can then be presented to the individual computers and servers as local disks. With only a few clicks of the mouse and with no additional physical hardware changes, these disks can be re-sized, moved, added, and deleted with ease. Furthermore, the centralization of these storage technologies allows for easier management and secure segregation of data within a business enterprise. As a result, significant performance gains can be achieved by spreading data across multiple drives.

The traditional computer forensic examiner who is comfortable with locating and preserving evidence from stand-alone computers that have locally attached hard drives may have difficulty in finding and preserving digital evidence located on shared storage technologies. In the case of encountering shared storage, a forensic examiner must first be able to identify the type of technology being utilized. For example, the presence of a host bus adapter card physically attached to the computer hardware will indicate to the forensic examiner that FC technology is being employed. Additionally, a forensic examiner must be able to develop a concise and practical strategy to obtain digital evidence from storage arrays, which may consist of terabytes or even petabytes of data; and may possibly need to be obtained without the assistance or knowledge of the local system administrator. As a result, the strategy developed must encompass the ability to target specific data, as it may be impractical and too time-consuming to duplicate the entire shared storage array.

Because shared storage technologies are often affiliated with virtualized environments, a forensic examiner must be able to identify and locate the virtual computer(s). The forensic examiner must have an understanding of the types of files generated, managed, and saved by each virtualized environment. For example, VMWare uses "VHD" virtual disk files to store virtual machine(s). A forensic examiner also must be able to identify specific information they are seeking, even if it is encapsulated within a virtual disk.

The purpose of this presentation is to address all of the above listed needs. An overview of the most commonly used virtualization software packages, including VMWare's VSphere, Microsoft's Hyper-V, and Linux solutions such as XEN, and the system requirements for implementing

shared storage technologies, will be provided. This overview will cover specific hardware and software configurations affiliated with each shared storage technology, how to identify them, and proposed strategies for gaining access to the shared storage areas. Based on the type of virtualization environment, proposed strategies will be identified and presented for the recovery of digital evidence from the associated virtual machines.

At the conclusion of the presentation, attendees will be more familiar with virtualization, shared storage, and strategies for recovering digital evidence from these environments.

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### **Shared Storage, Virtualization, Digital Evidence**

## **B13 Understanding Issues in Cloud Forensics: Two Hypothetical Case Studies**

*Josiah Dykstra, MS\*, 1739 Carriage Lamp Court, Severn, MD 21144; and Alan T. Sherman, PhD, University of Maryland, 1000 Hilltop Circle, Baltimore, MD 21250*

After attending this presentation, attendees will understand how cloud computing poses unique forensic challenges that require new methods of forensic acquisition, evidence preservation and chain of custody, and open problems for continued research.

This presentation will impact the forensic science community by laying out the significant issues associated with digital forensics for cloud computing and preparing the way for forensic investigation of the inevitable criminal targeting of cloud environments.

Crime committed using cloud computing resources and against cloud infrastructures is inevitable. Though real incidents have already taken place against cloud providers including Google, an absence of documentation indicates that no crimes using the cloud or targeting it directly have been publicized nor litigated thus far. Forensic investigators must understand that current tools and techniques are inadequate in the cloud environment where acquisition, examination and analysis will be in practice executed very differently than is done today. To illustrate these issues, two hypothetical crimes are fabricated and the forensic investigation is deconstructed against them.

Companies are embracing cloud technology to offload some of the cost, upkeep, and growth of equipment that they would otherwise have purchased themselves. Cloud infrastructure, with exceptional bandwidth, storage and computing power, offers an attractive prize for hackers. While many people have lamented how the users of the cloud and their data are protected, few of these discussions have considered the difficulty of responding to security breaches, including forensics and criminal prosecution. Furthermore, no case law exists on which to extrapolate the desire of the courts on the matter. Garfinkel recently suggested that "cloud computing in particular may make it impossible to perform basic forensic steps of data preservation and isolation on systems of forensic interest."<sup>1</sup>

To provide an update about the state of digital forensics for cloud-related crimes, the investigative response and forensic process of two hypothetical, but plausible, case studies of crimes tied to cloud computing are considered. While fictional, they describe computer crimes that are not uncommon today. Case Study one uses the cloud as an accessory to a crime. In this case, a criminal stores and distributes child pornography using the cloud. Case Study two targets the crime against the cloud. In this case, a criminal hacks into a cloud-based website and installs malicious code. These common crimes require a reinterpretation when set in a cloud computing environment. In both scenarios, the following themes emerge that differentiate these investigations from traditional digital forensics:

- Acquisition of forensic data is more difficult.
- Cooperation from cloud providers is paramount.
- Current forensic tools appear unsuited to process cloud data.
- Cloud data may lack key forensic metadata.

- Chain of custody is more complex.

These two case studies illustrate larger issues that exist beyond the scope of the specific examples. Forensic acquisition is a renewed challenge, one unsuited for today's tools, which will possibly be addressed by a combination of technological and legal approaches. We have begun to evaluate the ability of popular forensic tools to obtain evidence from a cloud environment. Cooperation with providers will empower consumers to understand their risks and give them leverage to prosecute crimes. The preservation and availability of forensically relevant metadata remains an open problem.

The issues of common crimes that vary from today only in their use of the cloud have been evaluated. This technology alone introduces peculiarities and open problems that demand immediate attention. Shown in this presentation are deficiencies in both law and technology can be addressed with proper advances.

#### Reference:

- <sup>1</sup> Garfinkel, Simson, "Digital Forensics Research: The Next 10 Years", DFRWS 2010, Portland, OR, August 2010

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#### Cloud Computing, Digital Forensics, Case Studies

## B14 Facebook®: Do You Leave a Trace? A Forensic Analysis of Facebook® Artifacts

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After attending this presentation, attendees will learn where Facebook® chat artifacts are stored in different browsers and what the specific format means. Furthermore, attendees will understand how Facebook® message artifacts are saved and which browsers keep full messages.

This presentation will impact the forensic science community by demonstrating how forensic scientists, specifically the digital forensics community, can obtain potential evidence quickly and effectively from Facebook® artifacts.

As the use of technological devices has increased and become widespread, the internet has become mainstream for global communications used by persons, informal groups, public organizations, corporations, and governments.<sup>1</sup> In turn, social networks have increased in popularity as a means of contact and communication. The generic structure involves creating a profile, making connections with existing friends, and meeting new people through the site.<sup>2</sup> In addition, social networks allow the user to upload photos, describe their interests, explain their work and education history, post a relationship status, reveal personal stories and activities, give their current location, and even plan events.<sup>2,3</sup>

Facebook® was originally designed in 2004 as a social networking site specifically for Harvard University students, but has been developed throughout the years for any user over the age of thirteen.<sup>3</sup> Presently, Facebook® is available for general use with more than 750 million active users, half of whom log on at least once every day.<sup>4</sup> It is up to the users to set their own privacy settings in order to control others' access to their personal profiles; and their choices in the resulting levels of privacy are based upon their trust of the website and their trust of other users.<sup>3</sup>

As society has become more technologically developed, crime has transitioned from the corporate world into the digitized world of cyberspace. Law enforcement agencies claim that at least 50% of cases have a digital component, and that the number is currently rising. In 2009, the internet Crime Complaint Center (IC3) reported 336,665 complaints of internet offenses. Since 2000, the number of complaints has been increasing each year by 24.5%. The internet is a method of communication that consists of noncommittal interactions; this facet creates an easy means to take advantage of users.<sup>5</sup> A variety of online assaults and crimes occur every day; bullying, harassment, theft of personal information, sexual grooming, encouragement to harm others and the self, racist attacks,

financial crimes, and fraud are just some of the various internet crimes that exist.<sup>5,6</sup>

Disclosure of certain personal information online, combined with other tools available for use on the internet, such as reverse directories, can aid fraudsters to obtain home phone numbers, full addresses, ages, and genders. These few pieces of information provide a means to acquire identity based information such as credit cards and driver's licenses, and in some cases make it possible to forge even more critical legal documents like passports.<sup>3</sup> It is important to recognize that these digital artifacts are contained within sources such as web pages, computer logs, internet newsgroups, and online chat rooms.<sup>1</sup>

A vast amount of the global population uses the internet and Facebook® each day for their activities. Unfortunately, some people are using these common places of communication for malicious and nefarious purposes. It is necessary for forensic investigators to have the ability to quickly and easily extract information from these sources so that evidence may be acquired as efficiently as possible. It should be feasible to recover Facebook® chat artifacts and discern a common pattern in order to quickly search for any previous chats. Furthermore, Facebook® messages may be able to be recovered, or parts of messages, in order to attain some information or at least create an apparent link between users.

In order to start off with pure profiles, three new user profiles were created on Facebook®. This was performed by first setting up three Gmail™ accounts from Google®; it was then possible to use these email addresses to create Facebook® profiles. Three virtual machines (VMs) were created (one per profile) for each browser that was to be tested. The "Windows 7 Original" VM was cloned and Internet Explorer® 8 was used to download each additional browser to be examined. This process was followed to set up three virtual machines each for Windows® Internet Explorer® 9, Mozilla Firefox® 4, Mozilla Firefox® 5, Google® Chrome 11, Google® Chrome 12, and Apple® Safari 5, totaling twenty-one virtual machines to use for the analysis. A *Single Chat Study*, a *Simultaneous Chat Study*, and a *Sent Message, New Inbox Message, and Already Read Message Study* were performed. A forensic duplicate image was taken from each VM using FTK® Imager and loaded into Forensic Toolkit® for examination.

#### References:

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#### Facebook®, Internet, Digital

## B15 Homicide: The Effects of Fraudulent Social Network Accounts

*Adanna N. Smith, MA\*, 1300 East Lafayette, Apartment 109-110, Detroit, MI 48207*

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After attending the presentation, attendees will be able to see the effect of fraudulent social network accounts (such as Facebook and Twitter) leading to child homicide and/or rape homicide as a result of meeting in

person for social purposes, prostitution, to buy drugs and other types of criminal acts.

This presentation will impact the forensic science community by highlighting, through research and comparative analyses, the demographics (race, socioeconomic status, location, religion, sexual preference, and other trends) that may be most affected by predators. The presentation will discuss and critique patterned escalation of deviant behavior of convicted sex offenders as well.

While most of the issues surrounding this new type of predator are still being researched and categorized, this session will identify some causes, effects, and impacts these predators have on the subset of targeted population evaluated in this study.

The presentation introduces computer forensics to attendees to provide the foundation for the chain of events leading the collection of evidence for homicides. Evidence such as fingerprints, pictures, and DNA will be discussed as additional mechanisms utilized to obtain a positive identification of the predators. The comparison of the systems in place to monitor child activity on the internet such as parental controls will be examined as well.

A differentiation between computer forensics and mobile phone forensics will be provided. It is important to examine forensic artifacts, such as exchanged e-mails, notifications received, and picture uploads to social sites or via MMS (Multimedia Messaging Service) from mobile phones that can lead to a meeting that results in homicide. Recent studies have shown that mobile phones are now being analyzed in investigations because many people check and respond to e-mails on their mobile phones. Because of this, investigators are unable to locate messages that could show insight on illegal activities on laptops or hard drives when they are confiscated.

This presentation acknowledges historical data related to chat rooms as they provide the framework of the evolution of social network use. It is important to note that data collection pertaining to investigations of crimes related to fraudulent social network accounts is still in the early stages of development. Because of this, there is a call for technological advances to assist law enforcement agencies to combat these types of deviant behavior.

Attendees will gain a fundamental appreciation and understanding of how multimedia can reveal pertinent information on homicide investigation. By studying demographics affected, attendees will obtain greater insight on the subset of targeted population through cognitive research and comparative analyses. The presentation will provide a description of how the advent of new multimedia technology affects the way homicide investigations are presently conducted. Useful information that can assist in both domestic and global homicide investigation by exploring how they intertwine will be provided. Collaborative efforts between domestic and global jurisdictions will be necessary to investigate and prosecute the predators. Finally, the collaboration between traditional forensic investigation and technological forensic investigation will be examined.

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**Homicide, Fraudulent, Social Media**

## **B16 TracHac: Non-Smartphone First Responder Forensic Tool**

*Marcus Rogers, PhD\*, Purdue University, Cyber Forensic Lab, 401 North Grant Street, West Lafayette, IN 47907; and Robert Winkworth, MSc, 656 Oval Drive, West Lafayette, IN 47907*

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After attending this presentation, attendees will become familiar with the current state of mobile forensics of non-smart phones and will be briefed on a custom hardware/firmware tool developed by the Purdue Cyber Forensic Lab for use with this class of mobile phones.

This presentation will impact the forensic scientific community by detailing the ability to acquire and analyze the data contained within mobile/cellphones. By presenting details regarding the development of a

tool to deal with the most commonly used disposable cellphones, the area of mobile phone forensics will be further matured.

The use of disposable cellphones by various criminals and terrorist groups is becoming increasingly more common. This should not be surprising, as this technology has outpaced any other class of technology including personal computers. Criminals and terrorists are very aware that law enforcement considers cellphones to be an important source of evidence and intelligence. As such, the use of low cost, non-contract, disposable cellphones are becoming increasingly common. It is assumed that these devices will be very difficult for law enforcement to connect to the criminal or terrorist and they will contain either a minimum of evidence or be impervious to the current automated cellphone forensic tools. As a result, government and law enforcement are struggling to develop low cost tools and procedures to deal with disposable cellphones. This task is further complicated by the need for these tools to be used in the field to close the gap between the discovery of actionable intelligence or evidence, and the interview of the suspect(s) involved.

This presentation is an overview of a research project conducted at Purdue University that was designed to investigate the feasibility of developing a tool that focused on low cost disposable cellphones (aka, TracFones®). The functional limitations of the tool were determined by the sponsoring agency. The tool was limited to being used with non-smart phones, being non-invasive and automated enough to be used by first responders with only a limited amount of digital forensic training.

These low cost disposable phones often have the limited functionality and as such they fall under the radar of most of the industry standard automated mobile forensic tools. The current project surveyed law enforcement in the southwest region of the USA regarding the most commonly seized disposable mobile phones. The results were then used to purchase a sample of these phones that ranged in price from \$20 - \$50. These phones were populated with known data and then tested against industry standard automated tools.

The results indicate that the automated tools could not process most of the phones as they did not appear in the tools list of supported phones. A proof of concept first responder hardware/firmware prototype for dealing with these phones was fabricated and tested. This presentation will discuss the specific results and procedures for testing the TracFones® against common industry mobile forensic tools as well as details of the fabrication and development of the Purdue Cyber Forensic TracHac tool itself.

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**Mobile Phones, Disposable Cellphones, TracFone®**

## **B17 Applied Predictive Behavioral Modeling: The Role of Behavioral Sciences in Digital Forensics**

*Marcus Rogers, PhD\*, and Kathryn C. Seigfried-Spellar, PhD\*, Purdue University, College of Technology, Knoy Hall of Technology, 401 North Grant Street, West Lafayette, IN 47907*

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After attending this presentation, attendees will be familiar with the current state of predictive modeling of cyber adversaries and cyber criminals using behavioral indicators and personality risk likelihood models. It will also provide those interested with a procedure and method to further this type of research.

This presentation will impact the forensic science community by providing understanding of the role that behavioral models can play in predicting adversarial behavior; digital forensics will be better able to take advantage of discoveries and tools from the other forensic sciences. It is also important policy decisions and responses are based on valid real world empirical evidence and not solely on theoretical abstract models that lack any real world credibility.

It has become obvious that purely technical solutions to try and deal with cyber adversaries in a *post hoc* fashion have failed. Business and

governments spend more money on cyber security than ever before, yet, if the most recent surveys are believed, the rate of cyber crime and other adversarial attacks is at an all time high. With the current economic crisis budgets are tight and as such it has become increasingly important that any technology expenditures be judged on their Return on Investment (ROI). This shift to an economic model has highlighted that technology alone will not mitigate the risk of cyber crime or cyber attacks.

The fields of predictive analysis and behavioral predictive modeling are now being applied to cyber security and cyber crime space. Several models have developed that, while appearing to be valid from an internal consistency approach, have failed when tested in real world settings. Most of these failed models rely on synthetic data sets, have improper factor loading and issues related to multicollinearity. Even more disturbing is the fact that these failed models are being used to inform government policy and to draft anticipated responses to cyber related events. A further disturbing finding is that in most cases the models were developed with little or no input from behavioral scientists and were based on the fallacy of game theory and behavioral economic: that people are logical rational actors. Human behavior is far more complex than envisioned in these rudimentary models and is more similar to the problem set used for Chaos theory namely irrational behavior.

The current project provides a meta-analysis of behavioral predictive analysis from the areas of insider threat modeling, hacker profiling and cyber terrorism/infowar. The research project focuses on a specific category of cyber criminal behavior (child pornography) and using real world data sets from law enforcement, tested various models to determine their goodness of fit and predictive validity. The Rogers-Seigfried (RS) predictive model was then tested against the same data sets to determine its fit and validity (research ongoing at time of submission)

The results of this research will be presented as well as suggestions for its investigative use and further development of the RS model. The meta-analytic procedure used in the study will also be discussed in order for other researchers to conduct similar studies.

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**Cyber Crime, Behavioral, Predictive Modeling**

## **B18 Should We Fear Peer-to-Peer? Some Basics in Peer-to-Peer Investigation**

*Walter T. Hart, MBA\*, 149 Hamerton Avenue, San Francisco, CA 94131*

After attending this presentation attendees will gain a better understanding of peer-to-peer file-sharing application installation options and artifacts, possible uses of the applications, artifacts created during use, and approaches to investigating cases involving the use of peer-to-peer file-sharing applications.

This presentation will impact the forensic science community by providing a basic understanding of peer-to-peer file-sharing in investigations involving digital evidence including risks, vulnerabilities, and opportunities.

Peer-to-peer networking and other file sharing programs have become increasingly popular with users of the internet for sharing both large and small files. Many small organizations will use the capabilities of peer-to-peer file-sharing to effectively share information about their organization without having to maintain a centralized server. These could include families, bowling leagues, social organizations, and virtually any other groups of individuals sharing the same interests or a need to access the same data. Larger organizations utilize peer-to-peer file-sharing to economically and rapidly deploy very large datasets. There are a number of legitimate business uses for peer-to-peer networking and distributed file sharing including rapid dissemination of patches for software and new distributions of games and their data.

Peer-to-peer file sharing has also become popular with distributors and others sharing illegal files or illegally sharing legal files such as intellectual property rights protected software and media including movies,

television shows, music, and almost any other copyrighted material that can be stored in a digital format. With rapid and widespread distribution of files, it is also possible to distribute viruses and other malware unchecked quickly. Without proper controls, this could provide entry into otherwise secure networks by unwitting users. These illegal uses of peer-to-peer networking have created a challenge in the digital forensic community due to the volume and perceived anonymity of the process. The number of available peer-to-peer applications and incredible volume of data being shared could easily overwhelm digital forensic examiners.

As is the case with many software applications, there are similarities between the many peer-to-peer applications but the differences and the forensic artifacts created are significant. This presentation will discuss many of these applications and some differences in the artifacts created. Examples will be given of the installation, configuration, and use of some common peer-to-peer file-sharing applications. This will include some of the standard, default installation options that both hamper and help investigators in these cases. Some general approaches to peer to peer investigative cases will be examined as well as the use of available tools to assist these cases. While generally accepted investigative techniques will uncover the presence of peer-to-peer installations, there are some specific tools directed specifically at investigating these products.

The presentation will also discuss smaller subset of file sharing applications appropriately called friend to friend as opposed to peer-to-peer as they are a group of applications that employs the ability to have some level of access control before users can share or download files. This subset of file sharing applications, while using some similar processes and technology, presents even greater challenges to the investigator as users frequently must "buy" their way into the group.

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**Peer-to-Peer, File Sharing, Friend-to-Friend**

## **B19 Look Ma! No Wires: Challenges Wireless Networking Presents to Investigators**

*Walter T. Hart, MBA\*, 149 Hamerton Avenue, San Francisco, CA 94131*

After attending this presentation, attendees will gain an understand of the scope of wireless networking technology, potential problems it creates for investigators, and what methods can be employed to help account for the presence of wireless networking technology in the field.

This presentation will impact the forensic science community by providing a basic understanding of the principles and challenges faced by investigators because of the widespread use of wireless networking technology.

Wireless networking is everywhere around us from our phones, our computers, our printers, our storage devices, and even our cars. While this makes life for most very convenient, it presents many challenges to network security managers and to investigators of wrongdoing using digital devices. In virtually all types of digital investigations, investigators must plan for, and account for the presence of wireless devices. These devices provide vulnerability, opportunity, as well as possible defenses to allegations of wrongdoing involving digital evidence. Wireless technology provides both a way in and a way out of what might otherwise be a controlled network environment. Many businesses both large and small, households, and government installations, may have wireless access points. Many of these may have multiple wireless access points with varying levels of security on each. While this makes access to the network resources, including the internet, very easy, it also provides possible access to unauthorized persons or organizations to internal resources and/or the internet. Securing a network while providing a convenient level of wireless access has proved to be challenging, even for experienced network administrators. Many home users, unaware of the need to secure their home network, do not even attempt to do so, leaving themselves at risk to the loss of personal data and/or unauthorized access to their resources.

Additionally, many resources within a network can now be accessed wirelessly such as printers, scanners, phone systems, and internal data servers. While convenient to be able to use these resources without having physical infrastructure in place, this also provides an opportunity for data compromise.

Basic examples will be given of wireless network scanning resources, how they can be utilized, when they might be utilized, and precautions and pitfalls to using these tools. This will include mapping of the wireless network environment while conducting an investigation. The manufacturing of inexpensive wireless network scanning equipment that can be utilized in investigations as well as an introduction to the capabilities of some commercially available equipment will be briefly discussed. While an effective wireless investigators kit may not require any of these, some basic understanding of the capability is beneficial. Examples will be given of the use of this technology in investigations as well as investigations where it was not employed, but might have been.

A brief discussion about wireless security in the home, home office, and/or small business environment will highlight the basics of what might be done to help secure these networks. Some typical wireless installation options for common home or home office type wireless equipment will be used as examples. This will allow an investigator to possibly ask better probative questions during interviews of network administrators, home office users, and/or subjects of the investigation.

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**Wireless, Network Vulnerability, Wi-Fi**



## C1 Study on the Effects of Surface Roughness on Blood Patterns

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After attending this presentation, attendees will understand the feasibility of using a contact angle as a parameter to represent the surface characteristic at the blood impact velocity estimation.

This presentation will impact the forensic science community by introducing a new parameter capable of representing surface characteristic at the blood impact velocity estimation.

Blood impact velocity estimation plays an important role in crime scene reconstruction, and it is mainly studied by correlating the impact velocity to the number of spines radiating from the periphery of circular blood stains and the droplet volume. Paper, drywall, wood, etc. have been considered regarding the surfaces that blood impacts without introducing quantitative parameters that represent surface characteristics. This study focuses on the effects of the surface condition on the blood pattern by introducing the contact angle as a parameter to represent the surface.

The surface roughness of six stainless steel specimens used for blood impact tests was altered using a sand blasting process. The ten point roughness  $R_z$  of the specimens is 16.3 $\mu\text{m}$ , 17.5 $\mu\text{m}$ , 19.6 $\mu\text{m}$ , 24.5 $\mu\text{m}$ , 29.3 $\mu\text{m}$ , and 38.1 $\mu\text{m}$ . The contact angle of each specimen was measured by using a sessile drop method and maintaining blood temperature at 37°C. Maintaining the blood temperature at 37°C through a thermal bath, the viscosity of the test blood was measured with a cone plate type viscometer. The blood was dropped vertically by using a syringe with an adjusted droplet volume of 20 $\mu\text{l}$ . The syringe was mounted onto an adjustable laboratory stand to allow for variation in release height from 10cm to 100cm. A computerized fluid dynamic (CFD) analysis was performed to study the mechanism of spine formation by using the Flow-3D program, which finds the boundary of two phases by the volume of fluid (VOF) method. The surface of the specimens was scanned using an ATOS-III system. The scanned surface image of the specimen was used as a boundary wall in the CFD analysis to study the surface effects on the blood pattern.

The measurement results of blood viscosity showed the shear thinning characteristic of a non-Newtonian fluid; that is, the viscosity decreases as the shear rate increases. The results showed deviation in comparison with the results yielded by the viscosity model equations of Carreau and Kensey due to individual differences in the blood composition. Additional study is required to determine whether individual deviations of blood can be generalized by introducing multiple variables acquired by many blood samples including the results of bio-chemical analyses. The contact angle increased linearly as the roughness increased, and variation among the blood samples was also observed. The spread factor was calculated as the ratio of the stain and the droplet diameter and was represented as a Reynolds number. It increased as the Reynolds number increased but decreased as roughness increased. From these observations, it was concluded that roughness affects the contact angle and the spread factor through flow resistance. This tendency was more clearly pronounced when the momentum of the droplet was less. From the droplet impact experiments and the CFD analysis, the difference in the spread factor of specimen  $R_z = 0 \mu\text{m}$  with the other specimens becoming larger as the Reynolds number decreased. There was no spine formation for the specimen of  $R_z = 0 \mu\text{m}$  and  $R_z = 17.5 \mu\text{m}$  but with the specimen of  $R_z =$

38.1  $\mu\text{m}$  at the droplet release height of 0.5 m. As the release height increased, there was spine formation for the specimen of  $R_z = 17.5 \mu\text{m}$  but not with the specimen of  $R_z = 0 \mu\text{m}$ . In the case of the specimen of  $R_z = 0 \mu\text{m}$ , there was no spine formation throughout the range of release height. From these observations, it could be concluded that the spine formation depended on the droplet velocity as well as on the surface roughness. The contact angle can be an additional parameter to represent the droplet impact velocity together with the spine number and spread factor. Experiments to make a general equation to describe the blood impact velocity by introducing the parameter of the contact angle for various surfaces were prepared.

**Blood Pattern, Contact Angle, VOF**

## C2 The Role of a Federally Funded Research Development Center (FFRDC) in Advancing Forensic Sciences

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After attending this presentation, attendees will learn the history of support and contribution to the engineering forensic sciences field by Aerospace Corporation personnel. The Aerospace Corporation (Aerospace) operates a Federally Funded Research and Development Center (FFRDC) that was chartered to support national security space (NSS) for over 50 years. This presentation will explain how the practical application of knowledge and experience derived from technically complex space-related problem solving has contributed specific evolutionary and revolutionary advances to support engineering and multijurisdictional forensic sciences.

This presentation will impact the forensic science community by highlighting how Aerospace will continue to play an important role in the development of new forensic procedures and investigation methodologies.

Because Aerospace is an engineering firm, much of the work is to protect and prevent as well as support case work and incident response. Specific examples of case-based technical support will illustrate the capability to work with criminalists and the potential to contribute expertise when the solution set requires specific knowledge, equipment, or instruments not resident in a mainstream, accredited forensic laboratory.

Aerospace has a history of support to the federal government in the fields of law enforcement and public safety. Aerospace personnel were involved in the re-investigation of the assassination of President Kennedy as well as other high profile cases. Methods for the recovery of the video from tapes that have been soaked in solvent and had parts melted have been developed. Aerospace developed one of the first "gunshot residue" tests and has contributed substantially to the "less than lethal" weapon and body armor fields. Methods for remotely detecting "drug cooks" have been developed and field-tested. This technology was used to support post 9/11 terrorist attack analyses. A hyperspectral instrument was flown over the site in an effort to confirm or refute the presence of asbestos in and around the wreckage area as well as the landfill where the debris was deposited. Because of Aerospace's assistance in the investigation of launch failures, there are Subject Matter Experts (SME) in the fields of explosives and the damage to various materials caused by explosions. This includes metallurgical analysis of failed materials, electron microscopy, and x-ray

analysis. Mass spectrometry analysis of bullet fragments has also been provided to various law enforcement agencies.

Aerospace ran a regional criminal justice technology support center that was operated for the DOJ. This center helped transition DOD technology to local agencies. Video tapes were processed by the Center's Imagery Exploitation Facility, leading to several arrests. Audio evidence was processed to extract voices from noisy audio tapes, including difficult dialects and computer aided voice identification. These cases covered a wide spectrum of criminal activity, ranging from employee theft to child molestation to murder. Aerospace personnel also implemented a computer crime facility to assist law enforcement in recovering, enhancing, and evaluating computer information. This facility is used to decipher evidence from confiscated computers – evidence often thought to be unretrievable. In addition, Aerospace helped develop GPS for the Air Force. This space based technology, small enough to be hidden, provides pin-point accuracy in tracking.

This support continues with the development of expertise in real-time forensics. Real-time forensics is a new field where rapid analyses support in-real-time situational awareness enables multijurisdictional decision making in excessively costly or life-and-death situations. This is becoming especially important in the area of Improvised Explosive Devices (IED)—both in their detection and their handling.

A proactive approach to real-time engineering forensics will be put forth. Combining real-time forensics with emerging concepts, and based upon lessons learned, will advance the capability to develop site-specific safety and security features that facilitate post-event response, recovery, and evidence collection, as well as to deter crime.

The presentation will conclude with a delineation of the path forward for using Aerospace's technical and process capability in alignment with recommendations of the National Academy of Sciences.<sup>1</sup>

#### Reference:

1. "Strengthening Forensic Science in the United States: A Path Forward," Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council, 2009, ISBN 0-309-13135-9.

#### Space Related Problem Solving, Rapid Analysis, Real Time Forensics

### C3 Finding the Genie in a Bottle

*Michael C. Hadka, PhD\*, Trillium Inc., 520 Peck Road, Downingtown, PA 19335; and James S. Smith, PhD, Trillium, Inc., 28 Graces Drive, Coatesville, PA 19320-1206*

The goal of this presentation is to describe how to use forensic science investigation/evaluation approaches on environmental release work where soil was impacted by past dumping practices.

This presentation will impact the forensic science community, by adding to the arsenal of petroleum hydrocarbon release site investigation techniques, using typically available or easily obtainable chemical analysis data to investigate the release. This technique has direct impact on who is the liable party for the release.

In 1994 during the removal of an underground heating oil tank behind a gasoline service station, a light non-aqueous phase liquid (LNAPL) described as a black viscous oil, was discovered floating on top of the groundwater. The service station at this location has been in operation since the 1930's. The underground heating oil tank, used to supply fuel for heating the station building, did not appear to be leaking when it was removed. Other potential sources of the LNAPL were the gasoline, diesel fuel, and kerosene stored in underground storage tanks (USTs) and dispensed at the service station. The service station building, USTs, and dispensers are located in the southeast corner of the property. About 100 yards of empty land separates the service station building from a tidal creek along the western boundary. The property is prone to flooding during extremely high tides. The groundwater is only about three feet below the ground surface. In the past, fill material was added to the east and north

sides of the service station to raise the elevation of the property. Additional LNAPL was found in twenty-two test pits located within this fill material.

Samples of the LNAPL were submitted for gas chromatographic analysis using a flame ionization detector (GC/FID). The GC/FID chromatogram of the LNAPL collected from a pit in the southwestern corner of the property is shown in Figure 1. The chromatogram is a lubrication-type oil (i.e., mineral oil) with the center of the unresolved complex mixture (UCM) in the C<sub>22</sub> to C<sub>23</sub> n-alkane retention window. The n-alkanes C<sub>17</sub> and C<sub>18</sub> are present in the chromatogram along with the isoprenoids pristane and phytane. LNAPL samples from the eastern portion of the site do not have the C<sub>17</sub> and C<sub>18</sub> n-alkanes indicating that more aerobic biodegradation has taken place. Finding the lubrication-type oil next to the service station would lead one to suspect that this oil was used crankcase oil from oil changes that were performed at the station; however, zinc, lead, chromium, and copper were either not detected or were present at only a few parts per million in the oil. Zinc compounds are added to motor oil for antiwear and anticorrosion properties. Lead, chromium, and copper are wear metals from the engine that contaminates used crankcase oils. The concentrations of these metals in the LNAPL were too low to be considered used crankcase oil.

Other indicators that the LNAPL was not used crankcase oil was the polynuclear aromatic hydrocarbons (PAHs) content. There are two sources of PAHs in used crankcase oil, petrogenic and pyrogenic. Petrogenic PAHs are from the crude oil used to refine the oil while pyrogenic PAHs are formed from combustion. For pyrogenic PAHs, the unsubstituted parent PAH predominates over the alkylated PAHs of the parent and the abundance of the alkylated PAHs decreases with the increasing level of alkylation. For petrogenic PAHs, the alkylated PAHs predominate over the parent PAH. In used crankcase oil, there will be an increase in pyrogenic PAHs as the result of combustion blow-by in the engine.<sup>1</sup> In the LNAPL samples, the alkylated PAHs predominated over the parent PAH indicating that the source of the PAHs was petrogenic and not from combustion.

The LNAPL found at the site was co-located with fill material containing trash, glass bottles, glass shards, and documents from a nearby glass bottle manufacturer. One of the documents found dated back to 1940. Other material identified in the fill that was associated glass bottle manufacturing including bottle molds, bottle slugs, mold swabs, bottle brushes, and oil cans used to squirt oil into the molds. The trademark molded into the bottom of the intact bottles belonged to the same glass bottle manufacturer identified in the documents found in the fill. Research into glass bottle manufacturing practices revealed that the molds used to form the glass bottles are coated with a releasing agent called a mold dope before the molten glass is added. A mold dope is an oil containing a small percentage of other materials such as sulfur, graphite, rubber, and even old shoe heels.<sup>2</sup> The oil used in commercial mold dopes is mineral oil.<sup>3</sup> The GC/FID chromatogram of one commercial mold dope contains mineral oil similar to the LNAPL including the n-alkanes, pristane and phytane. The mold dope entered the waste stream of the glass manufacturer from drippings collected in pans and on absorbents, in sweepings, from used mold swabs, rags, and discarded mold oil containers. Workers from the glass manufacturer transported these wastes along with general trash, used bricks from the ovens, and unusable glass in dump trucks where it was used as fill in the marsh next to the service station.

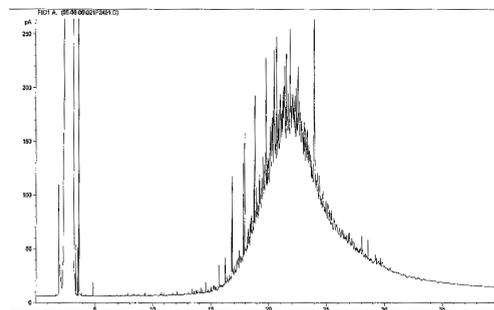


Figure 1

## References:

1. Wang, Z., and J. H. Christensen, *Chapter 17: Crude Oil and Refined Product Fingerprinting Applications*, In: R. D. Morrison and B. L. Murphy, eds., *Environmental Forensics Contaminant Specific Guide*, Elsevier, London, 2006.
2. Tooley, F. V., ed., *The Handbook of Glass Manufacture*, Volume 1, Books for Industry and The Glass Industry Magazine, New York, 1974.
3. Kampf, R. P., Lubricants for Shears, Lehrs, and Everything in Between, *Glass*, 2006, 83(6), 14.

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## Environmental Forensics, Lubricating Oil, LNAPL

## C4 Attorneys, Journalists, Environmental Forensic Scientists, and Four Words

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After attending this presentation, attendees will better understand the meanings of the terms theory and possibility as used by each of the experts involved in an environmental case.

This presentation will impact the forensic science community by highlighting the fact that scientific disciplines use different meanings for the same terms, which will cause confusion for the trier of fact.

In the realm of environmental forensic science, attorneys, journalists, forensic geologists, hydrogeologists, chemists, engineers, environmental scientists, and many other experts do not speak the same language. Yes, it is English; however, we define words differently which does not clarify the issue for the trier of fact. Instead it creates smoke and fosters junk science.

How often has the phrase, "it is the plaintiff's or defendant's theory been heard." A theory is a formula derived by inference from scientific data that explains a principle operating in nature. A theory implies a greater range of evidence and a greater likelihood of truth than a hypothesis. A theory allows one to make a prediction with a good chance of being correct. Environmental forensic science rarely, if ever, reaches this level of predictability.

Normally, environmental forensic science begins with a hypothesis. A hypothesis is a tentative assumption made in order to draw out and test its logical or empirical consequences. Environmental "whodunits" usually have insufficient evidence to provide more than a tentative explanation. Joseph P. Bono, MA, in the President's Message in the *Academy News*, September 2010, Volume 40, Issue 5, page 3, states "The six most questionable words used to formulate the justification for a conclusion by any forensic analyst are 'Based on my TRAINING AND EXPERIENCE.'" Numerous attorneys have been told that when those words are spoken at a deposition or in trial, they can count on the fact that the remaining portion of the sentence is being made up at the time. In other words, they are about to hear speculation. This brings me to two additional words to be defined.

It has been asked "isn't it possible?" more times than can be remembered. This word possible, should be eliminated from forensics. "Possible" is in the realm of expertise of a philosopher or a person of religion. As a forensic scientist, we do not have the expertise to determine the "possibility" of an explanation, hypothesis, or theory without testing to determine the probability of the hypothesis being asked.

It is the opinion "reasonable" means that the conclusion is based on logic, references, supported by and derived from a tested hypothesis, so that there is a good probability the hypothesis is correct or, at least, consistent with the facts. Instead, more often a lot of speculation is heard in the courtroom or, as some describe it, the "throw it against the wall and hope some of it sticks, and if it doesn't stick, throw some more" approach. Yes, we are back to "In my experience...." and "possibilities."

As a first step toward improving the situation, the deletion of the words "theory" and "possibility" in the language of environmental forensic science is recommended.

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## Possibilities and Probabilities, Hypothesis, Theory

## C5 The Use of Regulations, Codes, Standards, and Acceptable Practice in Forensic Architecture and Engineering

*Mark I. Marpet, PhD, PE\*, 14 Cowie Road, Chester, NJ 07930-9715*

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After attending this presentation, attendees will understand the proper (and improper) use of regulations, codes, standards, and acceptable practices in the evaluation and analysis of hazards and defects, with emphasis on premises liability.

This presentation will impact the forensic science community by showing how codes, standards, regulations, and the more amorphous "accepted practice" forms the corpus that allows a practitioner to evaluate whether the hazard of a particular feature is or is not acceptable. Those who attend the presentation will be in a better position to underpin any such evaluations with reasonable certainty, in a manner that can better withstand *Daubert* and *Frye* challenges.

In establishing a breach of duty between parties, it is essential to understand just what duty one party owes to another. One way to establish that is to look at what has been codified in the technical literature. This presentation discusses the use of Regulations, Codes, Standards, and Accepted Practice (RCSAP) in establishing duties between parties and discusses how they should be used and are misused by forensic engineers and architects.

There exist two ways in which RCSAP can be used: (a) directly, by showing a violation of a legally mandated and on-point RCSAP provision, generally the violation of a Regulation; and, (b) indirectly, by showing a non-binding but on-point violation of acceptable practice.

This paper will use staircase elements as examples.

Definitions:

**Regulations** - A Regulation<sup>1</sup> has the force of law behind it. It can be international, federal, state, or municipal in origin. Generally speaking, violations of state and federal statutes can be used to show a violation of duty or, at least, evidence of such a violation. (Check the case law in your venue.) International treaties and municipal ordinances are not generally considered to be capable of generating negligence but can be an element of a violation of acceptable practice.

**Codes** - A code is a document generated by and under a committee of interested parties. For example, the International Building Code has been developed under the auspices of the International Code Council, Inc. Codes are generally far more comprehensive than are standards. There are many different types of codes. For example, building codes relate to construction and alteration of a structure; existing structures codes relate to the maintenance of an already built structure. The violation of a temporally-appropriate code is evidence of a violation of acceptable practice. If the code is incorporated by reference into a regulation, it can also be a direct violation of a regulation.

**Standards** - A standard is a document generated by a group of interested parties. The typical *raison d'être* for standards is to assist in commerce. Standards are generally narrow in scope and standards can range from company-wide to industry-wide to international. Standards are voluntary unless the parties involved agree to be bound by them or the standard is incorporated by reference into a statute or regulation. Standards having the most impact are known as full-consensus standards, meaning that all interested stakeholders are allowed input and substantial agreement must be reached on all of the provisions. On-point temporally-appropriate but non-binding standards may well be evidence of acceptable practice.

**Acceptable Practice** - Acceptable practice is the concrete manifestation of the way things should have been done in the time period and geographical area of interest. It is a catch-all category. The absence of on-point regulations, codes, and standards does not and cannot suggest that anything goes. For example, if a building had been built before the existence of a building code for the geographic area, that doesn't suggest that anything is acceptable. One must look to acceptable practice.

**Appropriate use of RCSAP** - For a regulation code, or standard to be directly used, it must be strictly applied, temporally appropriate and on-point. To show a direct violation, Regulations Codes, and Standards (RCS) must be strictly applied. The use of the document must fall within the ambit of the scope of the document. The timeframe of the RCS must match that of the object under study. Given all of that, violations of not-mandated standards and codes are not *per se* regulatory violations. Unless mandated by law, no one is required to follow a standard or code; however, non-binding documents can give insight into acceptable practice.

Acceptable practice can be developed using (temporally and geographically) “nearby” building codes, by age-appropriate textbooks, by an architectural survey of similar-age buildings, and anything else that can place the analyst into the time and practice mindset of a competent practitioner. Acceptable practice is a more amorphous concept than are RCSs. The thing about Acceptable Practice is that it is far more flexible than is RCS, but requires one to do one’s homework.

Here’s an example. When one looks at the apartment buildings in New York City built in the early twentieth century, it is clear that the main exits, which typically have one or two steps down to the sidewalk, were simply not built with handrails as the code of the era would appear to require. (The 1916 Building Code of the City of New York states that (§154) exterior stairways “shall conform ... to the requirements ... for interior stairs.” Interior-stair requirements (§153.6) requires handrails on both sides” of a stairwell). But if an architectural element is evident in many or most of the built-in-the-same-era buildings, that strongly suggests how that element had been parsed in that era. (It is often the case that neighborhoods develop quickly after the infrastructural elements: water, sewers, electricity, and roads, fall into place. Thus, many of the original buildings in the same neighborhood are architecturally similar). It is clear that the architectural grammar of the teens and twenties of the twentieth century did not require handrails on the main entrances of buildings, notwithstanding what the literal wording of the code suggests. It’s not that architects were ignoring the code; rather, architects did not consider the one or two steps at main entrance of an apartment building to be an exterior staircase.

What acceptable practice is not is something that is based upon “in my experience.” In my experience doesn’t reach the level that *Frye* or *Daubert* requires: as one’s experience in isolation lacks both “general acceptance” and reproducibility. If there is no research backing up an opinion, no discussion in the literature of practice, nothing save someone’s opinion, that opinion should be looked at with circumspection. One obvious issue; how on earth can any person practicing today talk about “in my experience” with respect to a building built in the 1920s?

**Misuse of RCSAP:** *Conflating A Violation With Causality* - One thing that is important to understand is that a “violation” of a regulation, a code provision, or a standard, is not in-and-of-itself tantamount to negligence. One must also establish that the violation is causal in any damages. A classic example of conflation of a violation of a code provision with the issue of negligence concerns handrails in stairs. Specifically, building codes, which were originally developed to ensure fire safety, placed early emphasis on the need to have handrails on both sides of all but the narrowest of stairs, and intermediate handrails on wider stairs. The intermediate handrails are both to channel downward (eliminating side-to-side traffic) rushing-down-the-steps-in-emergency crowds of pedestrians and to provide handrails for at least half of the stair users. But one or two people exiting a building do not need intermediate handrails, because one or two pedestrians can simply avail themselves—if they choose to—of the handrails at the edges of the stair.

*Using Generic Standards To Establish A Specific Violation* - Most building codes have somewhere a section that states that a building must be “reasonably safe and without nuisance.” You must not rationalize that what you think is a hazard, therefore, violates that generic provision. In other words, because you think something is a hazard that doesn’t *per se* cause a violation of that generic “keep things safe” provision of a code. In short, the RCS should not be used merely to wrap your ideas in the mantle, the imprimatur, of Standard or Code. What you think is a hazard may well be

a hazard; but it does not become a hazard by citing to a generic “keep-things-hazard-free” RCS citation.

*Equating Quantity With Quality* - One does not need large numbers of RCS citations to prove a point. The experience here is that there is an inverse correlation between the quantity of standards cited and the relevance and utility of those standards. The plain fact is, two, two dozen, or two hundred not-on-point citations are not-at-all equal to one on-point citation. That said, if one is trying to show the state of accepted practice, the more temporally-appropriate citations, the better. If you are trying to show that large, square newel posts at the top and bottom of a staircase was the custom in 1900, one citation is good, two are better, and three are even better. If the citations are both local and geographically distant, that would be better yet.

Asserting that the absence of an on-point, temporally-appropriate standard implies that “anything goes” is nonsense. Dimensional rules that define how a staircase should be constructed date back to the late 1600s. There has been much written in the early practicum on stair dimensions. Carpentry texts from the late 1800s give suggested dimensions for risers and corresponding treads. Thus, the idea that, before codes had been published, any riser and tread dimensions would be acceptable is cynical nonsense. Again, in the absence of on-point RCS, acceptable practice must be researched. (Do your homework!)

*Being Dogmatic In Areas Of RCSAP Flux Is Counterproductive*  
From at least the 1930s through the 1980s, handrail heights were set at 30–34 inches above stair nosings. From the 1980s, based upon research by Brian Maki, handrail heights were raised to 34–38 inches. Handrails that also serve as stair guards are allowed to be 42 inches high. Given all that, it is absurd to assert that a building built in, say, 1950, is defective because its handrails were 36 inches tall. It is arguably problematic to suggest that a building designed in 2000 that has 33 inch handrail heights, is hazardous because it violates the 2000 building code. It is a code violation, to be sure. But it was acceptable for over fifty years, so it would be hard indeed to establish that handrail height as a causal element in a stair accident.

Similar situations exist when looking at riser and tread dimensions, stair widths, and so forth. Architectural elements that change over time imply that it is no “right” or “optimal” answer. In a situation where codes have changed over time, and within reason (that is, the parameter under discussion is within the range of variation of code and/or acceptable practice), a “code violation” doesn’t *per se* suggest a hazard.

**Reference:**

- <sup>1</sup> *Regulation* is used in a generic sense in this paper, covering treaties, CFR, statutes, ordinances, etc.

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**Forensic Engineering, Building Codes, Accepted Practice**

## **C6 Apportionment of CERCLA Liability - A Case Study**

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After attending this presentation, attendees will understand how CERCLA liability was apportioned following trial in one case, in a decision affirmed by the U.S. Supreme Court, as well as the implications of that case for demonstrating a reasonable basis for apportionment.

This presentation will impact the forensic science community by illustrating how relevant data are used by the courts.

*Burlington Northern v. United States*, 129 S.Ct. 1870 (2009), was the first CERCLA action in which a district court determined the degree to which different parties contributed to the contamination at a site and apportioned liability accordingly.

In 1960, Brown & Bryant (B&B) opened an agricultural chemical facility on its 3.8-acre parcel. B&B carried two soil fumigants, D-D and Nemagon, manufactured by Shell Oil Company (Shell), and a weed killer, dinoseb, made by Dow Chemical.

In 1975, B&B leased an adjacent 0.9-acre parcel from the predecessors of BNSF Railway Company and Union Pacific Railroad Company (the Railroads), on which they parked application rigs, kept dinoseb cans, and stored empty Nemagon containers.

The site made the National Priorities List in 1989. In 1996, the United States and California Department of Toxic Substances Control filed cost recovery actions against B&B, the Railroads, and Shell. A major focus of the trial was whether the environmental harm was capable of apportionment, including the underlying analytical data regarding contamination on the railroad parcel.

Restatement (Second) of Torts Section 433A provides that damages are to be apportioned among two or more causes where there are distinct harms, or where there is a reasonable basis for determining individual contributions to a single harm. The Railroads argued that either the limited soil contamination on their parcel was a separate harm from the extensive soil and groundwater contamination on B&B's property, or the site contamination constituted a single harm that could be apportioned based on the relative mass of contaminants in groundwater attributable to each parcel. The Railroads submitted expert testimony to support this basis for apportionment.

The government argued the contamination constituted an indivisible harm that could not be apportioned, claiming releases on the Railroad parcel contributed to the groundwater contamination. Because the evidence was too uncertain to quantify the respective contributions from the two parcels, the Railroads were arguably jointly and severally liable.

The government also argued that apportionment is discretionary, and to avoid injustice to the plaintiff agencies, the court should allocate (insolvent) B&B's orphan share to the other defendants.

Shell argued any D-D contamination associated with product deliveries had not impacted groundwater and that it was not liable for the "hot spot" of dinoseb contamination because dinoseb was not its product.

The district court found the soil and groundwater contamination at the site was a single harm. While acknowledging the Railroads' evidence for apportionment, the court did not find that evidence to be helpful because it was unable to translate the expert's formula for apportioning the harm into usable data. Instead, the court's approach considered the size of the Railroad parcel relative to the total site area, the length of time it was leased to B&B, and that releases of dinoseb and Nemagon on the Railroad parcel contributed to the contamination. The court calculated the Railroads' divisible share to be 6%, then added a 50% allowance for calculation errors and concluded the Railroads' divisible share was not more than 9% of the total site response costs.<sup>1</sup>

Citing evidence as to the estimated quantities of various types of D-D spills, the district court apportioned 6% of total site response costs to Shell.

Finally, the district court concluded that reallocating B&B's orphan share would be "manifestly inequitable."<sup>2</sup>

On appeal, the Ninth Circuit reversed the district court's apportionment rulings and imposed joint and several liability on the Railroads and Shell. While agreeing that, conceptually and with adequate information, the contamination traceable to the parties could be apportioned, the Ninth Circuit found that the numbers used by the district court bore an insufficient logical connection to the pertinent issue and lacked the necessary precision.

The Supreme Court reversed the Ninth Circuit and held that the district court's apportionment of 9% of the harm to the Railroads was supported by the evidence and consistent with applicable principles, concluding "that the facts contained in the record reasonably supported the apportionment of liability."<sup>3</sup>

The Court acknowledged the Ninth Circuit's criticisms, but determined that "it was reasonable for the court to use the size of the leased parcel and the duration of the lease as the starting point for its analysis."<sup>4</sup> The Court also found any miscalculation due to the district court's conclusion that Nemagon and dinoseb accounted for two-thirds of the contamination was harmless since the final apportionment determination included a 50% margin of error. Had the court limited its apportionment

calculation to the amount of time B&B used the Railroad parcel and that parcel's percentage of total site area, it would have reached the same result.

The Supreme Court applied a clearly erroneous standard of review to the district court's apportionment of liability to the Railroads, as reflected by how the Court framed the issue – whether the record provided a reasonable basis for the district court's decision.

The Court's decision demonstrates that a CERCLA defendant may meet its burden of proving a reasonable basis for apportionment by introducing evidence sufficient for the district court, as fact finder, to determine the degree to which different parties contributed to the harm, even while not accepting the defendant's own arguments and expert testimony as factual bases for apportionment.

Affirming the district court's apportionment determination as a reasonable approximation of the Railroads' contribution to the harm, the Court rejected the Ninth Circuit's demand for "sufficient data to establish the precise proportion of contamination" attributable to the Railroad parcel.<sup>5</sup>

The Ninth Circuit agreed with other courts that, with adequate information, divisibility may be established by volumetric, chronological, geographic, or other types of evidence.<sup>6</sup> The Supreme Court's decision establishes that such "simplest of considerations" may be sufficient to provide a reasonable basis for apportionment,<sup>7</sup> affirming that a calculation limited to the amount of time B&B used the Railroad parcel and that parcel's percentage of the site area adequately supported the district court's apportionment determination.

Courts have long acknowledged that CERCLA allows for apportionment while almost always imposing joint and several liability. *Burlington Northern* demonstrates that a district court has considerable discretion to weigh the evidence relevant to a defendant's contribution to site contamination and find that such evidence provides a reasonable basis for apportionment.

#### References:

- <sup>1</sup> *U.S. v. Atchison Topeka & Santa Fe Ry. Co.*, 2003 WL 25518047 (E.D. Cal. July 15, 2003) ¶¶ 474-478, 480-484, 488-489.
- <sup>2</sup> *Id.* ¶ 471.
- <sup>3</sup> 129 S.Ct. at 1882-83.
- <sup>4</sup> *Id.* at 1883.
- <sup>5</sup> *Id.* at 1882.
- <sup>6</sup> 520 F.3d 918, 936 n.18 (9<sup>th</sup> Cir. 2008).
- <sup>7</sup> 129 S.Ct. at 1883 (quoting 520 F.3d at 943).

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#### CERCLA, Apportionment, Liability

## C7 A Really Big Corrosion Litigation

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After attending this presentation, attendees will learn the basis of metal corrosion and the dezincification phenomena that occurs with some types of brass fittings. This presentation also will address how a very large corrosion litigation matter was successfully handled.

This presentation will impact the forensic science community by identifying a large and growing area of litigation brought on by the changing nature, due to materials costs, of the brass fittings.

In one housing area, 34,000 homes suffered or were going to suffer severe water leaks due to failure of their plumbing system. A number of corrosion engineers were employed by various parties including tubing and fitting manufacturers, building contractors, plumbers, insurance companies, and homeowners. The plumbing system in question used Kitec brass fittings in conjunction with PEX tubing. The PEX tubing is made by cross linking high density polyethylene. The tubing was PEX-Al-PEX with a layer of aluminum sandwiched between PEX layers to prevent diffusion of oxygen into the water system and, hopefully, prevent corrosion from occurring.

The brass fittings are attached to the PEX tubing by compression rings or clamps. The plumbing system failures involved uniform layer type dezincification, as opposed to pitting type dezincification, with subsequent loss of strength of the brass fittings.

In dezincification, the zinc leaches out, leaving behind a porous copper sponge. The resulting sponge fitting leaks at the connection points. The leached zinc causes build up of bulky mounds of zinc oxide in the water fitting, resulting in constricting and choking the normal water flow. This is referred to as meringue dezincification.

The failed brass fittings were manufactured from a high zinc duplex alloy of 35 - 40% Zinc. The potable water in that area was considered non-corrosive and had a neutral Langelier index, but it appeared to be aggressive to the high zinc brass fittings.

The brass fittings were failing and leaks were occurring in three to six years. Factors that increase dezincification:

- High temperature
- Low water speed (stagnant water)
- Low aeration
- Zn concentrations above 15% in the brass
- High chloride content in the water

The litigation issues focused on the following:

- The plumbing installation was approved by the governing agency.
- The zinc content at 35% was unusually high in the failed fittings.
- The water was considered non-corrosive.
- Fitting manufacturing processes were questionable.
- Poor plumbing installation practices causing physical stress on the fittings.
- The use of an aluminum layer in the tubing was a problem.

Examination of the Cu-Zn phase diagram showed that an intermetallic compound of Cu-Zn formed at high concentrations of Zn. The intermetallic compound was susceptible to dezincification under certain water conditions that normally were not considered corrosive to other metals.

An empirical plot developed in 1961 that correlated water composition in various parts of England with the occurrence of dezincification of brass fittings was found to provide an explanation of the corrosive nature of the water in the housing area of failures.

The "Turner Diagram" plotted chloride concentration versus  $\text{CaCO}_3$  hardness, and showed that the water used in the area of the homes in litigation was aggressive to brass. The water, in combination with the high zinc content in the fittings, was the cause of the dezincification.

Because copper costs four times more than zinc, more high zinc brasses will be found on the market. These "yellow" brasses will be susceptible to dezincification failure, in particular with soft water containing more than 40ppm chloride.

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### **Corrosion, Dezincification, Brass Fittings**

## **C8 Being Significant and Watching Your Figures**

*Denise A. Shepperd, BS\*, Trillium, Inc., 2014 Carol Drive, Wilmington, DE 19808*

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After attending this presentation, attendees will better understand the importance of the relationship between the accuracy of a measurement system and the number of significant figures used to report the results.

This presentation will impact the forensic science community by demonstrating the relationship between the demonstrable accuracy of a result and the defensibility of the data in legal proceedings.

A Google search of the term "significant digits" offers up a variety of definitions. They all boil down to this: significant digits are those digits in a number that can be defended based on the accuracy of the measurement system used to generate them.

Every analytical chemistry course includes a unit on precision and accuracy and a lesson about significant figures. Students are taught what

they are, how to use them, and why they are important. When this lesson is forgotten, the illusion of greater accuracy than the analytical methods can produce is proliferated. For instance, when using a calibration equation to calculate a result, any number of digits and decimals can be produced, but only those that are representative of the method's accuracy can be defended.

No analytical result can be more accurate than the factors used to determine it. In other words, if a balance that is accurate to 0.1 g is used to weigh a sample, a result of 0.11g cannot be defended because that result implies greater accuracy and precision than it was possible to achieve with the measurement system. Likewise, a calibration curve produced by analyzing standards at concentrations of 5.0, 10, 50, 100, and 200pg/mL cannot produce a defensible calculated result of 5.005pg/mL.

The last significant figure in an analytical measurement is the only figure that should express variance. For instance, a result of 1.6 reflects two significant figures and an error term of 0.1, meaning that the result could be 1.5, 1.6, or 1.7. The error term is expressed as 62 parts per thousand or 6.2%. If the same result is reported with three significant figures, as 1.62, a smaller error term is expressed (6.2 parts per thousand or 0.62%), meaning that the result could be 1.61, 1.62, or 1.63, but not 1.5 or 1.7. Supporting this level of accuracy and precision at this concentration range would require that the laboratory can routinely and demonstrably analyze two portions of a project-specific sample with duplicate agreement of less than one relative percent difference (RPD).

The second of the four non-exclusive factors that make up the criteria used to judge the validity of an expert opinion in a *Daubert* test asks, "Is there a known error rate or variability?"<sup>1</sup>

Every measurement has an error term. Any measurement system will produce a range of results for a single sample when that sample is repeatedly measured for a given analyte. The known or potential error term is a statistical measure of how similar (or different) these results are likely to be. In analytical chemistry, the method includes the entire analytical procedure used to identify and measure the concentration of an analyte in a sample matrix. Some sources of error are unavoidable, while some sources can be minimized by following good analytical practices and sound quality control procedures. The objective of any experimental process should be to produce the most accurate result possible, i.e., a result with the smallest possible error term. The use of good laboratory practices and sound quality control procedures is essential in a laboratory's effort to reduce the error term, but it cannot be eliminated. This measurement uncertainty is reflected in the number of significant figures used to report the results. It is critical to present results using only the significant figures that are appropriate to the data set. The error term implied by the significant figures must be supported by the available data and documentation and must be demonstrated to be repeatable for the results in the data set.

### **Reference:**

<sup>1</sup> *Daubert v. Merrell Dow Pharmaceuticals, Inc.* (1993) 509 U.S. 589  
**Significant Figure, Error Term, Defensibility**

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## **C9 Why Current Detection and Reporting Limits are Useless in Low Level Data Evaluation**

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After attending this presentation, attendees will have a better understanding of the basis of laboratory detection and reporting limits, and how those limits fail to provide the information necessary to evaluate low level data with a high degree of confidence.

This presentation will impact the forensic science community by providing data users reasons for the inadequacies of the current complex series of terms used for detection and reporting limits.

Currently, the environmental field is plagued with a number of acronyms for both detection limits (DL) and reporting limits (RL). For example, method detection limit (MDL), minimum quantitation limit (ML), level of detection (LD), limit of quantitation (LQ), estimated detection limit (EDL), practical quantitation limit (PQL), contract required quantitation limit (CRQL), etc. These are all derived either statistically, by contract, or from regulatory limits. None of the terms used are based on results from real world samples. The environmental laboratory industry is being required to report results down to the DL without justification or proof; thus reporting non-defendable data which the user may misinterpret. Laboratories have even been judged based on their MDLs (labs with lower DLs are better!). DLs and RLs are not the same and the terms should not be used interchangeably. The DL should be the concentration at which one can state with a known confidence level that an analyte is present. The RL should be the lowest concentration at which the analyte is determined within a known precision and accuracy range. Unfortunately, RLs in most cases are calculated as a factor times the DL, with no requirement to confirm for a specific sample. For example, a statistical approach (MDL) was originally developed by the USEPA to demonstrate that the methods being promulgated for regulated analytes had detection limits below the regulatory levels in discharge permits of Publicly Owned Waste Treatment facilities (POWTs) and receiving water bodies. The formula is  $MDL = 3.143$  times the standard deviation of the measured analyte concentration of seven replicate spiked laboratory water solutions. The USEPA minimum level of quantification (ML) was originally stated as ten times the standard deviation used to calculate the MDL in the described way. The ML is also commonly calculated as 3.1 times the MDL.

The original intent of the USEPA was to use the MDL procedure to determine detection limits of analyte(s) in the method user's samples. Instead, the MDL requirement has been interpreted such that the environmental laboratory industry is required to determine MDLs on all analytes, general matrix types, and instruments used, but not on specific samples.

This approach has led to unbelievably low DLs and RLs in laboratory reports and subsequently in project databases. How does one defend a beryllium in soil result of  $<0.01\text{mg/kg}$  when the associated matrix spike (MS) and matrix spike duplicate (MSD) data has 15% and 12% recovery with spiking at the  $1\text{mg/kg}$  level? The laboratory flagged the low recovery MS and MSD data as a matrix effect. This implies that the sample itself interferes with recovery of beryllium. Using the reporting limit of  $<0.01\text{mg/kg}$  would be in error and if the regulatory limit was  $0.05\text{mg/kg}$ , one would be reporting non-defendable data that truly is wrong. The data show that one can only state with any certainty a reporting limit of  $1\text{mg/kg}$ .

Now, if the same data in the above example were reported for the analysis of hexavalent chromium by Ion Chromatography in soil, then the results would be understandable and defensible. Hexavalent chromium is converted to trivalent chromium by this sample matrix and thus gives low recoveries for MS and MSD samples. This can be proven by having acceptable recovery of a post digestion spike sample.

Other examples of the pitfalls of DLs and RLs and their application will be presented. Recommendations will be made on a more realistic approach to a reporting limit and its confirmation, using appropriate quality control samples, what information should be provided to the laboratory to achieve useful data and most importantly, what questions to ask the laboratory to better understand the data.

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#### **Detection Limits, Reporting Limits, Quantitation Limits**

## **C10 On Confidence Intervals Used to Determine Background Concentrations**

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The goal of this presentation is to make attendees aware of the consequences of including and excluding outliers including non-detects when determining background concentrations.

This presentation will impact the forensic science community by showing what confidence intervals represent.

Determination of a background concentration is often a controversial exercise. It is further complicated by noisy data which requires a statistical expression of what the background concentration measurements mean, typically expressed as using a confidence interval.

A confidence interval simply expresses to probability that the mean value of the measurements will be less than a given value. When a finite set of measurements of the same quantity (background concentration in this instance) is made, the mean value represents the most likely (expected) value of that quantity. It is possible, however, that the mean value of a finite set of measurements does not represent the true mean of the entire population, in this instance all the possible samples that could be taken. To express the certainty with which we can say that the set of samples we do have represents the true value you would find with a much larger (or infinite) set of samples, it is customary to express the result in terms of a confidence interval.

When the measurements are consistent, that is, the standard deviation of the measurements are small, the confidence intervals are narrow. Specifically, if all the measurements are exactly the same, the standard deviation is zero, and it is extrapolated that no matter how many more samples are taken, the measured value would always remain the same. However, when the data are noisy and have a large standard deviation, the confidence intervals can be very large. For such data, the actual population of concentrations vary and exactly which subset of all the possible subsets we measure could give very different answers for the mean. As such, the large confidence interval expresses the fact that the true mean of the entire population may be poorly captured by the actual subset of measurements taken.

Special care should be taken with the treatment of extreme values, sometimes called outliers, as such values would increase the standard deviation of the data and hence the confidence intervals. On the low end of the concentration range are often non-detected values, while at the high end there may be outliers which may actually be the result of sample contamination or influence by the source being investigated.

Where background concentrations are relatively low, it is common to find a number of non-detects in the data set. Since concentrations are strictly positive and often show a log-normal distribution, selecting the specific concentration to use for the non-detect is very important. Half the detection limit is often used; however, when the detection limit varies among the samples, this may introduce an artificial variation in the sample set since the detection limit simply expresses an upper bound on the true concentration, while the actual concentration may show little variation among samples. Conversely, it is possible that the actual concentration varies greatly among the samples, but that this concentration is below the detection limit and that the same detection limit is reported for many samples. This would artificially reduce the variability.

This presentation discusses the statistical basis of confidence intervals, and demonstrates using examples the consequences of different ways used to treat these outliers.

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#### **Confidence Interval, Background Concentrations, Statistics**

## C11 Application of a Monte Carlo Analysis to the Johnson and Ettinger Soil Vapor Intrusion Model

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After attending this presentation attendees will be able to recognize the forensic distinctions between applying a Monte Carlo analysis to estimate the most likely impact of soil vapor intrusion as opposed to calculating unique solutions to the Johnson & Ettinger Vapor Intrusion model.

This presentation will impact the forensic science community by providing understanding of how models are often used to develop the justification for forensic investigations and actions. In order to apply the models correctly, the users must recognize when a Monte Carlo analysis is appropriate.

Models are used in environmental investigations to describe the site conditions using the best available data. Known values are combined with estimates of unknown values to develop a representation of the historical or current or future site conditions. Confidence in the model may be increased as the site description improves, either by acquiring more data, or by improving the sophistication of the model. However, an environmental model should be as simple as possible to utilize or else its complexity will overwhelm practitioners.

Federal guidance and many state regulations cite the Johnson and Ettinger soil vapor intrusion model to describe the interactions of environmental factors which could influence the migration of a volatile chemical in groundwater into soil vapor and thence into the indoor air of a building. The Johnson and Ettinger (J&E) model calculates an attenuation factor,  $\alpha$ , for the ratio of the subsurface soil vapor concentration versus the indoor air concentration due to vapor intrusion. Some of the J&E model inputs include chemical reference values, scientifically derived characteristic values, measured site specific values, and estimated site information. The J&E model's complexity is suggested by publications intended to simplify its use.<sup>1</sup> Confidence in the representativeness of the model is generally low when it is tested against a small number of samples from a site, but for large sites the average value of the modeled  $\alpha$  has been shown to approximately represent the average attenuation factor for large numbers of samples.<sup>2</sup>

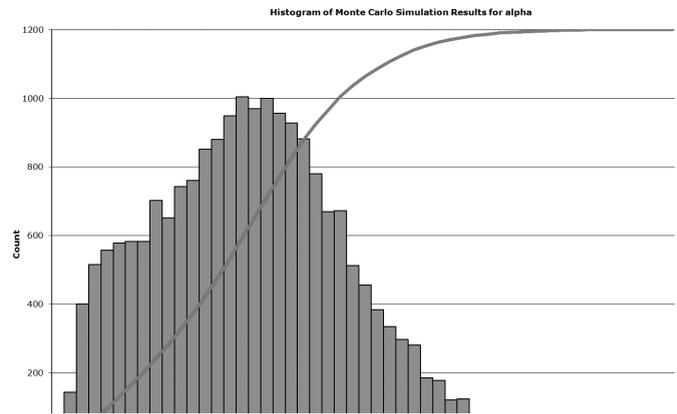
The failure of the J&E model to represent site conditions for small numbers of samples may be due to short-term fluctuations in some site factors that have very large effects on the model, but may not represent the average conditions found when analyzing many samples from the same site. The measured attenuation factors for a pair of samples could be predicted with choice selections of parameters in the J&E model, but using EPA recommended parameters probably will not generate a comparable result.

A Monte Carlo analysis using parameters from EPA and API vapor intrusion guidance gives a probability distribution for  $\alpha$ . Single determinations of  $\alpha$  may vary by several orders of magnitude depending on the values selected for the J&E model parameters, but the central tendency of the Monte Carlo probability distribution for  $\alpha$  has a much smaller range. As critical input factors for the model are varied, the overall impact on the central tendency of  $\alpha$  is less than the variation predicted from unique determinations of the model.

EPA has compiled a vapor intrusion database ([http://www.epa.gov/oswer/vaporintrusion/vi\\_data.html](http://www.epa.gov/oswer/vaporintrusion/vi_data.html)) which was queried for the average attenuation factor for all results for trichloroethylene detected in soil gas versus indoor air. The average attenuation factor calculated from the database (N=75) was  $1.8 \times 10^{-3}$  and the 50<sup>th</sup> percentile was  $1.2 \times 10^{-4}$  (range  $9.7 \times 10^{-7}$  to  $5.5 \times 10^{-2}$ ). A Monte Carlo simulation for TCE vapor intrusion using the J&E model gave an average  $\alpha$  of  $2.3 \times 10^{-4}$  which also was the same value as the 50<sup>th</sup> percentile of 20,000 iterations (range  $1.3 \times 10^{-5}$  to  $8.1 \times 10^{-4}$ ).

The Monte Carlo analysis gives a much narrower range of attenuation factors than are found in the EPA database of field measurements. However, the range of possible values of site specific parameters is limited for the Monte Carlo analysis, which tends to limit the range of values generated by the calculations. The mean value of the Monte Carlo analysis is comparable to the mean of all values in the EPA database, which is more precise than the range of estimated attenuation factors calculated in the EPA reference guidance.

A Monte Carlo analysis of the J&E model with a defined set of site specific characteristics will predict a small range of reasonable values of  $\alpha$ , while a deterministic approach to using the model to describe vapor intrusion at a site might not be any more accurate than a best guess. The Monte Carlo analysis can be used to predict the most likely site conditions.



### References:

1. Johnson 2002, *Identification of Critical Parameters for the Johnson and Ettinger (1991) Vapor Intrusion Model*, and, EPA 2000, *User's Guide for the Johnson and Ettinger (1991) Model for Subsurface Vapor Intrusion into Buildings*).
2. Hers et al 2003, *Evaluation of the Johnson and Ettinger Model for Prediction of Indoor Air Quality*

### Monte Carlo, Johnson & Ettinger Model, Soil Vapor Intrusion

## C12 Standardization of Risk Assessment: Why It Doesn't Always Work

James S. Smith, PhD\*, Oak Creek, Inc., 60 Oak Creek, Buxton, ME 04093-6616

After attending this presentation, attendees will become aware the potential pitfalls of using a standardized risk assessment approach in the characterization of human health risk at hazardous waste sites.

This presentation will impact the forensic science community by providing attendees with an appreciation for the potential shortcomings inherent in the use of a standardized approach to risk assessment at hazardous waste sites.

Over the last two decades, state and federal regulators have been working to standardize the conduct of human health risk assessments at hazardous waste sites. Regulators have long sought a process of human health risk assessment that is, quick, efficient, consistent, and easy to understand. The application of a standardized approach to risk assessment, however, does not always provide human health risk information that is useful to risk managers. This is because standardization of this process relies on the use of generic exposure parameters, equations, and other information in preference to site-specific information.

Actions taken to clean up Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Superfund sites are designed to be protective of human health and the environment. At such sites, a

baseline assessment of human health risk is an integral part of the Remedial Investigation/Feasibility Study (RI/FS). The goal of the RI/FS is to gather information sufficient to support an informed risk management decision regarding which remedy appears to be the most appropriate for reducing health risks. Specific U.S. EPA guidance relating to the performance of baseline risk assessments identifies them as “site-specific” assessments of human health risk that can “vary in both the detail and the extent to which qualitative and quantitative analyses are used,” characterizing the complexity and particular circumstances of a site. U.S. EPA experience has shown that Superfund sites are complex, characterized by heterogeneous wastes, extreme variability in contamination levels, and a variety of environmental settings and potential exposure pathways. Because the complete characterization of a site, necessary to reduce uncertainty, is not feasible or cost-effective, U.S. EPA adopted a streamlined approach to the RI/FS and the selection of appropriate remedies, making a policy decision “to use, wherever appropriate, standardized assumptions, equations, and values in the human health evaluation.” According to the U.S. EPA, this policy “has the added benefit of making the human health risk assessment easier to review, easier to understand, and more consistent from site to site.” The inherent problem of such streamlined standardized approach to assessment of human health risk is that it may not always provide useful information to risk managers about which remedy is most appropriate for reducing human health risk.

The Massachusetts Department of Environmental Protection (MassDEP) has taken a similar approach to the conduct of human health risk assessment with its ShortForms. Provided as a “shortcut” or “streamlined method for evaluating potential human health risk at hazardous waste sites” MassDEP provides ShortForms (i.e., computer spreadsheets) to calculate human health risk associated receptor exposure to environmental media. These ShortForms only require the operator to input an appropriate exposure point concentration (EPC) for the media of interest in order to quantify human health risks. Similar to other standardized approaches to human health risk assessment, the resulting estimates of human health risk may not always provide useful information to risk managers about which remedy is most appropriate for reducing human health risk.

While anyone might use a standardized approach to perform risk assessment, only qualified professionals are capable of determining whether the resulting health risk estimates are useful to risk managers in the selection of appropriate remedies. In this presentation, the potential for several standardized approaches to human health risk assessment used by state and federal regulators to result in inappropriate risk management decisions will be examined.

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#### **Risk, Assessment, Standardization**

### **C13 The Analysis of Gun Shot Residue Using Transmission Electron Microscopy**

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The goal of this presentation is to present to the forensic community information about how Transmission Electron Microscopy (TEM) coupled with Energy Dispersive X-ray Spectroscopy (EDS) can be instrumental in the analysis of nano-sized gun-shot residue (GSR).

This presentation will impact the forensic science community by showing the advantages of using TEM-EDS to identify nano-sized GSR particles released during the discharge of a firearm.

Routine GSR analysis is normally performed by Scanning Electron Microscopy (SEM). Classic GSR particles are usually defined as spherical particles, 1µm in size and larger, containing three main components, which are Pb, Ba, and Sb. Therefore, particles less than 1µm in size are usually overlooked during routine analysis for GSR by SEM.

For this presentation, an experiment was done using TEM-EDS to characterize the particles released during the discharge of a firearm. Samples of GSR were generated by firing five rounds of Browning High Powered Sellier and Bellot ammunition from a 9mm handgun in an enclosed facility. Air samples were collected simultaneously with the firing of each round of ammunition. The air pumps were set up adjacent to the muzzle and ejection port of the handgun. An ambient air sample was collected 12 hours after firing the initial five rounds of ammunition. A second set of GSR samples was generated by firing three rounds of Winclean ammunition also from a 9mm handgun in an enclosed facility. Air samples were also collected simultaneously with the firing of each round of the Winclean ammunition. The GSR samples were collected on 0.45 µm pore sized, 25mm diameter, Mixed Cellulose Ester (MCE) filters and prepared according to procedures described in the NIOSH 7402 method for TEM. TEM-EDS analysis of the filters showed that particles, both spherical and non-spherical, of varying sizes and elemental compositions, were released into the atmosphere during the discharge of each round of ammunition. A significant amount of the particles observed by TEM-EDS were less than 1µm in size. However, the spherical GSR particles containing Pb, Ba, and Sb, analyzed by TEM-EDS, ranged in size from greater than 1µm to less than 100nm. Analysis of the ambient air sample showed that nano-sized, spherical GSR particles containing Pb, Ba, and Sb were still suspended in the air 12 hours after the initial rounds of ammunition were discharged.

This study confirmed that many GSR particles less than 1µm in size are released into the atmosphere during the discharge of a firearm and these particles tend to remain suspended in the air for longer periods of time than the larger particles. Therefore, TEM-EDS can be instrumental in the analysis of nano-sized GSR particles especially in the absence of those greater than 1µm in size. Future research involves determining how long and under what conditions, nano-sized GSR particles can remain suspended in the air.

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#### **GSR, TEM, Nanoparticles**

### **C14 Chemistry Ignored — A Case Study**

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After attending this presentation, attendees will understand how the evaluation of chemical tracers in ground water can play an important role in a site investigation.

This presentation will impact the forensic science community by illustrating a valid and useful means of evaluating ground water data to interpret the source and migration of contaminant plumes that is readily available but not routinely used.

In an all too familiar scenario, drinking water wells in a residential neighborhood were found to be contaminated with low concentrations of chlorinated solvents; 1,1-dichloroethene (11DCE) was the initial red flag. A chemical release from a neighboring manufacturing facility (Facility A) was alleged, and, in the subsequent toxic tort litigation, the facility was named as a source of the contamination. After multiple site investigations were performed by at least five different consultants, the analytical data were compiled into a single data base. Evaluation of these data yielded an unexpected result: two plumes were identified, and only one was linked to Facility A.

While it was clear that both a “Northern Plume” and a “Southern Plume” were present, it was not clear where, or if, they overlapped. Since both plumes contained chlorinated solvents and ultimately migrated toward the affected neighborhood (Neighborhood A), this was an important determination. Initially the request was to review isotopic analysis data to determine if the plumes could be differentiated on this basis, but since isotopic data were only available for Facility A, there was nothing to

compare. Instead, the entire set of ground water data was reviewed with a focus on chlorinated solvent chemistry, and used the biodegradation and hydrolysis daughter products of the source contaminants as chemical tracers to map the plumes.

Results for three source contaminants (1,1,1-trichloroethane [TCA], trichloroethene [TCE], and tetrachloroethene [PCE]) and four degradation products (11DCE, 1,1-dichloroethane [11DCA], 1,2-dichloroethane [12DCA], and cis-1,2-dichloroethene [c12DCE]) were plotted separately. For simplicity, the plotted result for each sample location was estimated as an average over time and depth. From this first set of maps, several things were clear: (1) there was a significant source (Facility B) of PCE south and west of Facility A; (2) no PCE was detected above 10 ppb at Facility A; and, (3) both Facility A and Facility B were significant sources of 111TCA and 11DCA in the ground water. 111TCA was looked at more clearly.

111TCA hydrolyzes to form 11DCE. Because biodegradation is not involved, this chemical reaction in ground water affords an opportunity to estimate the age of the 111TCA (i.e., how long it has been dissolved in the ground water at that location) based on the relative concentrations of these two analytes, the temperature of the ground water, and the kinetics of the hydrolysis process. At the likely source of the Southern Plume, estimated 111TCA ages ranged from 14-20 years; immediately down gradient of this source (Neighborhood B), ages were estimated at 26-41 years. At Facility A, there were not enough data to calculate any 111TCA ages, but estimates ranging from 22-39 years, were calculated for Neighborhood A. A plot of the estimated ages shows a plume that travels east, then curves to the northeast, skirting Facility A entirely. This trend did not, however, convince the clients. So, it was tried again.

111TCA biodegrades in ground water to form 11DCA. The ratio of the 11DCA concentration to the total calculated 111TCA concentration (measured 111TCA + measured 11DCA + [4.545 x measured 11DCE]) allows us to evaluate the relative amount of 111TCA biodegradation that has occurred at each sample location. This plot showed a clear distinction between the two plumes: at Facility A, the ratios ranged from 0.27 to 0.62, while at Facility B they ranged from 0 to 0.08. Clearly, the 111TCA at Facility A is more biodegraded than the 111TCA at Facility B. At Neighborhood A, the ratios were a striking match to the 111TCA from Facility B, and it was concluded that Facility A (the Northern Plume) did not contribute to the contamination in Neighborhood A.

Unfortunately, the clients were not comfortable with the approach and conclusion, partly because results less than 10 parts per billion were excluded, where analytical error is highest, and partly because this was not a standard approach to using ground water tracers. Remaining confident; however, that the chemistry of chlorinated solvents in ground water is a reliable and readily available means of mapping plumes and identifying sources of contamination.

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### **Chlorinated Solvents, Groundwater, Chemical Tracer**

## **C15 The Road to the Supreme Court**

*James S. Smith, PhD\*, Trillium, Inc., 28 Graces Drive, Coatesville, PA 19320-1206*

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After this presentation, attendees will understand the chemistry behind the contamination at a contaminated railroad and agricultural chemical site and the reliability, or lack thereof, of the analytical data presented to the court.

This presentation will impact the forensic science community by illustrating the questionable nature of one set of data that was presented to, and ultimately used by, the court in apportioning CERCLA liability.

*Burlington Northern v. United States*, 129 S.Ct. 1870 (2009) was the first CERCLA action in which a district court determined, using the data presented as evidence, the degree to which the parties contributed to the contamination at a site and apportioned liability accordingly. In 1995, the railroad companies requested an evaluation of the analytical chemistry and

the fate and transport of agricultural chemicals at a site in Arvin, California. The chemicals of concern were:

- Dinoseb - a herbicide used for potato crops
- 1,2-Dichloropropane (1,2-DCP) - a nematocide used for root crops
- cis- and trans-1,3-Dichloropropene (1,3-DCPe) - a nematocide used for root crops
- 1,2,3-Trichloropropane (1,2,3-TCP) - an impurity in both 1,2-DCP and 1,3-DCPe

A small portion of the site was leased from the railroads by the agricultural commodity provider. Since the owners of the leased site were without funds, the USEPA, as plaintiff, was suing the railroads and the manufacturers of the chemicals of concern for investigative costs and remediation expenses.

The USEPA conducted a majority of the investigative sampling and analysis activities of soils and groundwater on and adjacent to the site. The analytical results for soils and groundwater indicated widespread contamination by the chemicals of concern. However, the soil results for the leased property contained only a few "hits" of relatively low concentration. The main difficulty with the EPA data was that its validity was highly questionable. There were two fundamental reasons for the EPA data to be of questionable value for their investigative purposes:

1. Sampling highly contaminated soil immediately prior to sampling a supposedly contaminant-free background soil boring. For example, a sample 400 feet off site at 45 feet below ground surface (bgs) contained dinoseb at 1,100ug/kg (ppb), but there were non-detects at 25 and 35 feet below the surface. The boring that was sampled prior to the background boring was in the most highly dinoseb-contaminated area of the site.
2. Analytical chemistry for the analysis of 1,2,3-TCP was done by the EPA with an on-site laboratory utilizing a gas chromatograph (GC). The calibration of the GC and the detection of 1,2,3-TCP in pure water blanks indicated that the reported values for 1,2,3-TCP in soils were unreliable at values below 100 ppb.

With the best data, there were only two soil samples containing dinoseb that were found on the railroad property. Both of these "hits" were not valid because the GC retention times were different from the calibration retention times. Thus, EPA did not have any valid data demonstrating contamination emanating from the railroad property.

Groundwater was about 70 feet bgs and moved southwest in the 1990s when these investigations were being done. The site's unlined wash basin was the main avenue for dissolved chemicals to be transported to the groundwater. Mounding of the groundwater beneath this wash basin and a down-gradient potato shipping wash basin pushed some of the chemicals of concern to the west and beneath the railroad property. This location of the chemicals in the groundwater was exploited by the EPA during trial, along with the language of "joint and several," all the way to the Supreme Court.

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### **CERCLA, Apportionment, Chemistry**

## **C16 Free Product Determination Using Ethylbenzene to Total Xylenes Ratio**

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After attending this presentation, attendees will understand a complimentary method to determine whether or not a petroleum free product is in the soil.

This presentation will impact the forensic science community by putting another tool in attendees toolbox for use at petroleum-contaminated sites.

During a 2005 kerosene UST assessment, concentrations of diesel range organics (DRO) (140,000 - 4,900,000 ug/kg) were reported in the

shallow samples. However, the absence or generally low levels of polynuclear aromatic hydrocarbons (PNAs) and volatile organic compounds (VOCs) associated with these samples do not support an association with free phase conditions.

During the 2009 and 2010 supplemental soil boring sampling activities, selected samples in which total VOCs did not exceed 75,000ug/kg were additionally analyzed for gasoline range organics (GRO) (C-6 to C-10). Based on the available data, however, no specific data trend or correlation of total VOCs versus GRO was identified. Because of the absence or very low levels of PNAs reported in these soil samples, no additional DRO analysis was deemed necessary in order to assess for diesel range free phase conditions.

An evaluation using the Ethylbenzene/Xylenes Ratio (EXR) Method (DeWitt, Smith, and Hoitash, 2008) determined that the residual contaminant levels reported in soil samples do not represent free phase conditions. Gasoline and middle distillates (ie., diesel, kerosene, and fuel oils) have composition ethylbenzene/xylenes ratios (EXRs) of approximately 0.20±0.05. Long term sources of contamination 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). Upon a release, bacteria rapidly uses available oxygen, driving the release environment anaerobic. Anaerobic biodegradation removes xylenes faster than ethylbenzene (Reinhard, Hopkins, and LeBron, 2005), thus EXRs increase with time. An EXR of approximately 0.25 or greater indicates anaerobic biodegradation (Smith and DeWitt, 2006). Release areas can act as continuing sources having extremely slow biodegradation resulting in continued elevated contaminants and EXR values typically greater than 0.15 and less than 0.25, but can vary pending site-specific conditions and release compositions. EXR data not showing an increasing trend and remaining near the range anticipated for product can indicate the presence of “free product” and/or significant source material (e.g., free phase conditions).

The EXR values for the cumulative data for the VOCs consistently reported (2005 to 2009) will be presented. In general, for the samples exhibiting benzene concentrations, which would be expected to be present if free phase conditions were acting as a continuing source of contaminants, the EXR values are above 0.25 (i.e., 0.33 to 1.71) indicating that biodegradation is occurring and that free phase conditions cannot be present. The highest VOCs concentrations in this group are represented by the former excavation sidewall sample S-17 (EXR - 0.58) and soil boring sample SB-3 (EXR - 0.35), which might be considered as having free phase conditions. However, the EXR values for samples S-17 and SB-3 and the lower levels of contaminants in the other samples are not representative of free phase conditions.

Based on the above weight of evidence approach and given the volume of removed source soils, it is not probable that free product or free phase conditions are present at the site.

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**EXR, Free Phase Petroleum Product, Soil**

## **C17 Determining the Primary Source and Time Period of Trichloroethylene Contamination in Groundwater — An Unusual Case History in Which TCE Apparently Moved Faster Than Its Degradation Compounds**

*John B. Robertson, BS\*, J.B. Robertson Consulting, 40107 North 3rd Street, Desert Hills, AZ 85086*

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The goal of this presentation is to show that some of the conventional thinking about the relative mobility of trichloroethylene and its degradation products in groundwater does not always apply.

This presentation will impact the forensic science community by showing how investigators sometimes need to think “out of the box” to

determine relative timing and locations of groundwater contamination sources.

A large area of trichloroethylene (TCE) contamination was discovered in groundwater in 1989 at a former industrial facility rural Kansas. The facility served as an Army Air Base during World War II (WWII) where heavy bombers were prepared for final deployment to European and Pacific war theaters. After the War, the facility was turned over to a local municipality and converted to a municipal airport and industrial park. A major aircraft manufacturing company then leased much of the property and conducted various manufacturing and assembly operations at the site from 1950 to the early 1960s.

After the discovery of groundwater contamination at the site in 1989, regulatory authorities determined that the aircraft manufacturing company was responsible, at least in part, for the contamination and ordered the company to characterize the extent of contamination and to implement appropriate remedial actions. The primary contaminant is the industrial chlorinated solvent TCE, but its chemical biodegradation products, 1,2-dichloroethene (DCE) and vinyl chloride (VC) are also significant contaminants. The groundwater contamination plume extends several miles down-gradient from the source areas. The contaminated aquifers are composed of three, relatively thin, flat-lying limestone strata.

During the course of conducting remedial investigations and cleanup actions, the aircraft manufacturing company filed a law suit against the federal government under the terms of Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), claiming that the U.S. Army caused a significant portion of the contamination during its activities at the base during WWII. Serving as an expert for the government to determine what portion, if any, of the contamination could be reasonably attributed to the Army’s activities.

An unusual aspect of the groundwater contamination plume was the fact that in the most distant, down-gradient portion of the plume, TCE is the dominant contaminant, with very little of the degradation products, DCE and VC. In contrast, near one of the two primary source areas, the degradation products are the dominant contaminants. This is unusual because in many aquifers, DCE and VC are more mobile than TCE and move faster because TCE is more retarded by adsorption to solid organic carbon matter in the aquifer matrix.

There were two primary source areas where TCE was initially released: a large hangar area called Hangar A and another larger hangar area, Hangar B. The plaintiff’s expert in this case argued that the explanation for the TCE-dominant contamination at the leading edge of the plume was caused by early releases of clean TCE during the Army’s activities at one of the two source areas (Hangar A) and that the DCE/VC dominance at that source area was due to the later releases of non-chlorinated solvents, fuels, and paint strippers probably during the aircraft manufacturing company’s activities. The aircraft company conducted TCE vapor degreasing operations at both hangar sites. No credible evidence could be found that the Army ever conducted TCE degreasing operations or other significant use of TCE at the site during its WWII operations.

After careful analysis it was shown that the unusual distribution of TCE, DCE and VC was due to the release of relatively clean TCE at Hangar B by the aircraft company and that undegraded TCE constituted the contamination at the leading edge of the plume. Later releases of TCE mingled with petroleum hydrocarbons by the aircraft company at Hangar A caused the DCE/VC-dominated contamination near that source area. Another factor effecting the contaminant distributions is the fact that matrix diffusion in the fractured limestone aquifers favor greater mobility of TCE over DCE and VC (the opposite effect of adsorptive retardation).

At trial, the court concurred with my analysis and concluded that the federal government had no liability for the contamination.

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**Groundwater, Contamination, Trichloroethylene**

## **C18 Molecular Level Compositional and Structural Characterization for the Deepwater Horizon Oil Spill By GC-GC and FT-ICR Mass Spectrometry**

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The goal of the presentation is to provide information on the first compositionally comprehensive inventory of the Deepwater Horizon crude oil, and monitor molecular level changes in the crude as a function of biotic and abiotic modifications from the wellhead to terminal point in the environment.

This presentation will impact the forensic science community by understanding how Ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) enables detailed compositional characterization of complex petroleum samples (all acidic, basic, aromatic and aliphatic species) at the level of molecular formula assignment. Comprehensive 2D gas chromatography (GCxGC) provides exhaustive structural characterization of nonpolar oil components below a carbon number of ~35. Such information is critical for source appointment and quantification of water washing and evaporative losses. Combined, the two techniques provide a wealth of compositional information that provides forensic fingerprinting of petroleum releases and monitors compositional changes at the molecular level. For the first time, comprehensive 2D GC and ultrahigh-resolution FT-ICR MS are combined to provide molecular level information on a real world oil spill.

An oil spill changes its chemical composition continuously, due to evaporation and dissolution. Furthermore, "weathering" (biotic and abiotic modifications) changes the composition of a crude oil and increases the relative abundance of polar species that are readily addressed by FT-ICR MS, but that are problematic for GC separations (requiring chemical derivatization prior to analysis). In work completed under an NSF RAPID Grant (#CHE-1049753), we have resolved and identified 72,000 acidic, basic, and nonpolar species in the Deepwater Horizon parent crude oil. Subsequent fractionations by micro-distillation and HPLC-2 have revealed thousands more species as well as structural information not provided by high resolution mass spectrometry alone and provide relevant fractions for more detailed analysis by FT-ICR MS and GC x GC. Collectively, the compositional results provided a detailed fingerprint and inventory of species released into the Gulf of Mexico. Water washing experiments have revealed water-soluble species that cannot be directly analyzed by gas chromatographic methods. Collaborative efforts reveal acidic/basic species compositional changes from the wellhead to being washed ashore in Pensacola, FL, where the compositional complexity of the basic species increased by 50%. Compositional analysis of tidal beach sediments at various depths exposes compositional changes due to weathering and sea water solubility, critical for enhanced model development. Most notably, the carbon number and aromaticity (double bond equivalents = rings plus double bonds to carbon) for the species identified below the initial oil contamination zone are lower than for the primary oiled sediment layer. Such compositional changes have been previously documented by our research in simulated oil spills performed in the laboratory (Stanford *et al.*, 2005). FT-ICR MS enables access to high boiling, polar species that have eluded environmental studies in the past due to their complexity and acidic/basic nature. That very character can now be exploited and combined with the highest performance FT-ICR mass spectrometry, to provide detailed compositional information for tens of thousands of acidic, basic and nonpolar species that lie within and far beyond the range of state-of-the-art gas chromatographic techniques. However, the resolution and structural analysis of lower boiling, nonpolar species (less than 35 carbons)

is critical to determine the identity of suspect crude oils and measure weathering phenomena of volatile/semi-volatile species not easily analyzed by FT-ICR MS. Work supported by NSF (CHE-10-49753 and DMR-06-54118).

**GC-GC, High Resolution Mass Spectrometry, Deepwater Horizon Oil Spill**

## **C19 Corrosion Evaluation of Lock and Dam Structure**

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After attending this presentation, attendees will understand the concept of using research type electrochemical techniques for rapid evaluation of corrosion problems involving steel and coatings in lock and dam structures.

The presentation will impact the forensic science community by providing forensic engineers with the ability to rapidly acquire corrosion and/or coating behavior data under the actual conditions of interest.

Literature research revealed the possibility of thermal sprayed coatings providing significant improvements over currently used vinyl coatings for U.S. Corps of Engineers locks and dams. This was confirmed by the following tests:

Electrochemical corrosion methods can be used to rapidly acquire data on the performance of metals and coatings in corrosive environments. Corrosion of metals involves transport of ions and electrons. DC current methods can measure corrosion rate and evaluate pitting and cracking tendencies in metals. Barrier type paint coatings produce a capacitor situation when placed on a steel substrate immersed in water. AC current techniques (electrochemical impedance spectroscopy or EIS) can utilize the effect to monitor moisture transport through coating and ultimately coating break down. Research using these techniques is almost always conducted in controlled laboratory environments. A lock miter gate is exposed to muddy, turbulent, and aerated river water. Tests using both DC and AC electrochemical techniques on actual muddy river water produced some startling results in the area of the current vinyl coatings behavior. Tests were conducted with samples of both vinyl (the current coating) and thermal sprayed coatings immersed in barrels containing aerated, intermittently agitated, muddy river water. (Clear river water was used for some samples.) Previous reported work has utilized small cylinders containing artificial sea water without aeration.

Steel plates coated with 20mil (500 micron) thick layers of thermally applied zinc, aluminum, and zinc -15% aluminum (125 micron surface profile) were supplied by two separate contractors and placed into a 55 gallon (210L) drum on a PVC pipe rack. Similar samples of three coat (51 microns) vinyl coat with vinyl zinc rich primer were also provided. One set of the vinyl samples was fully cured. A second set was placed into the water at 24 hour intervals after application to test the effect of not meeting cure requirements.

The AC EIS results on the vinyl coatings were surprising in how fast they exhibited total coating failure. Fully cured samples failed at a slower rate than freshly painted samples but they failed. Failure was indicated in the EIS tests by a drop of several orders of magnitude in measured impedance of the coating. This is evidence for moisture saturation of the coating. The vinyl zinc primer also failed to provide any protection potential. These observations raise serious concern for all of the coated steel structures using these vinyl coating systems.

Thermal spray coatings exhibited no attack or degradation during the tests. Corrosion rates were low thus confirming published work. Both the zinc and zinc-15 aluminum coatings provided good protection potentials to the steel substrate while the aluminum coating was marginal in this regard.

Separate corrosion tests on bare steel in the intermittently stirred, muddy, aerated river water produced very high corrosion rates. Tests on clear river water revealed lower corrosion rates. Prior work suggests that this was the result of iron bacteria.

These results show that thermally sprayed zinc and zinc-15 aluminum are attractive candidates for replacing the current vinyl coatings. Thermal sprayed coatings have no VOC's and are reported to yield at least three times the service life of vinyl coatings. Test results suggest that the vinyl coatings may not provide near the protection expected from them in locks/waters with heavy traffic and water turbulence. Replacing the vinyl coatings with thermal spray coatings has the potential to save enormous amounts of money because shutting a lock down for recoating shuts down the navigable river in many cases. Increasing recoat time by at least three times saves maintenance costs and lost revenue from river traffic.

The electrochemical techniques used for this work can be used in most applications where corrosion is a problem. They have been successfully used in cooling water systems, chemical processes, and other coating studies. They provide the forensic engineers with the ability to rapidly acquire corrosion and/or coating behavior data under the actual conditions of interest.

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**Corrosion, Corrosion Tests, Infrastructure**

## **C20 The Application of Scanning Electron Microscopy and Energy Dispersive Spectroscopy for Forensic and Failure Investigations**

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After attending this presentation, attendees will have a better understanding of the use and value of using SEM with EDS in investigating component and industrial and chemical process failures.

This presentation will impact the forensic science community by providing examples of approaches to use in failure investigations in a timely manner.

Scanning Electron Microscopy (SEM) with associated Energy Dispersive Spectroscopy (EDS) has been used extensively for industrial forensic applications at Materials and Chemistry Laboratory, Inc. (MCLinc), particularly to evaluate causes of failure in manufacturing and chemical processes. Failure investigations are typically initiated with a client interview to establish alloy type of the failed component, operating conditions, process excursions, and other historical data. The region of failure is then visually inspected. Proper collection and conditioning of samples for shipping has been found to be essential for preserving sample integrity prior to analysis. After visual inspection and photography, coupon sections are mapped out and separated to allow for comparison of sections near the region of failure and those isolated from the region of failure. The coupons are then embedded in epoxy in a 1.25 inch diameter mold, cured, and polished to 0.3µm. The mounts are then examined by Reflective Optical Microscopy and SEM. The polished cross sections are typically etched during the examinations to expose the grain structure of the alloys. Optical Microscopy is conducted to determine the general grain structure and/or inclusions while Electron Microscopy is used to examine fine details at higher magnification and obtain general chemistry and chemistry of features and/or precipitates within the grain boundaries. The grain structure observed in the failure region provides insight into the failure mechanism. Observed failures include thermal granular growth, intergranular attack, and general corrosive attack.

A recent investigation included the examination of a 3/8 inch Inconel 600 vessel which failed in hydrogen fluoride service. The results of the investigation demonstrated that sulfidation was the cause of the failure. It

was determined that a process variable caused the sulfur concentration in the feed stream to be higher than typical. Moreover, a mechanical issue involving a defective heater element caused elevated temperature at the failure region, hence, accelerating the rate of sulfidation. The low oxygen content of the process also contributed to the accelerated sulfidation. Photomicrographs and EDS spectra revealed a breakdown of the Cr<sub>2</sub>O<sub>3</sub> layer with the resulting transport of sulfur within the bulk alloy. Ni-S, Cr-S, and Cr depleted regions were also observed. This observation was consistent with published literature on sulfidation. Results of this investigation provided a mechanism for failure that is consistent with the operating conditions.

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**Failure Investigations, SEM-EDS, Chemical Processes**

## **C21 In-Situ Hardening of a Steel Tank: Carbon Diffusion Over 35 Years at Ambient Temperatures**

*Anastasia Micheals, MS\*, Forensic Materials Consulting, 1784 Sanchez Street, San Francisco, CA 94131-2741*

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After attending this presentation, attendees will understand the importance of diffusion in materials, particularly in surface hardening of steel.

This presentation will impact the forensic science community by presenting a case study where low temperature diffusion has a large impact on the properties of steel.

Municipal water storage tanks are reservoirs that ensure both adequate supply and pressure to customers during low and high usage periods. Typical materials used in ground-located water tanks are environmentally robust materials such as fiberglass, plastic, or stainless steel. Corrosion-sensitive carbon steel is also used. Modern methods to protect tanks from corrosion include silica glass coatings, applied to panels, processed at high temperature and then assembled. Historically, the choice for protecting carbon steel tanks from the combination of water and air that cause corrosion was a coating of coal tar pitch epoxy. Coal tar pitch is the semi-solid that remains after distillation of the coal tar by-product of coal gasification. Fairly inexpensive, it is water-resistant and long lasting. However, being a fossil fuel derivative, it is composed of a number of long chain hydrocarbons, phenols, and polycyclic aromatic hydrocarbons (PAH). Exact composition depends on the coal source material. Various PAHs are known carcinogens, mutagens and teratogens, so protective equipment must be used when applying or removing coal tar pitch.

During recoating of the interior of a 35-year old, 1.2 MG municipal water tank, the interior coal tar pitch epoxy coating was being removed by sandblasting; the goal was a white metal, or SSPC-SP 5, finish, so that a new coating could be applied to clean metal. Initial sandblasting was performed with silica, which resulted in unexpectedly low removal rates. Time was of the essence, as the tank needed to be on-line within a specified timeline, so the abrasive was switched to steel abrasive, a more expensive material. The blasting still took much longer than scheduled and anticipated.

A three inch diameter, 7/32 inches thick disk was cut from the roof of the tank. This disk was cut in half; **Piece A** was not cleaned in any way, and **Piece B** was sweep blasted with the steel abrasive. Optical microscopy, scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) was performed on both **A** and **B**.

Under the optical microscope, a cracked, black coal tar pitch epoxy coating was observed on un-cleaned **A** (Figure 1). In some areas, the coating had spalled away, and, in the presence of both air and water, red iron oxide scale formed. **A** was then examined by scanning electron microscopy, in a region containing both coating and corrosion (Figure 2). The corrosion has an appearance typical of iron oxide (Figure 3). The coal tar coating (Figure 4) shows some surface deposits. It also shows a typical surface crack in the coating.

Elemental analysis of *A* by EDS was unremarkable. Carbon, iron, and oxygen were the major elements present. Minor elements were silicon, aluminum, potassium, magnesium, calcium, sulfur and chlorine. The major elements are from the coal tar coating and steel tank material; the minor elements are likely deposits from the well water held in the tank.

Optical microscopy of *B* showed that the sweep blasting, while incomplete, had removed most of the coal tar coating (Figure 4). Residual red iron oxide was observed, as well as some black regions that were consistent in shape and density with the coal tar coating seen on the uncleaned piece. *B* was examined in the SEM. The surface was somewhat rough, indicating incomplete sweep blasting. EDS of the area again revealed no unexpected elements.

Spot elemental analysis was performed on an area of sweep blasted *B*, where a cross-section from the pitch coating to the bare steel was visible (Figure 6). Elemental measurements were made at three locations; pitch coating, mid coating, and bare steel. The analysis results are summarized in Table 1. The additional elements detected, likely deposits from groundwater, are silicon, calcium, aluminum, magnesium, potassium, sulfur, and chlorine.

Element	Atomic %		
	Spot 1	Spot 2	Spot 3
Carbon	50.1	33.2	1.7
Oxygen	34.9	31.8	22.6
Iron	0.4	30.1	65.3
Additional elements	14.6	4.9	10.4

Table 1. Concentration of elements found at three different spots on a cross-section of *Piece B*.

At Spot 1, the top of the coating, carbon from the coal tar pitch epoxy was detected. Hydrogen is also likely present, although the EDS technique cannot detect it. At Spot 2, a significant amount of iron was detected, in addition to carbon and oxygen. The ratio of iron to oxygen is approximately 1:1, which suggests iron oxide of the form FeO, a black oxide. The presence of carbon suggests carbon diffusion into the iron oxide. At Spot 3, the primary element present is iron. The oxygen present may be due to debris from the sweep blasting process. A very small amount of carbon is present as well.

*Piece A* is representative of the present condition of this 35-year-old tank and coal tar coating. Its condition is reasonable considering the age and environmental history of the system. With time and temperature fluctuations, volatile organic compounds evaporated from the pitch, causing it to lose elasticity. Cracks in the coating were then initiated and grew in the stiffened coating, as the pitch shrunk due to the loss of the volatile material, and as the steel substrate expanded or contracted with changing ambient temperature. Water then penetrated through the cracks to the steel, and formed ferrous oxide (FeO), the iron corrosion that forms in limited-oxygen environments. The corrosion propagated underneath the coating (Figure 7); the corrosion continued underneath the coating, allowing pieces to spall off. The iron, then exposed to oxygen from the air, converted from black FeO to red Fe<sub>2</sub>O<sub>3</sub>.

*Piece B* reveals a second process occurring, that of carbon diffusion. Carbon will readily diffuse into iron, at a rate dependent on temperature. The carbon gradient discovered in *Piece B* indicates that the carbon diffusion process has been taking place over the 35-year lifetime of the tank. Although the maximum ambient temperature was relatively low (~100° F), the long time allowed significant carbon diffusion to occur, causing surface hardening of the low carbon steel. This diffusion rate was verified by Fick's Second Law.

The goal of sandblasting was to remove the pitch layer, and the top surface of the steel, leaving a clean steel surface for recoating. The increase in hardness, from that of the expected low carbon steel material, to a high carbon steel of around 2.5%, resulted in exceptionally low material removal rates by sand blasting.

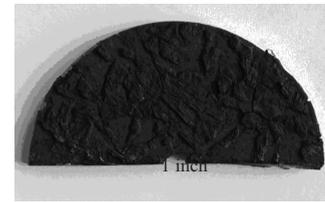


Figure 1. Water tank specimen, *Piece A*, no cleaning.

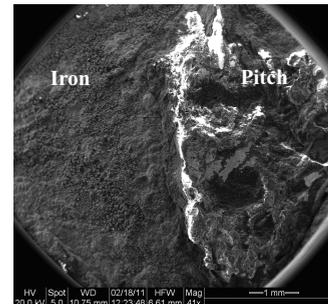


Figure 2. Electron micrograph of *A*, in a region with both red iron oxide corrosion and pitch epoxy coating.

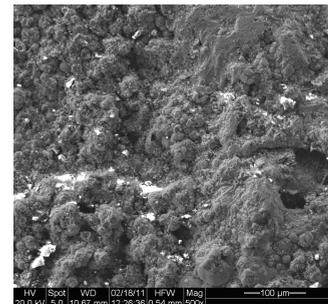


Figure 3. Electron micrograph of corrosion from *A*. The bubbles are typical of rust; EDS analysis confirmed the composition of the rust to be Fe<sub>2</sub>O<sub>3</sub>.

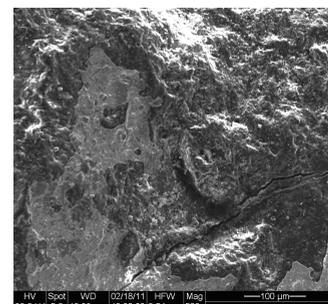


Figure 4. Electron micrograph of the pitch epoxy coating from *A*. The light areas are surface deposits. The crack is typical of those found in the coating.

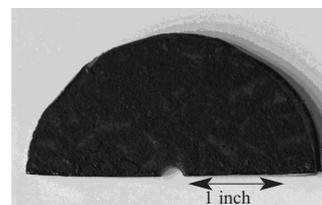


Figure 4. Water tank specimen, *Piece B*, sweep blasted.

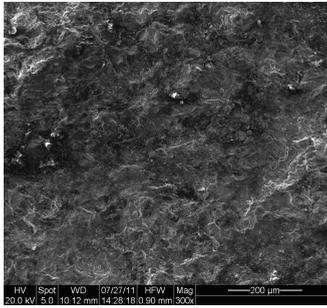


Figure 5. Electron micrograph of *B*. The rough surface is a result of sweep blasting with steel abrasive, which did not penetrate to the steel substrate.

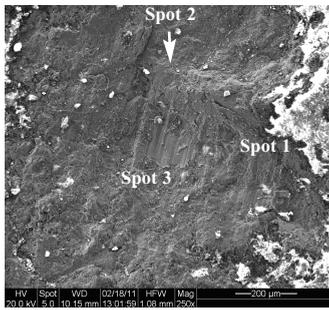


Figure 6. Area of EDS analysis.

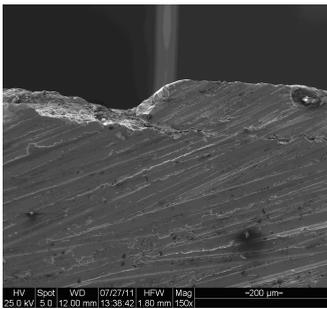


Figure 7. This cross section shows the propagation of corrosion beneath the coating.

#### Diffusion of Carbon in Steel, Oxidation, Corrosion

## C22 Review and Analysis of a Bowstring Wood Truss Roof Collapse During a Snow Event

*Daniel M. Honig, PE\*, Structures Consulting Engineers, PO Box 125, Swarthmore, PA 19081*

After attending this presentation, attendees will be introduced to a case where roof trusses in a renovated building collapsed at the time of an ice and snow event. The investigation into the cause of the collapse and the recommendation for remediation will be described.

This presentation will impact the forensic science community by illustrating the necessity of a proper and reasonable structural engineer review and evaluation of existing building conditions prior to a renovation project.

Distress often develops in individual truss members during normal load service because of the cumulative damage effects of the long-term loadings over the structure's life. Overstress issues due to inadequate allowances for accumulations of rain or drifting snow need to be reviewed and properly addressed when investigating bowstring roof trusses.

A building was affected by an ice and snow event, which occurred during a recent winter season. During the 1998/99 timeframe, a single story prior use supermarket was renovated, upgraded, and enlarged as a medical and professional office building. The construction type of the original

structure consisted of brick masonry bearing walls supporting heavy timber bowstring profile wood trusses. These trusses supported conventional wood framing bearing on the truss top chords, with ceiling joists supported by the truss bottom chords.

During the course of the significant winter precipitation event, three of the trusses located towards the middle of the original building section structurally failed and began to collapse. These roof trusses, which remained in place, were found to have had long term deflections, thereby causing some of the bottom chord split ring connectors to develop significant gaps between the connectors and the bottom chord elements.

Significant additional structural loadings were placed on the existing truss system as a result of the building renovation. A new roof profile created new snow drift zones adjacent to the intersection of a new rear two-story area and the existing bowstring truss roof as well as at new valleys on either side of a new semicircular entrance. Given the uniform profile of the bowstring trusses prior to the alterations, the existing bowstring wood trusses were not designed to support the additional loading created at the new snow drift zones.

The minimum due diligence of the design team, including the architect and structural engineer of record, during the modification of the existing building profile included taking into account any new additional loads. This required the design team to complete a detailed structural review of the existing building system. This structural review was not properly performed during the 1998/99 renovation work, and as a result additional loads and snow drift profiles were added to the existing roof without any structural reinforcement or alteration.

Based on computer analysis of a typical truss loading after the renovation project, some truss members had significant overloading stresses that exceeded those allowable under the uniform snow load, unbalanced snow load, and drifted snow load cases. Based on the analysis, the trusses were found to be significantly overloaded under the maximum anticipated service loads, as defined by the 2000 edition of the International Building Code and the ASCE-7 Subcode. In addition, top chord shear stresses were found to exceed allowable stresses, and the four longest diagonal struts were found to be too slender for the compressive forces.

Based upon field observations, evaluation, and follow-up structural analysis, it was confirmed that long term wood deterioration, shrinkage, and movement of the southern bottom chord ends must have occurred, thereby permitting the southern wall to shift outward and the roof trusses to move downward under the additional loading caused by uneven snow drifting. The trusses required strengthening in place by increasing the tensile capacity of the bottom chords, the shear capacity of the top chords at panel points, and the area of the slender diagonals.

Given that a proper structural survey and inventory was not completed prior to the building alterations, significant areas of concern during the renovation design phase were neglected. These renovation design omissions led to the collapse during the winter storm events.

A proper and complete structural evaluation of the bowstring wood truss framing system prior to the structural and building renovation program would have determined the scope of the structural deficiencies, and the overstress of the bowstring truss members then could have been addressed from a structural engineering standpoint. Given that far more severe storm events occurred prior to the renovation and collapse, it is reasonable to conclude that the renovation was made without the proper and expected structural engineering review and evaluation of the existing bowstring wood trusses.

#### Bowstring Wood Trusses, Snow Load, Structural Analysis

## C23 Hazards of Volcanic Eruption to Aviation

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The goal of this presentation is to provide information on both the chemical and physical properties of the ash plume from Eyjafjoll volcano. Information on the potential and actual hazards of ingesting volcanic particles into modern jet-turbine engines will be considered.

This presentation will impact the forensic science community by presenting recently determined scientific data on the chemical and physical nature of volcanic ash. That information will be valuable in litigating mechanical failures in aircraft jet engines.

Commercial aviation has been striving in recent years to improve the efficiency of their jet-turbine engines, especially after fuel costs have continued to sky-rocket. In order to offset the high cost of fuel, aircraft engine manufacturers have been striving to produce jet turbines that are more thermodynamically efficient.

The efficiency of a jet-turbine engine equals  $T_1 - T_2 / T_1$  where  $T_1$  and  $T_2$  are the absolute temperatures in the hot chamber and cold chamber respectively. The upper temperature limit in the combustion gas is dictated by the maximum temperature that the high-temperature turbine blades were designed to operate in. If the high temperature turbine blades are exposed to higher than the maximum design temperature, the blade alloy will be subjected to an "overtemp" condition, in which incipient melting of grain boundary metal will occur, and carbon will be solutioned in the metal grains. Incipient melting in superalloys usually starts at about 100°C below the alloy's actual melting point.

In order to obtain a more efficient engine performance, the blades in the first two high-pressure turbine discs are fabricated with hollow cores and numerous small air holes, approximately 1mm in diameter. Those small holes can direct a layer of highly pressurized cold air across the airfoil of those blades and thus permit the turbine blades to safely operate in combustion gas at an appreciably higher temperature. Unfortunately, if those engines are operated in dust-ridden conditions, such as sand storms or high concentrations of volcanic ash, those small air holes in the blade's airfoil can easily become blocked. Should blockage occur, it will then cause the operating temperature of HPT-1 and HPT-2 turbine blades to rapidly rise to an unsafe operating level.

Particulate matter, that is larger than that which can either pass through or block air holes in the turbine blades, can do appreciable damage to metal parts in jet turbine engines and lead to severe structural damage.

High-temperature oxidation of superalloys in "clean" combustion gas will produce mixed oxides of their major metal components, which are usually nickel and chromium. When the combustion gas carries extraneous gas and/or low melting point particulate material to the heated surface, a deposit-modified oxidation process will occur: called "hot corrosion."

To characterize the ash plume from the Eyjafjoll volcano, which erupted in Southern Iceland from April 14, 2010 to May 23, 2010, a sampling of volcanic ash was collected on April 30, 2010 from Thorvaldseyri Farm, which is located approximately 40 kilometers west of the city Vik. The actual eruption site was approximately 4.5 kilometers north of the farm.

Approximately 29 grams of volcanic ash was sieved and analyzed using a combination of polarized light microscopy (PLM), scanning electron microscopy-energy dispersive x-ray spectrometry (SEM-EDS) and transmission electron microscopy coupled with select area electron diffraction an energy dispersive x-ray spectrometry (AEM). PLM examination of the <63µm fraction reveals abundant volcanic glass fragments with mineral inclusions. The glass ranges from frothy colorless pumice particles to brown fragments, some exhibiting vesicular texture. Many fragments are magnetic, apparently due to the presence of microscopic inclusions of magnetite; ilmenite and/or titanomagnetite are

also indicated by SEM-EDS analysis of inclusions in the glass. Plagioclase, olivine and clinopyroxene are present as individual grains and as inclusions within the glass. SEM and AEM analysis were used to support the PLM analysis.

### Volcanic Ash, Jet Engine, Microscopy

## C24 BioMedical Engineering Methodological Protocol for Testing Real World Helmet Performance

*Laura L. Liptai, PhD\*, BioMedical Forensics, 1660 School Street, #103, Moraga, CA 94556*

After attending this presentation, attendees will understand how to objectively investigate helmet impacts and utilize proven protocols and techniques to test motorcycle helmets beyond standardized testing. The objective is to quantitatively compare and analyze impact performance of three types of DOT approved helmets and three types of Non-DOT approved helmets in real world impacts.

This presentation will impact the forensic science community by quantifying the dynamic differences between helmet choices in real world impacts beyond standardized testing methods. By increasing the knowledge base of engineers and scientists, helmets can be better designed to be most effective for the intended use.

Motorcyclists suffer serious trauma to the head more often than automotive occupants, primarily due to contact with non-yielding surfaces, such as asphalt and concrete, and/or direct impact from other motorized vehicles. A motorcycle helmet is the principal protection to the head from an impact once the occupant is ejected. In the United States, consumer helmets are initially divided into two distinct categories: DOT (Department of Transportation) approved and non-DOT approved (sometimes referred to as novelty helmets). For a helmet to be approved by the Department of Transportation it must pass a series of tests administered by the Department of Transportation, and once done so, the helmet will display a "DOT" sticker. DOT approved helmets must meet a series of performance testing requirements including impact, penetration, and retention tests. In terms of impact performance testing, the motorcycle helmet is dropped in a guided freefall at a set range of velocities onto a hemispherical or flat steel anvil, where data collected must not exceed critical accelerations of 400 g's maximum, 200 g's for two milliseconds, and 150 g's for four milliseconds. (Federal Motor Vehicle Safety Standard (FMVSS) No. 218). The testing methods used in this research project differ from the above because the helmet performance testing is conducted at higher impact velocities, different principal directions of force, and impact onto real world surfaces compared to the standardized testing.

To quantitatively compare the impact performance of the motorcycle helmets, including the energy dissipation characteristics, the forensic engineering method was utilized. The forensic engineering method is composed of five steps: (1) occurrence of precedent event; (2) define forensic engineering problem; (3) collect data; (4) analyze data; and, (5) develop and evaluate findings. The dynamics of the real world incident is studied thoroughly using longstanding forensic methods. The remaining four steps are then iteratively evaluated. The engineering problem definition in this instance is to quantitatively compare the impact performance, including the energy dissipation characteristics, of a sample of DOT approved helmets and a sample of non-DOT helmets at real world energy levels.

Six helmet types were tested; three types of DOT approved helmets and three types of non-DOT helmets. The impact tests were conducted using an inverted pendulum system with a helmeted Hybrid-III head-form. When dropped, the helmets contacted the exemplar surface (helmet performance can potentially be altered by the properties of the contact surface). Accelerometer data was acquired with a data acquisition system. The data from the sensors was filtered in accordance with SAE standard J211.

Four exemplars were tested for each of the six helmet types at two different velocities, yielding a total of 48 impact tests. For each test, a new helmet was fitted to the Hybrid III head-form and the chinstrap of the helmet was secured per the manufacturer's instructions. The real world principal direction of force can be utilized in the experimental design to replicate the subject head impact region. To initiate each test, an inverted pendulum protocol with Hybrid III head-form and helmet was raised to replicate the subject impact velocity ranges. Helmet performance was revealed.

The results quantify the performance by category and model, and proved to be consistent with the principals of rotational and translational energy, collinear contact of two bodies, and impact deformation. The higher delta t resulted in substantially lower peak accelerations for the DOT approved helmets when compared to the non-DOT approved helmets.

#### **Motorcycle, Helmet, Head**

## **C25 How Far Has the Federal Government Advanced Toward Meeting the Recommendations of the National Academy of Sciences (NAS) Report and How Does the United States Effort to Strengthen Forensic Science Compare With That in Other Countries?**

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After attending this presentation, attendees will be updated regarding Federal efforts in meeting recommendations of the National Academy of Sciences Report.

This presentation will impact the forensic science community by alerting listeners to the progress of efforts to upgrade forensic science in the United States.

Most AAFS members, regardless of our disciplines, will be affected either directly or indirectly by the reverberations flowing from the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*. A number of federal entities are working on various initiatives linked to the NAS Report. Among these are: The White House Subcommittee on Forensic Science, the U.S. Senate Judiciary Committee, and the National Institute of Standards and

Technology (NIST). These entities will be shaping the future of forensic science, as it is practiced in the United States.

Today's session has been structured in an effort to answer a variety of questions. Among these questions are:

- Who are the participants, what have they done, and what are they going to do?
- What are the relationships between the participants?
- What constructive role can we, AAFS members play in this process?

Presenting in this session will be distinguished speakers from the AAFS, the Senate Judiciary Committee, the White House Subcommittee on Forensic Science, the NIST, the NAS, and the Innocence Project. Descriptions of the experiences of the international community in this area will also be presented. Speakers will describe the forensic science activities in Canada, the United Kingdom, and Australia. There are several issues of keen interest to all:

- How should forensic practitioners be certified? Should they be tested, and if so how and by whom? Should they be grandfathered if they have already been certified by an earlier regime?
- What methodology should be used to accredit forensic laboratories? Who should do the accreditation, the federal government, professional organizations, the Judiciary, or the private sector?
- Will any of the money allocated to the above processes make its way outside the federal system to fund the private sector to help with these activities?

Underlying the above issues is the even more important question of validity. The validity of the procedures currently used in many forensic "practices" (methods) has been called into question. Among these methods are DNA mixture analysis, fingerprint identification, and the analysis of hair, bite marks, fibers, shoeprints, soil, and tool marks. Shaken baby syndrome methods and diagnoses are also being questioned. What procedures will be employed to validate these methods? Will this be done by the Government or outside parties? No matter who does the validation work, how can we be sure that they will be done in a scientifically rigorous manner? In this session one speaker will discuss his disagreement with how many of the above techniques have been applied in the past.

Judge Domitrovich will describe how judges can improve their gatekeeping roles regarding the admissibility of relevant and reliable scientific evidence, as well as what resources are available to educate and train judges?

These issues will suffuse today's proceedings. Each talk will be followed by a 10 minute question & answer period.

AAFS President Dr. Ubelaker will begin the session by describing the respective responsibilities assumed by the disparate federal entities that are addressing the 2009 National Academy of Sciences Report mandated by Congress: *Strengthening Forensic Science in the United States: A Path Forward* (NAS Report). As AAFS spokesperson and as a member himself of the White House Subcommittee on Forensic Sciences, President Ubelaker has had a front-row seat as both an observer and participant in the actions taken by these entities in addressing the NAS Report. His presentation to the Joint Session will summarize his views of what has been accomplished, where he thinks we are headed, and what the AAFS role should be in strengthening forensic science in the United States.

There can be no better source of information regarding the activities of the White House Subcommittee on Forensic Science and of its interactions with other federal agencies than Mr. Melson and Mr. Stolorow. They have been involved in creating within the Executive Branch of the federal government a structure that in some regards tracks the structure that the Leahy bill seeks to create. Of particular interest will be their observations regarding the comparison and contrast of the two approaches, differences that in part reflect the different natures of the Executive and Legislative Branches of the federal government. In addition to these comments, they are expected to also present their own views on validating

practitioners, accrediting laboratories, and determining the validation of forensic theories and techniques. Mr. Melson is the Forensic Science Advisor at the Department of Justice Office of Legal Policy. Mr. Stolorow is Director of the NIST Office of Law Enforcement Standards.

Dr. Gallagher is the Director of the one of the most important scientific bodies within the federal government: the National Institute of Standards and Technology (NIST). Reflecting this, and the bill's intent to emphasize science, the Leahy bill calls on NIST resources and personnel for a major part of the new forensic structure it envisions. It will be of great interest to learn how he expects this expansion to take place and whether he sees NIST as awarding outside grants for forensic research.

Mr. Bookbinder and Mr. Park, senior staff members of the Senate Judiciary Committee chaired by Senator Patrick Leahy. Senator Leahy began working in 2009 on a legislative approach to addressing the issues in forensic science highlighted in the NAS Report. In assisting the Senator to approach this task, Mr. Bookbinder and Mr. Park sought to confer with all segments of the forensic community and, over an 18-month period, sat down, separately and in concert, with forensic scientists, crime lab directors, defense attorneys, law enforcement officials, Innocence Project members, and others. AAFS members were well represented at these gatherings. Through this process and guided by political realities, they developed the bill introduced in January 2011 by Sen. Leahy under the caption *Forensic Science Reform Act of 2011*. Regardless of whether this bill in some form becomes law by the time of the 2012 AAFS Annual Meeting, an account of considerable interest and help to anyone hoping for legislative solutions to the needs of the forensic community will be provided.

Since its creation in 1999, Dr. Mazza has been the Director of the NAS Committee on Science, Technology, and Law, the body that created and oversaw the ad hoc Committee responsible for the NAS Report. Associated with the NAS since 1995, she also worked during 1999-2000 with the White House Office of Science and Technology Policy. With this broad background in science policy and the specific experience involved in taking part in the preparation of the NAS Report, she is in an excellent position to discuss the generation of that report, the history behind it, and the manner in which its findings have been understood and misunderstood, construed and misconstrued.

The key recommendation of the NAS Report is that a National Institute of Forensic Science (NIFS) be created as an independent agency of the federal government. Although the Leahy bill does not go so far as to call for the creation of such an agency, it does envision an Office of Forensic Science within the Department of Justice. Therefore, it should be illuminating to learn the experience of Australia in setting up and operating its National Institute of Forensic Science. Mr. Ross, as one of the creators and presently the Director of the NIFS serving Australia and New Zealand, is an excellent person to describe the problems in creating such an organization, as well as the benefits that it has provided to those it serves. NIFS currently resides within the Australia New Zealand Police Advisory Agency as a Directorate.

Dr. Milroy, a forensic pathologist based in Canada after an extensive career in the United Kingdom, where he was a member of the National Forensic Science Service, has for many years been an active participant in the Evidence-Based Medicine movement. He will address the efforts in the United Kingdom and Canada to police forensic science practitioners and laboratories, including thinking in those countries regarding oversight committees. It is anticipated that he will discuss the recent dismantling of the forensic-practitioner registry developed over many years in the United Kingdom, thereby providing valuable insight to those looking toward the certification of forensic practitioners in other countries.

Mr. Neufeld is co-founder and co-director of the Innocence Project, which, directly or indirectly, has been responsible for the DNA exonerations of more than half of the nearly 300 persons wrongly convicted of homicides and/or sexual assaults in the United States. In most cases these individuals served long sentences for crimes which forensic DNA proved they had not committed. In seventeen cases, they had been sentenced to death. The post conviction DNA exonerations and the revelation that in a substantial

number of the underlying trials, forensic analysts called by the prosecution provided invalid testimony at trial, were important factors in the NAS investigation of forensics and cited in the final 2009 NAS Report. As a consequence, Mr. Neufeld and his associates at the Innocence Project have been expected to play a major role, along with other entities, in the government led collaborative undertaking to enhance forensics. He will detail some of forensics' systemic problems and offer appropriate remediation. As a corollary to this, he will comment on various improvements proposed by Congress and agencies of the Executive Branch.

AAFS Board of Directors member Ms. DesPortes and former AAFS President Dr. Bohan plan to provide a summary of the day's proceedings augmented by their own experiences since the release of the NAS Report. They will advise audience members of the opportunities for involvement in the initiatives described by the other program presenters.

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**Forensic Science, Forensic, NAS**

## **C26 Development of a Scanning Electron Microscopy Screening Method for World Trade Center Dust**

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After attending this presentation, attendees will be knowledgeable about the procedures and methods used to develop a scanning electron microscopy signature screening method for World Trade Center (WTC) dust.

This presentation will impact the forensic science community by providing a detailed description of an environmental forensic challenge, the methodology used to address the problem, and the results of an inter-laboratory study designed to test a new analytical method.

Dust from the September 11, 2001, collapse of the WTC penetrated indoor areas of lower Manhattan. Apartments, offices, and public buildings were contaminated with the debris. In 2005, in response to continuing public health concerns about dust inhalation, including the "World Trade Center Cough," the U.S. Environmental Protection Agency and the U.S. Geological Survey (USGS) were tasked with developing a screening method to detect low-levels of WTC dust in the presence of indoor dusts from other sources.

The development of a WTC dust signature was complicated by the dilution of the dust by urban background dust accumulated over the period of four years. The composition of the analyzed dust was highly variable because of contributions from a number of sources unrelated to the WTC collapse on September 11, such as building damage, construction debris, and other common urban dust sources. In addition, variations in the composition of the WTC dust over the affected area due to exposure to moisture, distance from the WTC site, and elevation were evaluated.

Analysis performed by USGS by scanning electron microscopy with energy dispersive spectrometry (SEM/EDS) determined that the major components of the less-than-150-micrometer fraction of the WTC dust were gypsum/anhydrite, phases compatible with crushed concrete, and man-made vitreous fibers (MMVF), including a calcium-aluminum-silicon glass, soda-lime glass, rock wool, and slag wool. These components were also present in urban dusts not impacted from the event, but in different ratios than dusts from the WTC. Slag wool, specifically, was found to be present in WTC dust at much higher concentrations (20-25 % by area) than in urban background dusts ( $10^{-3}$  to  $10^{-5}$  wt. %). Additionally, the slag wool

identified in the WTC dust was distinguishable from rock wool found in most urban dusts by iron content. The definitions used for slag wool and rock wool were those used by the Thermal Insulation Manufacturer's Association.

A SEM/EDS point counting method was developed to determine the amount of slag wool (fibers/milligram) in collected dusts. The method described a procedure that included splitting, ashing, and sieving the collected dust. From each split, a suspension of the dust in alcohol was prepared and an aliquot was placed on a SEM substrate. Sources of measurement uncertainty were evaluated and the major source of variability was found to be directly related to the small number of slag wool fibers present on the sample stub. Suitable reference materials containing known amounts of characterized WTC dust must be used to obtain accurate results.

A subsequent inter-laboratory evaluation of the method was implemented by eight laboratories analyzing a number of dust samples consisting of background dust and background dust spiked with material affected by the WTC collapse. The inter-laboratory study results illustrated that the method was able to distinguish WTC affected dust at the 5% level by weight from 22 out of 25 background dust samples.

**Scanning Electron Microscopy, World Trade Center Dust, Method Development**

## **C27 Lessons Learned About Spray Fireproofing Systems From the World Trade Center Collapse**

*Roger G. Morse, Barch\*, Morse Zehnter Associates, Rensselaer Technology Park, 165 Jordan Road, Troy, NY 12140*

After attending this presentation, attendees will: (1) understand how to investigate fireproofing systems; (2) will know about the fireproofing failures that contributed to the WTC collapse, standards developed for spray fireproofing; and, (3) will learn about needed changes to building codes and standards for installation of fireproofing materials.

This presentation will impact the forensic science community by improving the understanding of failure modes for spray fireproofing systems.

Over a ten year period, during investigation of the spray fireproofing systems, many spray fireproofing deficiencies were discovered in the World Trade Center (WTC) towers. Failures occurred due to design errors, installation problems, and maintenance issues. Some of the observed failures affected those highly stressed members that failed causing the collapse of the towers including: the connection between the long span joists supporting floors and the outside walls, the long span joists themselves, and interior core columns. It should be remembered that the towers stood despite the damage inflicted by the planes. It was only after the fires started by the planes that burned long enough to weaken the structural steel that the towers fell. This presentation will describe the investigative techniques used and provide photographic documentation of the observed problems. Experiences during application of spray applied materials during construction of the World Trade Center towers, as well as other buildings, led to development of standards regarding the suitability of spray fireproofing materials for use in buildings and their initial installation. Unfortunately, spray fireproofing materials are relatively fragile and become damaged during later construction activities; and then during occupancy of a building. Additional standards are needed to insure the continued utility of fireproofing systems following their initial application. The importance of these issues has been underscored by the WTC collapse.

Mineral fiber spray materials in common use since early in the Twentieth Century as acoustical treatments were made completely from inorganic materials so they were fire-resistant and were also good insulators. In 1917, ASTM developed a full scale fire test, now standard E119, to determine if structural elements would survive a fire, and if so for how long. A vigorous mineral fiber industry, looking for new markets,

applied sprayed mineral fiber acoustical materials to structural steel elements and subjected them to the ASTM E119 fire test. It was discovered that when tested, these materials were sufficiently rugged, temperature resistant, and survived a fire long enough to meet building code requirements. This discovery made high rise construction more efficient and less costly helping to spur the post-war high-rise building revolution into high gear, including the building of the World Trade Center towers.

However, all this was not without problems. Serious application problems with spray fireproofing materials existed at the time of construction of the WTC towers. Sometimes spray mineral fiber materials would fail to stick to the surface of the steel members and simply fall off. This happened to the spray material on the core columns, the columns that bucked in Tower one, where the fire protection fell off in story high sheets exposing the naked steel to fire conditions. Sometimes the spray material would be too thin or have insufficient density to adequately insulate and protect the steel from fire. In the WTC towers, spray fireproofing was found to be either completely missing or too thin to protect critical structural members in many locations, such as the highly stressed ends of the long span joists and their connection at the outside walls, the location where the floors in the fire areas of the towers fell helping to initiate a progressive collapse. In 1977, years after the WTC towers were completed; ASTM developed standard E-736 that set forth tests for adhesion and cohesion, and Standard E-605 to measure thickness and density. Had these standards been in place at the time of the WTC construction it is likely that the observed deficiencies would have never occurred.

In 1980, standards were developed to test the suitability of a spray applied material for use for fire protection of steel; however, the work on standards and regulation governing the use of spray fireproofing materials is not complete. Buildings that pre-date the standards, damage during construction and occupancy still need to be addressed.

**World Trade Center Collapse, Fireproofing, Building codes**

## **C28 A Review of the World Trade Center (WTC) Dust Screening Procedure**

*Richard S. Brown, MS\*, MVA Scientific Consultants, 3300 Breckinridge Boulevard, Suite 400, Duluth, GA 30096-893*

After attending this presentation, attendees will gain insight into the process used by EPA and others to develop a procedure to screen dust samples for the presence of particles unique to the collapse of the World Trade Center after the September 11, 2001 terrorist attack.

This presentation will impact the forensic science community by showing the practical limitations built into the method and based on the instrumentation chosen to carry out the analysis ultimately prevented the method from being adopted by the EPA.

It was evident during a meeting of experts sponsored by the EPA that a substantial amount of time, labor and resources had been invested in the "Dust Screening Procedure" developed by USGS and EPA scientists and staff in their attempt to produce a cost effective analytical procedure to determine the presence of WTC dust particles in settled dust samples that could be followed by a commercial laboratory. During the day and a half "Dust Screening Procedure" evaluation meeting, the purpose of the procedure, the details of the procedure, and discussions about the procedure were presented. This proved to be an excellent way to reveal any potential problems with the procedure and suggest ways to correct them. A set of 32 samples would ultimately be distributed to the eight participating laboratories, (three government and five commercial) for analysis according to the procedure adopted with any modifications. The results would be reported back to EPA for compilation.

Scanning electron microscopy-energy dispersive x-ray spectrometry (SEM-EDS) was the method of choice. The SEM-EDS analysis was manual and labor intensive, using x-ray maps or to perform quantitative analysis of particles dispersed in a field of view. This technique was based

on an approach that could be applied to polished sections but was not appropriate for the analysis of particulate dispersed on a sample stub. Automated scanning electron microscopy, a technique that has been available to the forensic community for over 25 years, was discussed, but the fact that automated SEM-EDS was not generally available to the participating laboratories prevented its incorporation into the method. Polarized light microscopy (PLM), the work-horse of settled dust sample characterization, was not well represented. Many of the participating laboratories also did not have this capability (government labs included) and there was little confidence within the government agencies that PLM would be able to produce verifiable and reproducible data.

Time requirements of the analytical procedures incorporated into the method were such that collecting the x-ray maps (or x-ray images) required over 6.5 hours of instrument time. This did not include the time required for off line processing of collected digital images, manual SEM-EDS measurement and analysis of any glass fibers that may have been present and the time required to generate a report. The allocation of instrument time, analyst time and sample turnaround time were not well considered in the planning stages of the method development. The procedure that was eventually adopted took well over an eight hour work day to complete a single sample analysis by SEM-EDS. Instruments capable of automated analysis using motorized stages and available software were not an option due to the limited availability of such automated instrumentation in the government labs and in many of the commercial laboratories that participated in the study.

Overall, the study was limited by a singular approach to the analysis of a very complex sample. The "Dust Screening Procedure" was the result of existing USGS capabilities and was limited to the analytical capabilities available at USGS and the experience of the analysts with respect to multi-component dust sample analysis. Had the participating laboratories been allowed the opportunity to modify the procedure based on the sample constituents and known and widely accepted analytical methods, a more streamlined and thus cost-effective procedure may have been produced and ultimately accepted by the EPA.

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**WTC Dust, SEM-EDS, Microscopy**

## **C29 Update on Dust Particulate Analysis From the World Trade Center Disaster of September 11, 2001**

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The goal of this presentation is to provide an update 10 years later to the forensic community on the microscopical analysis of dust particles from the World Trade Center Disaster of September 11, 2001.

This presentation will impact the forensic science community by showing how forensic microscopical studies display the differences between WTC dust and other dusts from other sources in indoor environments thereby helping to provide the scientific information necessary for judicial decisions.

After the September 11, 2001 attack on the World Trade Center buildings in New York City dust samples were collected from a variety of locations both inside and outside of buildings. Microscopical analysis of dust samples showed that the dust was composed primarily of construction debris containing glass fibers, plaster and cement particles as well as soot, wood particles, paper, and cotton fibers.<sup>1-3</sup> Similar findings have been published by the U.S. Geological Survey.

Based on a number of samples and a number of different types of analyses, the general composition of the WTC dust was found to be:

- Glass fibers (primarily mineral wool) - 35 – 40 %,
- Gypsum particles - 25 – 30 %,
- Cement/Calcium-containing particles - 10 – 15 %,
- Cellulose (paper, cotton, wood fibers) - 5 – 10 %,

- Combustion Products (soot and char) - 1 – 10 %,
- Crystalline Silica ~ 6 %,
- Asbestos (primarily chrysotile with some amosite and tremolite) - < 1 – 2 %,
- Other Material Classes (paint, metal, vermiculite, glass shards) - <1 % per class.

Elongated particles of calcium/sulfur/silicon were also found. The source of these particles has not been determined. They may have been generated from wallboard gypsum and ceiling tile mineral wool under high temperature conditions.

All the classes of components in the WTC dust have been found in other residential and office dust samples but the population of small particles containing a combination of a high amount of glass fiber, a high amount of construction debris material (plaster/cement) and obvious presence of combustion product particles (both char and soot) serves as a distinguishing characteristic of WTC dust when compared to most typical residential or office dusts. Pieces of asbestos large enough to be seen with the light microscope are also a characteristic of some WTC dust samples because large asbestos particles are not seen in normal building dust samples. With the exception of the elongated calcium/sulfur/silicon particles, all the types of particles in the WTC dusts have been reported as associated with normal dusts. At this time, no single particle type is accepted by the scientific community as a signature particle for WTC dust.

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**WTC, Dust, Microscopy**

## **C30 Luminance as a Metric for Accident Reconstruction and Avoidance Analyses of Headlamp-Only Vehicle/Pedestrian Collisions**

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The goal of this presentation is to present a scientifically-valid luminance-based method for evaluating visibility distance to dark-clad pedestrians under headlamp-only illumination.

This presentation will impact the forensic science community by showing how identification luminance can be used to determine identification distances to pedestrians for the reconstruction and avoidance analyses of many common types of nighttime vehicle/pedestrian collisions.

Photometry is the measurement of quantities associated with light. Two primary quantities of light that can be measured directly are illuminance (measured in footcandles or lux) and luminance (measured in footlamberts or candelas/meter squared). Where illuminance is the density of luminous flux (light) incident on a surface, luminance is the quantity of light reflected or emitted off the surface in a given direction.

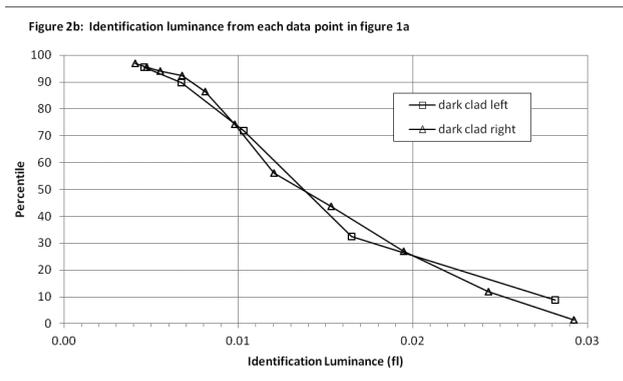
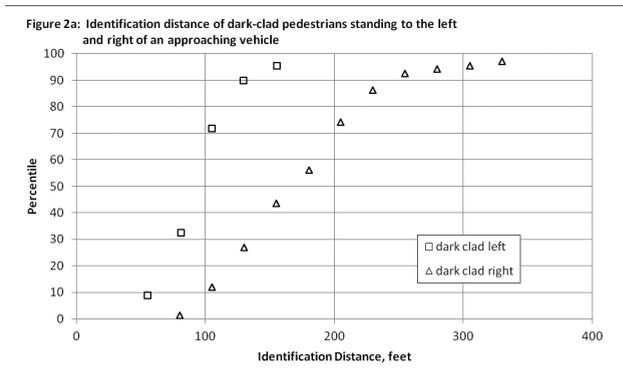
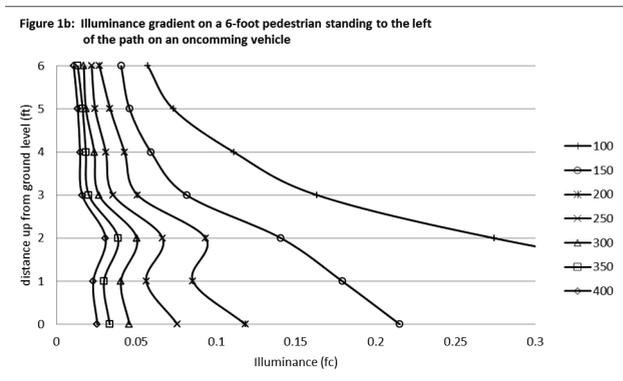
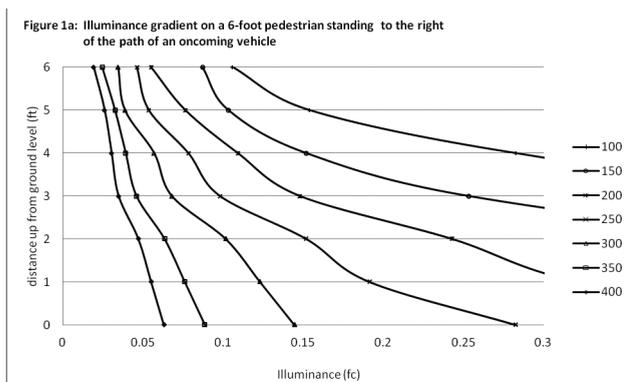
For practical forensic applications, the luminance of an object or pedestrian is a function of the amount of light falling onto the surface, the reflectance of the surface, and depending on the type of reflection, the relative orientation and position of the surface with respect to both the light source and the observer. In the case of headlamp-only automobile/pedestrian collisions, light falling onto the pedestrian is exclusively from the headlamps of the colliding vehicle, measured as illuminance.

The illuminance gradient falling on a pedestrian from an approaching vehicle is shown in figures 1a and 1b for a pedestrian standing to the left and right side of the path of the vehicle, respectively, for distances from the approaching vehicle in 50 foot increments from 400 to 100 feet. The figures are demonstrative of how illuminance gradients vary for pedestrians on the left compared with those on the right, as the vehicle approaches.

Whether the pedestrian is visually detected, recognized and then perceived by an approaching driver depends on the extent to which the pedestrian is more or less luminous than the background upon which he is visualized. To detect an object means to discover or determine its presence. A detected object that is also a hazard, however, may not necessarily be recognized as a hazard. To see the object, simply means to perceive it by the eye or by vision. To perceive it means to become aware of its presence through the senses (here by vision). An object is visible if it is capable of being seen. An object is conspicuous if it attracts or tends to attract the attention of an observer so as to be readily discovered by vision. Conversely, an object is inconspicuous if it is not readily noticeable or discoverable by vision. The term conspicuity, then, is the capacity of an object to stand out in relation to its background so as to be readily discovered by vision.

Figures 2a and 2b are cumulative probability plots of identification distance and identification luminance of a dark-clad pedestrian under U.S. low-beam headlamp illumination for pedestrians standing to the left and right of the vehicles path. How these figures were derived is described elsewhere.<sup>1</sup>

The results of this analysis allow the accident reconstructionist to determine visibility distance from luminance measurements or calculations for many common collisions involving vehicles operating with low beam headlamps and dark-clad pedestrians. A luminance-based analysis permits interpolation and extrapolation to be made for different pedestrian positions in the roadway and different clothing reflectance. Additionally, with a luminance-based analysis driver/observer statistics and expectancy considerations can be quantified.



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**Accident Reconstruction, Visibility, Illumination**

**C31 Threshold Energy for Vehicle Damage in Rear Impact Collisions**

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After attending this presentation, attendees will have a greater understanding of the relationship between speed, energy, and damage in collisions.

This presentation will impact the forensic science community by enabling attendees to more accurately reconstruct and analyze low-speed no-damage collisions.

Over the last two decades, more than a thousand full scale vehicle-to-vehicle collision tests have been performed by a variety of investigators and organizations. In addition, an even greater number of full scale vehicle-to-barrier crash tests have been performed. These tests have provided substantial amounts of data regarding damage and energy absorption in bumper-to-bumper impacts. The data has revealed that vehicles absorb significant amounts of energy before the onset of visible damage.

For the purposes of this paper, cosmetic damage is defined as scuffing or scraping and can be considered to be similar to minor parking lot contact damage. Bumper damage is defined as damage to the bumper plates, brackets, or assemblies, including bending or misalignment provided it is limited to the bumper assembly. Structural damage is defined as damage to the sheet metal or frame of the vehicle outside the bumper assembly area.

The theoretical background for the investigation is based on the principles of conservation of momentum, conservation of energy, and restitution. The relevant energy into a rear impact is the kinetic energy possessed by the vehicles. Based on conservation of energy, the energy into the collision must be accounted for in the energy transferred to the struck vehicle in the form of increased velocity, the energy dissipated in the components of the vehicles and the kinetic energy retained in the striking vehicle. Additional energy is dissipated in the form of noise and heat but these have not been shown to be significant in terms of reconstructing the impact.

Mathematically, the principle can be represented by:

$$KE(V1)_{pre-impact} + KE(V2)_{pre-impact} = KE(V1)_{post-impact} + KE(V2)_{post-impact} + C_{rush} E_{energy}(V1) + C_{rush} E_{energy}(V2)$$

In addition to the conservation of energy, momentum must also be conserved. The relationship of the final distribution of the momentum between the vehicles is a function of the restitution in the collision. In order to determine the total energy absorbed by the vehicles, the equations for conservation of momentum and kinetic energy were solved simultaneously using the appropriate restitution values.

In the course of the research,<sup>1-15</sup> 15 SAE technical papers, IIHS test data, other fully documented unpublished test results and NHTSA research dealing with full scale bumper impacts were reviewed. Of these, 10 papers were considered to possess sufficient data to perform an analysis of the energy absorption in vehicle-to-vehicle collisions. These papers covered 163 vehicle-to-vehicle, front-to-rear collisions. IIHS and NHTSA crash test data were considered in order to compare vehicle-to-vehicle collisions with vehicle-to-barrier collisions.

Due to test constraints, many of the published tests found used the same vehicles repeatedly. Often the tests were performed with human biomechanical considerations and did not continue until vehicle damage occurred. Additionally, many of the vehicles experienced both front and rear impacts. For the purpose of determining energy absorption, frontal and rear impacts on the same vehicle were considered as separate events although total energy absorption values from all impacts for each vehicle were considered. Table 1 provides a summary of the test data reviewed and the results of each set of collisions.

One concern with the data was the over representation of 1980s and 1990s vehicles. To evaluate the effect of using older vehicles, IIHS barrier impacts were researched.<sup>16-20</sup> Comparing older vehicles with newer models revealed that, in general, the cost of repair in the IIHS crash test has been decreasing since 1995, the earliest year reported. Nineteen vehicles were reported for which direct comparisons of crash test damage between a new model and an older model existed. Six examples were found where repair cost increased for the newer model. Only two of these increases were significant; comparisons between the 1997 and 1999 Mazda Protégé and the 1999 and 2004 Nissan Quest. Both increases correlated with significant model changes. If the vehicles suffered similar damage there would be an escalation expected for inflation. The decrease in repair costs can be attributed to improved vehicle body and bumper structure resulting in greater resistance to damage.

A comparison to barrier impacts revealed that damage to vehicles increased significantly when striking an immovable barrier. Table 2 provides a comparison using NHTSA crash tests for 1982 and 1984 Honda

Accords.<sup>21</sup> In this case, a vehicle-to-vehicle collision at 60.1mph. resulted in only 90% of the crush seen in a vehicle-to-barrier collision at 34.8 mph., despite the vehicle-to-vehicle collision having 298% of the kinetic energy of the vehicle-to-barrier collision. The data show that damage to vehicles is much greater when colliding with a barrier rather than another vehicle, even at lower speeds.

Analysis of the data revealed that the onset of damage to the bumper systems beyond minor cosmetic damage typically did not occur until over 10,000 foot pounds of energy had been absorbed. Damage beyond the bumper system typically did not appear until energy values approaching 15,000 foot pounds were dissipated. The highest single impact with no damage dissipated 7,642 foot pounds of energy. These values cannot be considered the upper limit since some vehicles had cumulative energy absorption in excess of 30,000 foot pounds with no damage.

The data revealed that from a reconstruction standpoint, significant amounts of energy are absorbed in vehicle-to-vehicle, bumper-to-bumper collisions before the onset of damage to the vehicles. By using the energy threshold values determined in this paper, it should be possible to determine minimum impact speeds by combining momentum, energy and restitution (MER). MER analysis has been described in the past by some authors,<sup>22-24</sup> but has always been limited by the lack of data regarding the initial threshold energy values.

Bullet Vehicle	Target Vehicle	Total Energy Absorbed (ft-lbs)	Bumper Damage Onset (ft-lbs)	Structural Damage Onset (ft-lbs)
1984 Ford Mustang	1982 Toyota Celica	6,967	No Damage	No Damage
1986 Honda Accord	1988 Mazda 929	15,602	10,340 <sup>1</sup>	15,601 <sup>2</sup>
1988 Mazda 929	1986 Honda Accord	2,021	No Damage	No Damage
1988 Mazda 929	1989 Chevrolet Cavalier	10,646 <sup>3</sup>	No Damage	No Damage
1989 Chevrolet Cavalier	1985 Chevrolet Celebrity	15,352	5,184 <sup>4</sup>	8,651 <sup>5</sup>
1982 Ford Escort	1981 Ford Escort	1,863	No Damage	No Damage
1983 Ford Escort	1981 Ford Escort	28,304	18,568 <sup>6</sup>	No Damage
1987 Volvo 244	1976 Volvo 242	32,002	No Damage	No Damage
1984 GMC C-1500	1986 Dodge 300	7,060	No Damage	No Damage
1984 GMC C-1500	1984 Buick Regal	28,136 <sup>7</sup>	No Damage	No Damage
1988 Ford Festiva	1988 Ford Festiva	535	No Damage	No Damage
1993 Ford Festiva	1988 Ford Festiva	3,449	No Damage	No Damage
1988 Ford Festiva	1983 Ford Festiva	2,058 <sup>8</sup>	No Damage	No Damage
1988 Ford Festiva	1988 Ford Festiva	1,273	No Damage	No Damage
1986 Dodge 800	1984 Ford Wagon	2,951	No Damage	No Damage
1984 GMC 1500	1984 Ford Wagon	12,947	No Damage	No Damage
1984 Ford Wagon	1984 GMC 1500	4,243	No Damage	No Damage
1984 Buick Regal	1986 Dodge 800	4,337 <sup>9</sup>	No Damage	No Damage
1986 Dodge 800	1984 Buick Regal	7,355 <sup>10</sup>	No Damage	No Damage
1984 GMC 1500	1984 Buick Regal	1,127 <sup>11</sup>	No Damage	No Damage
1984 Ford Wagon	1984 GMC 1500	4,988 <sup>12,13</sup>	No Damage	No Damage
1981 Volvo 240DL	1990 Honda Accord	88,023	No Damage	No Damage
1981 Ford Escort	1982 Ford Escort	29,935	No Damage	No Damage

Table 1: Onset of bumper and structural damage for reported collisions

- Damage was to both vehicles
- Damage was to Accord. Total absorbed energy for collisions with the 929 was 17,623 with only damage to bumper assembly.
- Total absorbed energy for collisions with the Mazda 929 was 28,269 ft-lbs with no reported damage.
- Damage was to the Celebrity. Total absorbed energy for collisions with the Cavalier was 10,053 ft-lbs with no reported damage.
- Damage was to the Cavalier. Total absorbed energy for collisions with the Cavalier was 14,520 ft-lbs with small buckle to fender.
- Total absorbed energy for collisions with the 1981 Escort was 20,432 ft-lbs.
- Total absorbed energy for collisions with the GMC C 1500 was 35,916 ft-lbs with no reported damage.
- Each of the four vehicles in this series was involved in two collisions. Total absorbed energy for collisions with the 1993 Festiva was 5,508 ft-lbs with no reported damage.
- Total absorbed energy for collisions with the Dodge was 14,643 ft-lbs with no reported damage and one test omitted.
- Combining both tests results in 11,692 ft-lbs with no reported damage.
- Total absorbed energy for collisions with the Buick Regal was 12,819 ft-lbs with no reported damage and one test omitted.
- Total absorbed energy for collisions with the Ford was 26,027 ft-lbs with no reported damage.
- Total absorbed energy for collisions with the GMC was 24,203 ft-lbs with no reported damage.

Test Type	Closing Speed in m.p.h.	Average Crush in mm	% Average Crush	% Kinetic Energy
VTB	34.8	637	100	100
VTV	60.1	571	90	298
VTV	55.6	567	89	255
VTV	54.9	570	89	249

Table 2: Damage comparison between vehicle-to-barrier (VTB) and vehicle-to-vehicle (VTV) collisions for 1982 and 1984 Honda Accords

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## Collision, Low Speed, No Damage

### C32 Method for Predicting Rear Impact Force Levels Associated with Bumper Override and Sheet Metal Crush

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The goal of this presentation is to provide an accurate analytical reconstruction tool to estimate crash force levels associated with rear-impacts that involve "bumper override" and large "sheet-metal crush damage".

This presentation will impact the forensic science community by providing a simplified means for evaluating "average-peak-G" force levels needed to assess vehicle rear-impact seat performance as related to

occupant loads and injury risk for both front seated adults, and rear seated children located behind, when large amounts of “offset sheet metal override crush” are experienced.

In many rear-impact accidents the front bumper of a large striking vehicle, like a pick-up or tractor-trailer type vehicle, eventually “overrides” the stiffer rear bumper structure of a smaller struck vehicle, like a minivan or sedan, and then induces a significant amount of “less-stiff” “sheet-metal crush” deformation that tends to complicate reconstruction of “average-peak-G” force levels experienced by the struck vehicle. The accuracy of these “average-peak-G” force levels is important in assessing the struck vehicle occupant interactions and performance of safety devices such as “seat systems” and “restraints” needed to hold front occupants securely in place and free from contact impact with rear occupants, like children, or non-yielding structures located behind. Unfortunately, most reconstruction programs and methods rely on high “linear-stiffness” parameters obtained from “moving-rigid-barrier” rear impact tests that simultaneously engage the “stiffer” bumper floor structure and the “less stiff” vehicle body sheet metal structure. The use of such “simultaneously” measured “linear-stiffness” parameters, especially in cases where “bumper override” and larger amounts of offset “sheet-metal” body-crush takes place, tends to “over-estimate” the impacted vehicle “peak force” levels, thus complicating the injury risk assessment of “seat system and restraint” safety performance.

In the current study, both “vehicle-to-vehicle” dynamic crash tests and quasi-static “bumper-override” and offset “sheet-metal” body crush tests were run to delineate and assess the stiffness effects associated with large amounts of offset “sheet-metal” body crush characteristics. The “bumper-override” and offset “sheet-metal” body crush “quasi-static” tests were run on a complete minivan vehicle and a sedan where a “barrier face” simulating the “front of a tractor-trailer vehicle” was laterally offset to overlap only about one-half of the test vehicle and was then slowly pushed into the top edge of the vehicle rear-bumper, causing the vehicle to “squat” on the rear suspension, allowing the “barrier face” to “only engage and crush” the sheet-metal body structure of the test vehicle. The measured forces and deformations from these offset “sheet-metal” body crush tests were used to calculate energy levels that were then compared to energy calculations made using the traditional “linear-stiffness” parameters obtained from “moving-rigid-barrier” rear impact tests. These comparisons indicated that the offset “sheet-metal” body crush energy was actually only about one-half the energy levels obtained from the traditional “linear-stiffness” parameters.

Next, “vehicle-to-vehicle” rear-impact crash tests were run on a popular family minivan and a sedan vehicle where the vehicles were impacted by a 20,000kg tractor-trailer rig traveling at about 40kph and inducing large amounts of offset “sheet-metal” body crush. In addition, “vehicle-to-vehicle” tests were also run on the minivan with impact from a pick-up and a mid-size truck that also induced offset “sheet-metal” body crush. Next, the struck vehicle crush energy from the above tests was analyzed in two parts by using the traditional “linear-stiffness” parameters obtained from “moving-rigid-barrier” rear impact tests to estimate crush energy for the “bumper engaged” portion of the crush, and then the offset “sheet-metal” body crush “energy” was calculated and adjusted as per the quasi-static test results. Both energy contributions were next added together and then used to calculate “fixed barrier” equivalent velocities for the struck vehicles of the crash experiments. The average of the “fixed barrier” velocity was then divided into the maximum measured total crush to get an estimate of crash pulse duration. Finally, a trapezoidal crash pulse shape was assumed with equal 1/3-time increments for “rise,” “dwell,” and “decline” portions of the pulse. The area under the assumed pulse was then equated to the “fixed barrier” velocity to obtain an estimate of the “average-peak-G” force levels. This value was then compared to the measured maximum slope gradient of the experimental crash “speed-change” curve. The values of calculated “average-peak-G” force levels compared within about 10 percent of the measured maximum slope gradient for the tests

examined. The method has application to cases with large amounts of offset “sheet-metal” body crush.

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#### **Rear-Impact, Bumper Override, Average-Peak-G**

### **C33 Landfill Operations and Vehicle Rearward Visibility Hazards: A Case Study**

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After attending this presentation, attendees will learn some of the hazards involved in landfill operations to include those involved with vehicle visibility issues and other hazards created by the lack of adequate procedures and training.

This presentation will impact the forensic science community by presenting a case study of a largely unreported or under reported industrial hazard situation.

An active “landfill face” is perhaps one of the more hazardous work environments in industry wherein workers and industrial vehicles share the same work space. The active landfill face is a work environment whose topography is continually and continuously changing. Mounds of debris and garbage are delivered and dumped by waste hauler trucks on the landfill face or tipping area as directed by the “waste spotter” or just “spotter.” Additionally numerous large noisy earth moving vehicles also under the direction of the spotter are active on the landfill face to both move the debris and to pack it down. The subject track loader which weighs more than 29 tons is a large machine and because of its size will present visibility limitations to the operator especially when travelling in reverse. This is significant given that it is known and foreseeable that these vehicles will travel in reverse approximately 50% of the time during normal operational conditions.

The case study reviewed herein, involves a track loader commonly referred to as a bulldozer working on an active landfill face and travelling in reverse wherein the bulldozer driver was inadequately trained. Additionally, the bulldozer back-up alarm while installed and operating was mounted behind portions of the grill support structure. There was closed circuit TV system provided with the bulldozer to guide the operator while travelling in reverse. The accident occurred while the bulldozer was travelling in reverse over a hillock of debris striking the spotter and amputating both his legs. Although having a back-up alarm its effectiveness was considerably diminished given the loud ambient noise generated by the vehicle’s large diesel engine and the blocked mounting location of the back-up alarm.

Given that the spotter works in an especially hazardous environment it is imperative that any landfill operation provide the spotter with a detailed work description and thorough training. In this case study there were no written procedures in place and no training provided for the spotter. Therefore, there were no procedures or any training in place for the spotter and the bulldozer operator to communicate as to their location and intended movement. Even though the landfill operation had a person identified as the landfill’s safety director, the person so identified admitted that he had no training in safety, that he was not really responsible for safety and that indeed there was no knowledgeable individual in the company responsible for safety at the landfill operation at the time of the accident. This situation is especially egregious when noting that the subject bulldozer was with an optional “waste handler” package, a configuration that was specifically designed for use in sanitary landfills and other waste handling situations. Therefore at the time of sale, the track loader manufacturer knew of the intended use of the bulldozer and that it would be travelling backwards approximately 50% of the time and when travelling in reverse the vehicle operator would have limited visibility of the vehicle’s immediate surroundings. Assessments of the vehicle visibility was made by the manufacturer on the basis of purely humanistic subjective and static driver

evaluations without taking into account the dynamic visibility issues arising with the vehicle travelling in reverse on the crowded and busy active landfill face. The subject bulldozer was not equipped with any of the commercially available in-cab camera systems nor even any outside mirrors to mitigate against the vehicles rearward visibility limitations.

#### Landfill, Visibility, Procedures

### C34 Experimental Model for the Determination of Coefficients Penetrating through the Skin of Bullets from Different Gauges of Short Weapons: A Preliminary Study Partially

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After attending this presentation, attendees will: (1) understand some principles of forensic ballistics; (2) the necessary elements for the application on terminal ballistics; and, (3) its experimental paths.

This presentation will impact the forensic science community by describing the key aspect of scientific research in terminal ballistics on a biological target. These experimental applications will be useful for a better and more reliable reconstruction of violent crimes made with firearms in the future.

Before a bullet can cause significant injury, it must be able to pierce the skin. The penetration of a projectile into the skin is different from other tissues because the drilling process requires a relatively high impact velocity. The knowledge of the minimum speed of penetration of the skin is important data for forensic medicine and forensic ballistics to evaluate the penetration ability of offensive missiles or to determine the operating range of the use of a firearm.

It is important to consider that the diversity of materials and the different behavior of individual bullets, depending on their structure and the speed at impact, do not allow the use of a mathematical model generally, but only empirical formulas. On the one hand, some high-speed projectiles deform easily on impact and, on the other hand, some bullets do not rapidly transfer their energy to the target. The starting point for calculating the penetration of the projectile in most materials is its kinetic energy. The penetration of bullets into iron (armor, etc.) has been the subject of extensive studies in the military. From this research, a formula has been developed by Krupp. The penetration of spruce wood is commonly taken as an indicator of the penetration effectiveness of a bullet. It can be calculated with the formula Weigel.

For the study of bone penetration, the formula used is:

$$P = 0.44 \frac{G (V-60)^2}{C \cdot 100}$$

V = Velocity at impact

G = weight in grams of the projectile

C = mm caliber bullet

The speed limit at which a projectile is able to pierce human skin has been studied for pistol bullets or spherical balls, using the formula of Sellier:

$$V \text{ lim} = 125 \frac{I}{Ds} + 22$$

DS: sectional density of the projectile (G / S)

G: the bullet weight in grams

S: section of the projectile sq. cm

**EXPERIMENTAL STUDY:** A study was performed to check the validity of the Sellier formula and search for a coefficient of penetration for projectiles involving "no Round Nose". The usefulness of the formula Sellier in terminal ballistics, is its ability to determine - with a close approximation - the speed that a bullet loses in crossing a biological target/human. The study was performed to determine exactly how fast a bullet lost speed as it passed through a biological target, to define the effects of terminal ballistics on the target material (interior ballistics), and clarify many aspects of exterior ballistics of the bullet that caused the gunshot wound (distance firing, identification of such ...). The purpose of the study was to identify a factor of one or two pure numbers which, when inserted in the formula of Sellier, which respectively include a factor (125) or addendum (22), may be representative of the particular morphology of the projectile. In fact, the Sellier is only applicable for round nose bullets, ie round toe, and many times there is a case study on the use of bullets of different morphology [Hollow Point (hollow point), wad cutters (flat tip) truncated cone (conical), metal piercings]. This change, which will be performed on that of the two pure numbers that experimentally vary more depending on the specific type used, would be the attempted penetration coefficient, which in fact is closely connected to the shape of the bullet.

With the testing done, the equation can be rewritten in the form of Sellier and solve the two equations X verifying which of the two numbers that we have replaced the X with several more. The number varies more to become the "C pen"

$$\text{Experimental limit } V = x \cdot 1 + 22 \\ Ds$$

or

$$\text{Experimental Limit} = 125 \cdot V \cdot 1 + x \\ Ds$$

#### Verification of the Applicability of the Theoretical Sellier Formula

**MATERIALS AND METHODS:** To make the velocity measurements two ballistic CED M2 chronographs were used which were managed by a microprocessor at 48 MHz with a measuring range from 50 fps to 7000 fps, which can store over 1000 speed measurements.

The two chronographs were placed in tandem with each other by interposing the target on first obtaining the impact speed of the projectile and the second speed output from the target.

The target was made on a young pig slaughtered the same morning of the tests with a skin thickness of 0.2 cm, comparable to the human model.

**CONCLUSIONS:** Although it is believed that the measurements taken in this first trial are not yet sufficient for the determination of the requested drag coefficient, the trend appears to confirm the validity of the results of the first scenario. The balls from the .32 caliber S & W and the .38 caliber Special lost speed in crossing the simulator at much higher levels than theoretical prediction, and that seems congruent with the obvious lower penetration of these projectiles by the morphology of flat and cylindrical tip section similar to the gauge.

However, there were some inconsistencies in the data, especially in the results obtained for the two series of shots in caliber Br 7.65 mm, which could indicate excessive accuracy of this first experimental model used.

The weapons used in this study were from TSN training center of Messina. These weapons had been fired several thousand times and consequently had very worn barrels. Some variability in speeds measured at the mouth of the weapons appear to contradict the noted condition. Hollow point bullets were not tested using the skin simulant.

To continue this work, which looks promising, it will be necessary to refine the experimental model especially in relation to the medium simulating the biological target. Future experiments should include a tissue of known thickness, which is able to simulate the response of human muscle to a ballistic penetration agent.

#### Terminal Ballistics, Biologic Paths, Forensic Pathology

## C35 Comparison of DOT Certified Motorcycle Helmets and Novelty Helmets in Head Drop Tests

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After attending this presentation, attendees will understand the differences in injury reduction performance of motorcycle helmets that are certified to meet Department of Transportation (DOT) requirements and novelty helmets that do not meet these requirements.

This presentation will impact the forensic science community by demonstrating the differences in the injury reducing performance of two types of similarly looking and similarly priced helmets that do not provide similar head impact protection.

Currently in the United States, laws requiring all motorcyclists to wear a helmet are in place in 20 states and the District of Columbia, while 27 states require only some motorcyclists to wear a helmet. Only three states have no motorcycle helmet use law. Most helmet laws require motorcycle riders to use a helmet that meets the DOT Federal Motor Vehicle Safety Standard (FMVSS) 218. FMVSS 218 requires that in a 138.4cm drop test (5.2m/s) onto a hemispherical anvil, and on a 182.9cm drop test (6.0 m/s impact speed) onto a flat anvil, the peak head acceleration shall not exceed 400g. If the peak head acceleration exceeds 200g, the duration at 200g's must be less than 2ms, and if the peak head acceleration exceeds 150g, the duration must be less than 4ms.

Helmets that are similar in form to a motorcycle helmet designed for on-road use, but that are not certified by their manufacturer to meet the requirements of FMVSS 218 are often referred to as "novelty" helmets. According to the National Highway Traffic Safety Administration (NHTSA), the 2006 NOPUS survey, a probability-based observational survey of motorcycle helmet use in the United States, found that 14 percent of motorcycle riders use helmets that do not comply with FMVSS 218. To combat this problem, NHTSA issued a final rule on May 13, 2011 that changes the labeling requirements on helmets, making it more difficult to sell helmets with markings that resemble current DOT labeling; however, novelty helmets remain a safety hazard.

In the current study, the performance of a DOT certified half helmet and a similar looking novelty helmet were evaluated using a drop tower system. Six drop tests were conducted using a novelty helmet and a DOT certified half helmet. The helmets were placed on a Hybrid III dummy head that was instrumented with a tri-axial accelerometer at its approximate static center of gravity. The head was suspended from a drop tower and dropped onto an asphalt test bed. It was suspended such that the head was free to rotate on impact. The drop height was 152.4 cm, to simulate the height of an average-sized rider on a cruiser-type motorcycle. All data was recorded at 10 kHz. Axis orientation and data filters were used in accordance with SAE J211 Recommended Practice. For the novelty drop tests, the peak resultant head accelerations were 451g, 358g, and 473g for the left, right, and top impacts, respectively. The corresponding HIC<sub>36</sub> values for the novelty drop tests were 3677, 2260, and 4201. For the DOT drop tests, the peak resultant head accelerations were 143g, 142g, and 243g, for the left, right and top impacts, respectively. The corresponding HIC<sub>36</sub> values for the DOT drop tests were 739, 595, and 1681.

For this test series, the DOT certified helmet met the DOT criteria described above in all three impacts; however, the novelty did not meet the criteria for any impact. The peak resultant g's from the novelty helmets were 1.9 to 3.2 times higher than those of the DOT certified helmets, depending on head impact orientation. The HIC<sub>36</sub> for the novelty helmets ranged from 2.5 to five times that for the DOT certified tests, again depending on head impact orientation. These results show that the DOT

helmet was at least twice as effective at reducing the potential for head injury when compared to the novelty helmet.

A study by Scher et al., (SAE#2009-01-0248) was similar to the present study with the exception that the head orientation was fixed during impact, so that all head motion was constrained to one direction, similar to FMVSS 218 testing. On average, for the Scher study, the peak resultant g's from novelty helmets were 2.6 times those of DOT certified half helmets, and on average the HIC<sub>15</sub> for novelty helmets was 2.9 times those of DOT certified half helmets. Thus, based on the average data presented by Scher, both studies showed similar reductions in head accelerations and HICs when using a DOT certified helmet rather than a novelty helmet.

The present study appeared to have higher magnitudes for peak acceleration and HIC for all drop tests when compared to the Scher study. However, because only average data was presented in the Scher study, and because the present study was limited to six tests, direct correlation cannot be accomplished. Possible explanations for the discrepancy between the studies include a constrained head versus a freely rotating head and head impact orientation. Further testing and more information regarding the Scher testing are needed to determine the basis for this discrepancy.

**DOT Certified Motorcycle Helmet, Novelty Helmet, Head Drop Test**

## C36 Frontal Crash Buckle Pretensioner Activation Causing Seat Belt Retractor Skip-Lock Failure

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After attending this presentation, attendees will be more informed about a parameter called webbing jerk that can have a detrimental affect on a seat belt retractor's ability to lock as intended in a frontal crash.

This presentation will impact the forensic science community by detailing how the activation of a seat belt buckle pretensioner can cause a retractor skip-lock condition. Case studies will be presented. The presentation will heighten the sense of awareness with regard to understanding seemingly conflicting evidence. On one hand, the seat belt assembly reveals artifacts indicating use in a crash. But, on the other hand, the injuries sustained are not those consistent with a properly seat belted occupant.

**Introduction:** Many modern seat belt assemblies utilize pretensioners to enhance the ability of seat belts to mitigate or prevent injury by removing excess webbing slack and cinching the seat belts on the occupants. By tightening the seat belts, the occupants are more tightly coupled to their vehicle and are able to enjoy the safety benefit of better ride down.

Seat belt pretensioners can be found in the retractor or buckle. When activated, the retractor pretensioner tightens the seat belt by rotating the retractor reel. When the buckle pretensioner is activated, the seat belt is tightened by pulling the buckle and latch plate closer to the inboard anchor.

It has been suspected that certain levels of webbing jerk (rate change of acceleration), upon activation of a buckle pretensioner, can cause retractor to skip-lock. That is, the retractor does not lock before an unintended amount of webbing payout, or does not lock at all. Thus, under certain crash conditions rather than enhancing occupant safety, the buckle pretensioner can induce a skip-lock, thereby negating the safety benefit of seat belt use.

In the past, laboratory tests have demonstrated buckle pretensioner characteristics that produce higher levels of webbing jerk can induce the skip-lock phenomenon. To augment the laboratory results, two examples of skip-lock retractor failure in the field resulting in unrestrained occupant injury patterns to seat belted occupants are presented.

**FMVSS:** Federal Motor Vehicle Safety Standard 209 is the compliance standard for occupant restraint systems. Section S4.3(j)(1)

states an emergency-locking retractor of a type 1 or type 2 seat belt assembly, when tested in accordance with specified procedures, shall lock before the webbing extends 25 mm when the retractor is subjected to an acceleration of 7 m/s<sup>2</sup> (0.7g). However, it has been shown that certain seat belt assemblies, those with buckle pretensioners, fail to perform within the intent of this section, and at worse, will fail to perform as specified in the standard in real world collisions.

**Case 1 Overview:** A southbound Nissan Sentra, while crossing a highway, was broadsided by a westbound Plymouth Neon. After impact, the Plymouth came to rest facing westbound in the eastbound lane with no lights. Minutes later, a 2000 Ford Focus, traveling at about 59 mph, struck the Plymouth head-on. The Ford sustained an average of approximately 16 inches of crush, corresponding to a velocity change of about 30mph with a Principle Direction of Force (PDOF) of 12 o'clock. The driver's seat belt was a type 2 assembly featuring a continuous loop webbing, single emergency locking retractor, and a seat-fixed, end-release pretensioning buckle.

Upon impact the Ford's airbag did not deploy and the driver sustained facial lacerations when his head struck and broke the windshield. Inspection of the Ford's interior showed outward bowing of the windshield with bits of hair and tissue embedded in the fracture lines. The steering wheel assembly was loose due to complete separation of the shear capsules. The instrument panel on either side of the steering column was deformed forward. These artifacts, or witness marks, are generally consistent with the forward translation of an unrestrained driver in a frontal crash of this magnitude.

However, inspection of the driver's seat belt revealed that the driver's buckle pretensioner had activated indicating that it had been buckled. In addition, the plastic coating of the D-ring, the plastic opening in the B-pillar trim plastic, and the latch plate showed physical evidence consistent with the webbing rapidly moving through these components. This physical evidence indicates the driver's seat belt was worn, but the emergency locking retractor did not lock, effectively rendering the driver unrestrained.

Review of NHTSA's ODI database did not reveal any defect investigations or recalls for the front seat belts of the 2000 Ford Focus; however, 80 percent of consumer complaints that were related to seat belt or retractor performance in frontal collisions indicated that the seat belt retractor failed to lock.

**Case 2 Overview:** A three-vehicle collision was initiated when a pickup truck lost control on a rain-soaked highway with a posted speed limit of 70 mph. The pickup truck crossed into the oncoming traffic lane, clipped the left side of a vehicle and then collided broadside with the front of 2000 Cadillac DeVille. The resulting velocity change to the Cadillac was approximately 20 mph with a PDOF of 12 o'clock.

During the second impact, the pickup truck subsequently rode up onto the hood of the Cadillac, pushing the hood rearward toward the windshield. Because the impact was offset to the right front of the Cadillac, the collision resulted in a higher degree of deformation to the front passenger area. The pickup truck may have also engaged the windshield header of the Cadillac, as there was localized intrusion to this region. The Cadillac was equipped with front seat type 2 assemblies that are completely seat integrated, with dual emergency-locking retractors and a pretensioning buckle for the driver and front passenger.

Upon impact the Cadillac's airbags did not deploy and the front passenger sustained serious injuries including multiple facial fractures, a basilar skull fracture, and a comminuted left femur fracture. This injury pattern is consistent with an unrestrained occupant in a frontal impact of this magnitude. Inspection of the Cadillac revealed that the driver and front passenger were in fact seat belted and both their buckle pretensioners activated in response to the crash, but the passenger's emergency locking retractor did not lock, effectively rendering her unrestrained.

**Cadillac Product Safety Recall:** In November 2000, the passenger lap belt retractor did not remain locked during an in-house 30 mph frontal barrier test. A visual inspection of the retractor revealed a fracture of the lock pawl and shaft tooth, artifacts indicating a partial engagement of the lock pawl to the shaft, or retractor skip-lock.

From December 2000 until March 2001, the seat belt supplier statically tested thirty samples. The conclusion of these tests showed that the damage to the retractor noted in November was an effect—not a cause.

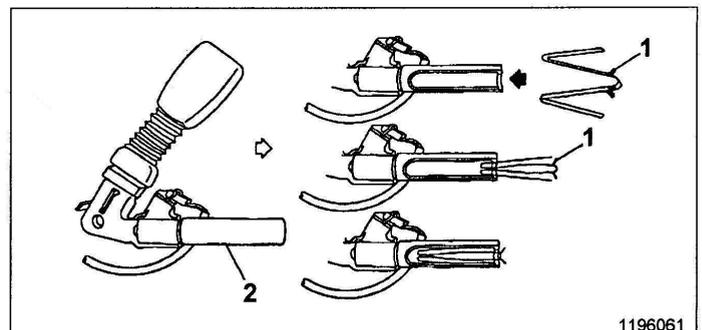
In April 2001, a driver shoulder belt retractor did not remain locked during an in-house 35 mph sled test. Visual inspection revealed a partial engagement of the lock pawl to the shaft.

From April to September 2001, the supplier modified the lock pawl processing to reduce die roll on its tip and improve the area of the lock surface. Static tests were performed to evaluate processing modifications and no significant differences in performance were noted.

In January through April 2002, a sled test series was conducted using seat mounted high-speed cameras to observe the retractor performance and loading profiles, but no suspect retractor behavior was observable. The test protocol was then modified to increase its capability to apply a controlled input to the retractor. It was determined that the velocity of the webbing alone did not duplicate the incident retractor performance. Through data analysis, focus was directed to webbing acceleration onset, i.e., jerk.

A final test series characterized the retractor lock performance over a range of locking pawl pre-positions and buckle pretensioner output (jerk) levels. A trend in retractor lock behavior was determined to be directly related to jerk. As the jerk level increases, the lock behavior transitions from lock on to the design intended tooth to a later lock which may result in a partial engagement of the intended tooth or a lock on a subsequent tooth.

**The fix:** A revised buckle pretensioner with a reduced micro-gas generator was introduced into the production of the 2002 Cadillac DeVille on May 15, 2002. However, over 269,000 vehicles, produced between May 1999 and May 2002, were subject to recall. Service Bulletins were sent to dealers describing a method to repair the defective buckle pretensioners. The procedure calls for a retainer to be inserted into the pretensioner cylinder (as depicted in the illustration). Once the retainer is seated, when the pyrotechnic micro-gas generator activates, the retainer slows the piston's travel within the cylinder, thereby reducing the level of jerk previously measured.



**Conclusion:** This study identified two cases where the physical evidence observed on the lap and shoulder belt indicated the system was worn at the time of collision. However, the resulting occupant kinematics and increased forward excursion lead to unexpected upper torso, head and facial injuries. These field examples of retractor failure augment the laboratory results that demonstrated that a jerk condition induced by buckle pretensioner activation could cause a retractor skip-lock condition that resulted in unwanted webbing payout. A vehicle product safety recall was issued for one of the vehicles.

#### Seat Belt Failure, Skip-Lock, Buckle Pretensioner

## **C37 Rear Impact Vehicle Occupant Ejection and Seat Belt Slack: Comparison of Upright, Reclined, and Collapsed Seats in Field Investigations and Laboratory Tests**

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The goal of this presentation is to inform the engineering, medical and legal community of the phenomenon of seat deflection in foreseeable rear impacts causing vehicle anchored seat belts to predictably fail. This phenomenon has been published in the technical literature since the 1960's but all efforts to enact safety regulations to prevent these failures have been resisted. Alternative designs exist which can prevent these failures.

This presentation will impact the forensic science community by showing how implications of this safety hazard affect all forms of seated transportation, not just ground vehicles.

This study analyzed field and laboratory investigations of rear impacts of passenger cars, utility vehicles, and pickup trucks where belt restrained occupants were partially or completely ejected from their designated upright seating position, and in some instances completely ejected from the vehicle. Hazardous slackening of locked-up vehicle-anchored seat belts was created by collapsing seats, even if seat belt pretensioner systems had been activated by another impact prior to the rear crash. Rear impact crashes analyzed in this study showed severe to fatal injury to front and/or rear seat occupants as a result of seat failure and resultant seat belt failure caused by slackened belts. Changes in velocity ranging from approximately 15 to 30 mph with impact vectors between 160 and 200 degrees. In several crashes there were two belted front seat occupants, with one remaining in a reasonably upright seat and one in a collapsed seat. In all instances the occupant of the collapsed seat was either seriously injured or killed, and the occupant of the upright seat incurred only minor to no injuries. The vehicle anchored seat belt combined with a collapsed seat, was not effective in preventing rearward occupant displacement and/or partial or complete ejection. The slackened belt and poor occupant geometry increased the hazards of submarining under the lap and torso belt. This is consistent with vehicle crash test research published since the 1960's, as well as prior research by Pozzi et. al. conducted and published since the 1970's.

Current research involved further quantifying this belt slackening phenomenon and establishing minimum threshold values for seat movement induced belt slack allowing occupant ejection. Adult male and female human surrogate static testing evaluated vehicle anchored seat belts combined with upright and reclined seats in various vehicles. Approximate seat belt slack was measured for various levels of seat displacement. Approximately 10-12 degrees of static rearward seat movement slackens vehicle anchored seat belts enough to allow rearward ejection of a belted occupant. Slack is exacerbated with dynamic rearward movement of seat belt buckles, seat foam compression, etc. These belt slackening effects were minimized or eliminated on Belt Integrated Seats (BIS). Rearward static load test comparisons using a rigid human upper torso were made between OEM and Modified OEM seats as well as production BIS to establish occupant crash load capacities to the point of allowing occupant ramping out of the seat. Sled and/or crash tests were also conducted to evaluate side-by-side comparisons of various OEM and alternative seat and seat belt designs.

In all rear impact tests in the foregoing velocity range where a weak collapsing seat (600-1,600 lb load capacity) was combined with a vehicle anchored seat belt, the occupant was not effectively restrained or protected. The weakest seats allowed the greatest remaining occupant load contributing to ejection and injury potential. Lap belt loads as the dummy

was moving rearward were negligible, and peak vehicle anchored belt loads always occurred on rebound, long after the occupant struck vehicle rear interior structures or a rear seat occupant. The poorest belt performance and greatest slack occurred with vehicle-anchored lap/torso belts that utilized pass-through latch plates. In all tests where a seat remained reasonably upright, the belt remained in significantly more effective contact with the occupant pelvis and upper torso to provide restraint as intended. BIS are significantly stronger, so they resist rearward deformation. BIS upper torso restraints move with the seat so there is no belt slack generated, and proper occupant-to-belt geometry is maintained far better under the same collision conditions than in collapsing seats and vehicle anchored belts.

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**Crashworthiness, Seatbelt, Seat**

## **C38 Using Surface Volta Potential Measurements to Visualize Fingerprints on Metals**

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After attending this presentation, attendees will understand how Volta potential measurements are made, leading to the visualization of latent fingerprints on metals. The advantages of this new method will be discussed together with the strategies to further improve the clarity of the fingerprints using Vacuum Metal Deposition (VMD).

This presentation will impact the forensic science community by revealing how a non-contact vibrating capacitor technique can visualize fingerprints on metals without the use of any chemicals, without damaging DNA evidence and with no surface contact.

The fingerprint pattern left on a metal surface often causes corrosion of the underlying metal due to the salt (sodium chloride) content of sweat. The presence of salt on the surface causes the Volta potential to be lowered at fingerprint ridge marks in comparison to the background metal potential. The scanning probe machine is used to measure Volta potential of the metal surface to a comfortable resolution of 500 dots per inch to reveal the latent fingerprint.

The clarity of the fingerprint depends mainly on the corrosive interaction of the fingerprint with the metal. Metals such as iron or brass often give clear images of continuous ridge detail whereas stainless steels or pure zinc are more challenging surfaces due to their higher resistance to corrosion. The image clarity is also affected by the quality of the machine and settings used e.g. resolution, probe dimensions, and scanning height.

The potential contrast between the fingerprint and the background metal can be increased by using the Vacuum Metal Deposition (VMD) technique to apply either silver or gold/zinc coatings that preferentially deposit on the metal. If the fingerprinted metal is noble e.g., copper then the gold/zinc coating is used but if the fingerprinted metal is reactive e.g. zinc then a silver coating is applied.

The typical exhibit that is analyzed is relatively small and flat e.g. a 5cm x 5cm plate or is a piece cut out from a larger exhibit. The machine is equipped with a stage moved by x, y, z and rotational motors allowing moderately non-planar surfaces to be scanned. Cartridge cases of various calibers can conveniently be accommodated and imaged for fingerprint patterns by first establishing a topographic profile for the exhibit followed by volta-potential mapping where a constant probe-to sample spacing is maintained. Examples will be presented of partial print recovery on spent cartridge casings both from laboratory studies and ongoing criminal investigations by United Kingdom police forces.

In summary, this presentation shows how the Scanning Kelvin Probe works and gives examples of fingerprint images obtained on various metal surfaces. The presentation goes on to discuss the use of Vacuum Metal Deposition to further enhance the images.

## References:

1. Williams, G. and N. McMurray (2007). "Latent fingerprint visualization using a scanning Kelvin probe." *Forensic Science International* 167(2-3): 102-109.
2. Williams, G., H. N. McMurray, et al. (2001). "Latent fingerprint detection using a scanning Kelvin microprobe." *Journal of Forensic Sciences* 46(5): 1085-1092.

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## Fingerprints, Metals, Corrosion

### C39 Visualization of Latent Fingerprints Using Columnar Thin Films

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After attending this presentation, attendees will have gained knowledge and insight into a new latent fingerprint development technique that does not rely on mechanical or chemical interactions, but instead on surface topology of latent fingerprint ridge detail. Information on optimization of conformal-evaporated-film-by-rotation (CEFR) development for latent fingerprint visualization on multiple substrates as well as comparisons to traditional techniques using split fingerprints and depletion studies will be presented.

This presentation will impact the forensic science community by allowing for an additional option for latent fingerprint development where traditional methods are not ideal or inapplicable. Forensically relevant surfaces for which either typical development methods are ill-suited or multiple techniques are necessary will now have an additional one-step technique available for forensic investigators to utilize.

Similar to vacuum metal deposition, columnar thin film (CTF) technology is a form of physical vapor deposition under vacuum conditions: thermal evaporation of a source material and subsequent condensation of the vapor create a thin film on a substrate that rotates above the vapor flux. Nano-scale CTFs have been employed in optical applications and in materials engineering for more than a century. Typically, surface defects, such as dust or debris, are highly problematic as once a thin film begins to develop on a substrate, the defect results in a non-uniformity, with the underlying defect propagating through the growing film. This phenomenon is, however, ideal for replicating latent fingerprint ridge detail as the topography or surface texture is essentially copied as the film grows. This results in observable contrast between the fingerprint ridge detail and the underlying substrate.

A systematic study was carried out in order to determine the optimal deposition parameters necessary to visualize the best contrast and clarity of developed fingerprint ridge detail. The clarity of development was such that level three details could be resolved. CTF formation was found to rely on: base vacuum pressure during deposition, the average angle of the vapor flux relative to the substrate plane, substrate rotation rate, evaporant material deposition rate, and final film thickness.

Subsequent to the determination of optimal development parameters, the new technique was applied to forensically relevant substrates for which current development techniques are either undesirable or inapplicable. Surfaces such as brass, stainless steel, and various plastics, woods, and adhesive tapes underwent CEFR development using multiple evaporant materials. The evaporant materials utilized included: chalcogenide glass, gold, germanium oxide, nickel, and magnesium fluoride. Each evaporant material was employed on each substrate to determine optimal development conditions for differing surfaces. Split fingerprints were also placed on each substrate, with one half of each print being developed using the optimized CEFR technique, and the other half being developed with

traditional techniques such as regular, magnetic, and fluorescent powder dusting, cyanoacrylate fuming, or other techniques recommended for that surface.

The optimal vacuum conditions were determined to be: base pressure of 0.1 mTorr, vapor flux angle of 10 deg, rotation rate of 3 rps, deposition rate of 1 nm/s, and a variable final film thickness of 50-1000 nm depending on the combination of the evaporant material and the substrate under investigation. Multiple evaporant materials were found to be optimal, depending on the underlying substrate. Chalcogenide glass, gold, and nickel all produced development with high contrast and clarity on the substrates investigated, while germanium oxide and magnesium fluoride did not produce optimal results.

Current research is still investigating and assessing the advantages of CEFR technique over traditional fingerprint development techniques on various surfaces, as well as the sensitivity of the technique compared to traditional techniques. The optimal base vacuum pressure found greatly reduces the requirements of a vacuum evaporation chamber necessary for this technology, which allows for increased use of this technique for on-scene development of latent fingerprints in the field. This also greatly reduces equipment cost and increases availability of the new development technique to crime laboratories and law enforcement agencies.

This work was supported by grant support from the U.S. Department of Justice.

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## Latent Fingerprints, Conformal-Evaporated-Film-by-Rotation Technique, Columnar Thin Film

### C40 Use of Automated Image Analysis Techniques to Determine Impact Velocities in Bloodstain Pattern Analysis

*Daniel Attinger, PhD\*, Iowa State University, Mechanical Engineering, 2025 Black Engineering, Ames, IA 50011-2161; and Zhenguo Li, PhD, Pei-Lun Hsieh, MS, and Shih-Fu Chang, PhD, Columbia University, Department of Electrical Engineering, New York, NY 10027*

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After attending this presentation, attendees will understand some principles of automated image analysis and its application to the determination of impact velocity in bloodstain pattern analysis.

This presentation will impact the forensic science community by providing automated methods to determine normal impact velocities of bloodstain patterns. This will enhance the capabilities of bloodstain pattern analysis and crime scene reconstruction.

Bloodstain patterns are deterministic signs of the blood drop volume, impact velocity, and impact angle. Bloodstain pattern analysis is however not straightforward, because the physical relation between the drop impact and the resulting bloodstain is complex and non-linear. For instance, the formation of bloodstains involves a complex interplay of fluid mechanics, heat and mass transfer, the presence of a complex fluid with a deforming free surface, and a solid substrate with specific roughness and wettability. In 2005, Hulse-Smith, Mehdizadeh, and Chandra showed in the *Journal of Forensic Sciences* that the number of spines (or fingers or rays) at the periphery of a bloodstain could be used together with the stain size to determine both the impact velocity and the initial drop size for the case of mm-size droplets impacting perpendicularly to a target surface. The determination of the impact velocity and the drop size is relevant to the determination of the region of origin of the blood spatter, if parabolic trajectories are to be reconstructed. The hypothesis behind the work presented here is that automated image analysis techniques can be reliably used to count the number of spines and correlate it to the impact velocities.

To this end, automated image analysis algorithms are designed and implemented in the Matlab® programming language, with the ability to accurately detect the edge and centroid of a drop, from where the fingering or ring shape of the drop is described. Given a photograph of a bloodstain, the boundary of the drop is first extracted. This is done by segmenting the

image and detecting the boundary between the drop and the background. Then the boundary in the 2D image is converted into a 1D curve using a distance transform, where the coordinates of a point in the boundary are mapped to its distance to the center of the boundary points. The bumps on the boundary are well preserved in this 1D curve. The number of spines is then obtained by detecting and counting the peaks in the 1D curve, using standard calculus methods.

For mm-size drops impacting at measured velocities between two and five m/s, the number of spines determined by automated image analysis techniques was found to be as reliable as the number of spines counted manually. The method of Hulse-Smith and coworkers was then applied to determine from the number of spines and from the stain size the impact velocity, which was found to be within 20% of the measured experimental velocities. High-density particleboard wood and wallpaper substrates were used, and 100 drops of human blood were tested on each substrate. Other applications of the automated image analysis and pattern recognition methods are discussed, such as the ability to measure the ellipticity of a stain, or the ability to interpret three-dimensional profilometry measurements of bloodstains. The role of the knowledge of drop size and impact velocity on the reconstruction of parabolic trajectories is also discussed.

This project was supported by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

#### Bloodstain, Image Analysis, Parabolic Trajectories

### C41 A Comparison of Tool Marks From Knives, Saws, Axes, and Loppers Used for Dismemberment and Some Comments on Forces

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The goal of this presentation is to discuss the types of tool marks left on bone surfaces from knives, saws, axes, and loppers used in dismemberment and to show the characteristic features of the different types of tool marks. Some comments about the levels of force required to achieve dismemberment with the different types of weapons will be made so that a forensic pathologist might have a clear idea of the level of force required with particular implements.

This presentation will impact the forensic science community by showing how implements used in dismemberment leave tool marks. This is particularly important for forensic pathologists but also for forensic scientists and engineers who are asked to provide expert opinions on the force required for dismemberment with the various weapons.

Tool marks are the marks left in a softer material when a harder material is used to cut or strike them. Several types of tool mark are possible: imprints left by the indentation of the tool into the softer surface such as when a knife point embeds in bone; or striations that are left by the edge of the tool by either a sawing or cutting action. Tool marks that leave an imprint or indentation leave a “negative” imprint of the tool itself in the material and as such the imprints can be used to help determine the size and shape of the tool’s tip. If the hardness of the bone is known, the size of the impression can be used to calculate the force required (Figure 1). Striations are parallel lines that are caused by a tool’s blade either cutting or sawing the material. For sawing, the marks are parallel to the blade’s length, for cutting the orientation of the marks depends on the direction of cutting.

Figure 2 shows the typical marks that are left on a bone surface from sawing for example. One goal of the work in this paper is to better understand how tool marks arise in bone or cartilage from the various tools that are typically used for dismembering bodies.

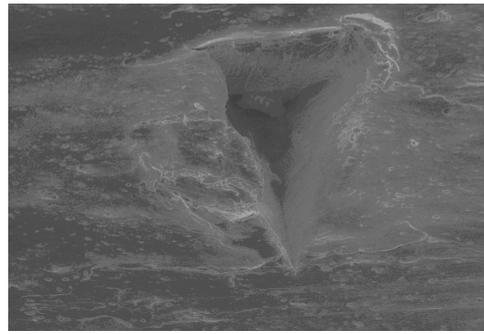


Figure 1: Imprint left in a deer bone from a knife, the size of the impression can be used to estimate the force required.

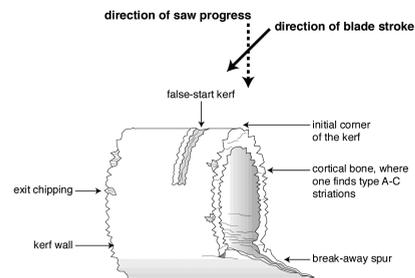


Figure 2: Schematic showing tool marks left on bone from sawing that can be used to identify the tool that made them.

The goal of tool mark analysis in forensic medicine is to compare the marks left on bone or cartilage with the suspected tool or instrument under conditions as close as possible to the conditions under which the original tool marks were made.

Tool mark experts try to match the mark found on the bone or cartilage to weapons that are thought to have made the mark. This can be done by making new marks from the suspect weapon and comparing the dimensions and markings with the ones that were characterized from the bone or cartilage. Traditionally, the impressions or striations are compared with a 3D stereo comparison microscope which allows both samples to be examined at the same time and regions where the marks correspond can be determined from carefully matching the positions of the samples. If a good match is found, over a sufficient area then the marks are deemed to have been made by the same instrument. This technique is used for matching marks from saws, screwdrivers, chisels, knives, hammers etc.

For marks on wood, metal, and polymers, tool marks are often clear and well retained. Bone is also a relatively hard, stiff material and this will also retain good marks. Cartilage is relatively soft and low in stiffness and the marks retained can be less clear. Both cartilage and bone have to be defleshed and dried before tool mark analysis can be performed. Tool mark analysis on bone and cartilage is more challenging because of these factors.

Currently, for marks left with knives, for example, an expert can usually say with confidence in court that a knife *may* have produced the wound, *could* be the murder weapon, or that it is possible to eliminate a particular knife from the enquiry. It is often more difficult to say that a mark *was definitely* made by a particular knife.

The presentation will show a range of examples of the marks left by different tools and show how the forces involved can be determined in some instances and how “uniqueness” can sometimes be determined from the striations left on the surfaces.

#### Tool Marks, Dismemberment, Force

## C42 Soft Computing Application to Anthropological Automatic Characterization in Forensics

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The goal of this presentation is to discuss technical methodologies to support forensic anthropology for the identification of the perpetrators of robberies in stores, using video surveillance cameras shots.

This presentation will impact the forensic science community by showing how a modern approach, using a soft computing methodology, can be utilized. In particular, the anthropological correlation is made using fuzzy logic. This allows combining the power typical of non-linear computing methods with the possibility to represent reality using a language similar to the human one.

Forensic anthropology can be used, today, for the identification of the perpetrators of robberies in stores, using video surveillance cameras shots. Literature reports that the most relevant anthropometrical methods are mainly based on: (1) proportional ratio between height of human figure and of internal furniture items and those displayed on recorded imagery; and (2) observation of subjects characteristics, such as postural poses caused by skeletal pathologies and/or facial details, so that they can be highlighted even though individuals have the face distorted by sunglasses, balaclava, etc.

In many cases, anthropometrical investigation used a fundamental ability to identify people, using video surveillance equipment. Some considerations have to be made: (1) the horizon line (image upper margin) and the ground line (image lower margin) of the perspective representation are drawn on the image shots; (2) The distance between the two lines confirms that the camera vision is from top to bottom; and, (3) The observer position (camera lens) is highlighted when the lines, arranged on the furniture items, reach the horizon line in a point, where the vertexes of the optical cones are.

The human eye perceives things through an imaginary optical cone or visual pyramid, making objects appear greater in the foreground than those in the background. The dimensions of the internal reference structures (furniture) of the crime scene are shown as colored segments. Those drawn on the human figure in the same picture are equivalent to the measurement of the height of the accused subject. It is the anthropological and anatomical correlation that, when defined by the somatic type of the examined subject, makes him absolutely different from others that superficially examined might be considered similar.

A soft computing methodology was proposed to help with the described procedures. In particular, the final anthropological correlation is made using fuzzy logic. The related principle is known as the incompatibility principle. It states that, as the complexity of system increases, the possibility to obtain a precise description of it in quantitative terms decreases. The use of fuzzy logic allows combining the power, typical of non-linear computing methods, with the possibility to represent reality using a language similar to the human one. It appears as a simulating theory to represent phenomena, more or less complex, through a definition of a certain number of fuzzy sets, elaborated with appropriate connectives. The cause-effect connections, regulating the process, are described through fuzzy implications (rules).

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**Anthropological Characterization, Fuzzy Logic, Soft Computing**

## C43 Design and Development of a Dynamometer for Quantifying Force Related to Stabbings

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The goal of this presentation is to show how dynamometers have a role to play in relating force levels in stabbings to injuries.

This presentation will impact the forensic science community by presenting the design and novel use of a dynamometer that can be used to relate the forces used in stabbings to common actions such as a punch or push. The ultimate goal is to help those in the legal system (judges/barristers/jurors) understand how a force required for stabbing relates to a real life situation, i.e., does the force equate to a hard punch or a slap? The paper discusses whether a scale can be developed that can allow a better appreciation of how engineering data relates to common perceptions about level of force.

In the United Kingdom, murder by stabbing is the most common form of murder. Previously, the forces required for stabbing with knives were considered and shown that the tip radius is important for defining the sharpness of the stabbing implement. Recent work has showed that for blunter instruments such as screwdrivers, the cross-sectional area is important for determining the forces required for penetrating a silicone rubber/foam skin analogue. More recent work has considered how much force is required to penetrate a skin simulant with broken glass bottles and also how much the victim's clothing influences the force required for penetration with knives. Work so far has focused on the force required for penetration of a skin simulant in an effort to identify the most important factors in relation to penetration. The mechanism of knife penetration is controlled initially by the tip sharpness as a knife penetrates the skin, once penetration has occurred, the edge sharpness becomes important for further penetration. Figure 1 shows the sequence of events as a knife penetrates a foam block. In A, the knife is just at the point of contact with the foam; in B, the foam is deflecting elastically but the foam is not penetrated; and, C shows a knife where the tip has penetrated the foam block. This current work furthers the understanding of knife wounding by focusing on the quantification of forces involved in 'real life' stab events.

A series of experiments were conducted using a skin simulant and a dynamometer. A series of everyday actions (such as a push) were carried out and also a series of stabbing actions (such as a thrust or overhand) using a purpose built dynamometer, by a group of volunteers of varying body types, ages, etc. As contact is made with the skin simulant the dynamometer records the load. For the everyday actions the maximum force is recorded during the impact. We will take similar data for the knife penetrating the skin simulant. Previously, stabbing simulations have been conducted using instrumented knives, these have the disadvantage that the knife has been modified to include the instrumentation which makes it more difficult for the person stabbing to use a natural action. In this work, the instrumentation is in the dynamometer and the knife was as manufactured without the additional encumbrance of the instrumentation. This should provide more realistic data to be recorded.

The results should provide quantified force data for both everyday actions and stabbing by volunteers that are representative of the population. These results allow a comparison of forces and are the first stage in an effort to compose a scale that could be used by forensic practitioners in court to explain the forces involved in stabbings to jurors more clearly.

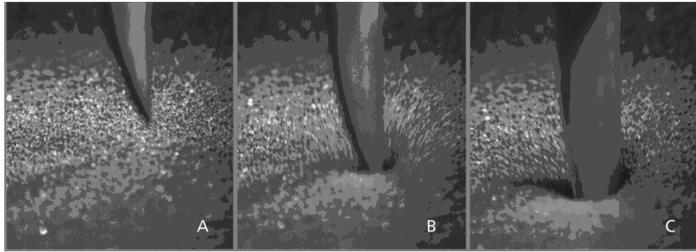


Figure 1: The sequence of events in a knife penetrating a foam block. A shows the tip as it first contacts the foam. B shows elastic deformation of the foam block. C shows both elastic deformation of the block but also the knife penetrating the foam.

**Stabbing, Force, Knife**

**C44 Effect of Surface Tension on the Ability of a Selection of Tribometers to Rank and Differentiate Standard Reference Surfaces**

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After attending this presentation, attendees will understand: (1) the significance of surface tension on the slip measuring process; (2) the importance of quantifying and standardizing measurement variables under wet conditions; and, (3) the effect of surface tension on machine bias.

This presentation will impact the forensic science community by demonstrating the effect of surface tension on wet slip resistance measurements and machine bias.

**Method:** ASTM F2508 was used to measure the effect of variations stemming from the use of surfactants on test results using the same reference materials with a Slip-Test Mark II portable inclinable articulated strut tribometer (PIAST).

Slip resistance and coefficient of friction measurements have been made since the time of Leonardo Da Vinci. In more recent history, efforts to make such measurements meaningful in the context of human ambulation have resulted in a series of test surfaces being subjected to human ambulation studies. The results of those ambulation studies using a select population of young adults produced a suite of walkway surfaces that when wet, ranged in slip resistance from very low (granite) to high (unglazed tile). ASTM F2508 correlated the human-ambulation-study results to tribometer measurements. Slip resistance measurements under water-wet conditions sometimes produce readings that are higher than when that same surface is tested under dry conditions. These results were often considered to be as anomalous, but recently, both normative analysis and carefully controlled tests demonstrated this to be the case. Certain European slip resistance test methods, e.g., DIN-Ramp studies, routinely use surfactants to minimize the effects of surface tension, a factor in the wet-test/dry-test anomaly.

To determine the effect of slip resistance on ranking and discriminating the reference surfaces (Surface), the ASTM F2508-11 specified adjunct tiles from ASTM International: ADJF2508-T4 (Granite), ADJF2508-T2 (Glazed tile), ADJF2508-T1 (Vinyl Composition Tile (VCT)), and ADJF2508-T3 (Unglazed tile) were obtained for this study. These four SURFACES were tested.

Two Surfactants, sodium lauryl sulfate (SLS) and nonionic surfactant were used to reduce the surface tension of water. Tests were conducted using distilled water and the two surfactants were each tested at two concentrations: 0.05% SLS ((SLS-Low) this is the concentration mandated in ASTM F2508 for cleaning the reference surfaces), 0.10% SLS ((SLS-High) this is the concentration mandated in the DIN Ramp protocol), 0.05% Triton X-100 ((Triton-Low) this is the concentration mandated in ASTM

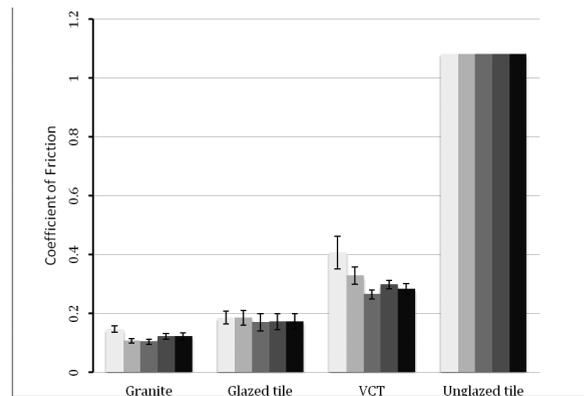
F2508 for testing the granite reference surface), and 0.10% Triton X-100 (Triton-High). Distilled water (Water) was also used, for a total of five Surfactant conditions.

Forty tests for each Surface/Surfactant Condition set were conducted: five replications in each of eight directions 45° apart (Direction). A total of 800 tests were conducted (5 replications x 8 Directions x 4 Surfaces x 5 Surfactants). The tests results were analyzed using a screening ANOVA, with Surface, Surfactant, and Direction as the factors. Direction was found to be not-at-all significant (p-value >40%); Surface and Surfactant were both highly significant (p-values of 0.00% and 0.02% respectively).

The results were aggregated over the not-significant factors, giving the following results, where the first number is the mean value and the second is the standard deviation:

	Granite	Glazed tile	VCT	Glazed tile
Water	0.146/0.01	0.185/0.021	0.406/0.055	≥ 1.08/0
SLS-low	0.106/0.008	0.185/0.025	0.327/0.029	≥ 1.08/0
SLS-High	0.103/0.009	0.169/0.029	0.264/0.015	≥ 1.08/0
Triton Low	0.121/0.009	0.171/0.028	0.297/0.014	≥ 1.08/0
Triton-High	0.122/0.012	0.172/0.026	0.282/0.019	≥ 1.08/0

The bar graph below is clustered by SURFACE, from the most slippery on the left to the most tractive on the right:



The individual bars in each cluster, from left to right (light to dark) are Water, SLS-Low, SLS-High, Triton-Low, and Triton-High. The error bars at the top of the bars are ±one standard deviation. The zero-length error bars for the unglazed tile are a result of the PIAST having reached its maximum reading (1.08); thus there was zero variation. (The results on Unglazed Tile are not to be used.)

The conclusion from the testing is that it matters less what surfactant one uses, but one should use a surfactant, especially in Granite and VCT. Worth noting is that the subset of the results for a given Surfactant: 160 tests, defines the F2508-11 validation sequence.

**Surface Tension, Slip Resistance, Reference Surfaces**

**C45 Progress in the Characterization of Barefoot Pedestrian Friction**

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After attending this session, attendees will appreciate the difficulties inherent in using current methods for measuring barefoot slip resistance. The current state of the art in barefoot tribometry will be discussed, and this paper will focus on improvements in the development of biofidelic test feet.

This presentation will impact the forensic science community by giving current knowledge in barefoot-pedestrian friction metrology. This is significant because it can help determine whether or not a given walkway surface is or is not hazardous to barefoot pedestrians.

Barefoot slip resistance has not received the attention – either through standards, research, or on-site testing – as has the slip resistance of footwear. However, slips and falls in bathrooms, tubs, and showers occur frequently and result in significant injury. The materials in a typical bathroom/shower enclosure are non-resilient which can increase the severity of injury upon contact. Characterization of the friction between the plantar aspect of the heel and a contaminated floor surface would assist architects and interior designers in choosing appropriate materials for those environments where pedestrians can be expected to be barefoot.

The ASTM F462 Standard Consumer Safety Specifications for Slip-Resistant Bathing Facilities, first approved in 1979, used a smooth Silicone Rubber (Silastic 382) test foot in a soapy-water-filled bathtub to determine the slip-safety of a bathtub.<sup>1</sup> More recently, barefoot pedestrian slip resistance has been evaluated using an inclinable ramp to measure the slip resistance of a barefoot pedestrian on a water contaminated walkway surface (DIN 51097 (1992)).<sup>2</sup> Medoff, et al. developed an inclinable Step Meter using an *in vivo* test subject to measure the slip resistance of footwear/walkway combinations, dry and wet.<sup>3</sup> This Step Meter was used with barefoot test subjects, “stepping” onto a varying angle, water-contaminated walkway surface.<sup>4</sup> When the motion of the lower leg was both constrained and passive, it was found that the results were consistent and repeatable. Recently a number of researchers have published studies on the coefficient of friction of a bare foot sliding against marble floor (Sariisik, Ali).<sup>5,6</sup> The Sariisik study used the DIN 51097 Ramp test, with different roughness values of wet marble, while the Ali study was focused on level walking with bare feet on marble with different concentrations of detergent in the water-covered marble flooring.

Medoff, et al.<sup>7</sup> measured the available friction, as determined by a barefoot pedestrian walking on a smooth, water-contaminated, instrumented walking surface, and compared these results with walkway-safety tribometry (WST) measured values on this same water contaminated surface using a smooth silastic 382 test foot. It was found that the tribometer with this silastic test foot was unable to characterize the slip resistance under these conditions, giving essentially zero values for the slip resistance. Siegmund, et al. measured utilized friction when entering and exiting a dry and wet bathtub, barefoot.<sup>8</sup> The bathtub base (porcelain on steel) was modified by adding “slip resistant medallions.” They found a wide variation in utilized friction (0.102 to 0.442).

A problem with barefoot WST is that the test feet currently in use lack biofidelity. Our long-term goal is to develop a biofidelic WST test foot. In this paper, we use non-contact optical techniques to characterize the surface morphology of the plantar aspect of the heel.

**Methods:** Skin morphology was characterized as follows. Plaster casts were prepared of the plantar aspect of the heels of six adults (3M, 3F, ages 21-54). Plaster1 was prepared and placed in a shallow container. The seated subjects’ heels were pressed down into the plaster, with the subjects remaining seated until the plaster set (approximately 30 minutes). Subjects’ heels were then removed from the plaster, and the plaster was permitted to fully harden for 24 hours.

Positive models of the heel were then prepared as follows: Room Temperature Vulcanizing Rubber2 (similar to Silastic 382 medical grade silicone rubber) was mixed according to manufacturer’s instructions, poured into the plaster molds, and permitted to harden. This created heel-surface replicates with the associated skin friction-ridge patterns.

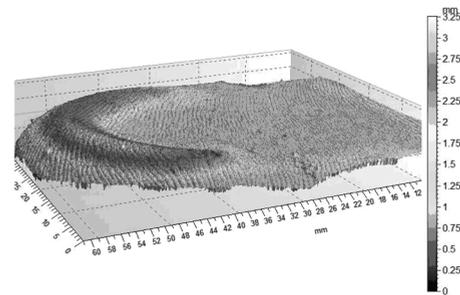
The surface morphology of two sample heels was measured using interferometry. These systems use a white light source, where light is focused through an objective lens onto the specimen, and the reflected light is captured. By focusing the lens, the distance from the objective to the sample is captured. Two companies were sent samples for analysis.

The first sample, sent to Zygo,<sup>3</sup> was scanned with a NewView™ 7300 system. This system uses an interferometric objective mounted in a closed loop piezo-scanning device that moves vertically (in the Z direction) over the sample. Data is collected from a CCD camera and processed. The phase relationships of individual components of the white light spectrum in the interferogram are analyzed by Zygo’s frequency-domain analysis,

resulting in a surface map with ultra-high Z resolution, independent of the objective magnification, with up to one angstrom resolution.

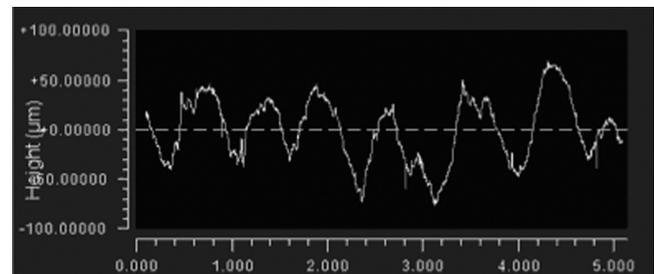
A second sample, sent to Nanovea,<sup>4</sup> was scanned with their ST400 Optical Profiler. This system uses “axial chromatism,” where white light passes through an objective lens having a high degree of chromatic aberration. (The refractive index of the objective lens varies in relation to the wavelength of the light. In effect, each separate wavelength of the incident white light re-focuses at a different distance from the lens). Due to the confocal configuration of the system, only the focused wavelength will pass through the spatial filter with high efficiency, thus causing all other wavelengths to be out of focus. The spectral analysis is done using a diffraction grating. This technique separates each wavelength and allows direct correspondence to the Z height position. With a measurement range of 10mm, this system has a Z resolution of 280nm and an accuracy of 900nm, with lateral resolution of 2.6 μm.

**Results:** Overall picture of the 3-D friction-ridge pattern. The three-dimensional graph just below gives an overall view of a typical plantar heel friction-ridge pattern. It has not yet been filtered through a high-pass filter, which would eliminate the low-frequency, i.e., gross height variations.

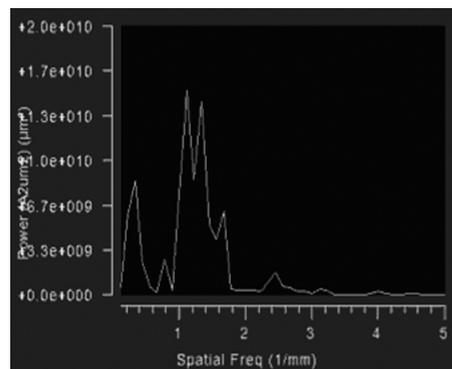


The friction-ridge pattern impressed upon the gross height variations can be seen. A typical cross-sectional result.

Figure 2, below, shows one cross-section of the heel, showing the actual dimensions of a friction-ridge pattern. The ridge-pattern period is roughly 0.8mm with a peak-to-trough extent of roughly 0.1mm. The pattern is very roughly sinusoidal.



In figure 3, below, translation to the frequency domain shows the spacing of the friction ridges (the period) to be between 0.75–0.9mm.



#### Future Research Directions

**The Big Picture:** The goal is to produce test feet that, when used with

conventional WST instruments, mimic the Available friction of a pedestrian ambulating with bare feet. The friction-ridge pattern in the plantar surface of the foot has been optimized over evolutionary millennia, forming an essential component of the friction picture. Presently, test feet used to characterize barefoot friction have been smooth. To compensate for the lack of biofidelity inherent in smooth-surfaced test feet, artificially low threshold values for acceptable available friction have been utilized. If the friction-ridge pattern were able to be characterized and implemented in test feet, this would go a long way to making test feet biofidelic, and hopefully eliminate the need for artificially low threshold values.

**Mathematical Modeling of the Friction Ridge Patterns:** It would be better to develop a generic friction-ridge pattern, versus having someone's friction ridge pattern serve as the standard pattern (in much the same way as the inch was formerly defined as the size of some king's thumb). One approach would be to mill a pattern of grooves in a mold that corresponded to an idealized groove pattern as generated by, say, the averaged-over-multiple subjects' first (or first and second) terms of the Fourier transform of the friction-ridge pattern.

**Multi-Phase "Hardness" Characterization:** Another important area for friction research is the development of a biofidelic model for the hardness of the friction-ridge pattern. The plantar surface of the heel is comprised of various biologic structures, viz., the epidermis, which contains the friction-ridge pattern, the endodermis, fat pad, the fascia, and the calcaneus (the heel bone). It is rather unlikely, to say the least, that this layered structure could be reasonably characterized by a homogenous material. It is speculated that, at the very minimum, a three-layer test foot is necessary: an outer layer representing the friction-ridge pattern, an inner layer representing the fat pad, and a backing plate representing the calcaneus.

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**Forensic Science, Walkway-Safety Tribometry, Barefoot Pedestrian**

## **C46 Estimation of TASER® ECD Discharge Duration Based on Surface Morphology Changes**

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After attending this presentation, the attendees will become familiar with analysis techniques that could be used in forensics investigation to correlate the physical evidence to the electrical activity of the Taser ECD. It will become apparent that the changes in surface morphology (physical evidence) indicate the duration of discharge of a Taser ECD.

This presentation will impact the forensic science community by providing a means to analyze and interpret physical evidence that investigators in the past may have neglected to analyze and correlate it to actions taken during an event where a Taser ECD was used. Electrical arcing creates changes in surface morphology that could be used in forensics analysis. This novel approach uses changes in surface appearance and its morphology to estimate the duration of ECD. i.e., it uses physical evidence that was formed by discharging Taser ECD to relate it to discharge duration.

TASER Electronic Control Devices (ECD) are used commonly for self defense and for law enforcement. An ECD is designed to deliver electric current to a subject's body with the purpose of temporarily incapacitating an individual. Activation of the ECD's trigger delivers a burst of energy at the output and to the subject. For example, a TASER X26® ECD is rated to deliver a 1,200 V pulse at the rate of approximately 19 pulses a second (each pulse lasting approximately less than 150 microseconds). The energy flows into the subject's body through the insulated wires and metal probes that penetrate the skin or adhere to the clothing of the subject. The attachment method between the wires and the metal probes creates an air gap between the wire tip and the metal probe's body that must be bridged on both probes to complete and then maintain the electrical circuit. Due to the high voltage pulse, the air gap is bridged by an electrical arc which develops between the wire tip and the metal probe's body. This arcing activity results in visible changes to the wire tip and the metal probe's surface exposed to the arcing. Such surface morphology changes can be useful as physical evidence that may indicate the ECD discharge duration.

ECD discharge tests were performed for a number of time durations. A minimum of three tests were conducted for each discharge duration using two probes connected to a 600 Ohm ( $\Omega$ ) resistor. The metal probe surfaces exposed to electrical arcing during the tests were then analyzed using scanning electron microscopy (SEM) and surface profilometry to determine the correlation between the surface morphology changes and the ECD discharge duration.

SEM analysis indicated that the exposed probe surface appeared visibly more damaged with an increase in the ECD discharge duration. In addition, surface profilometry analysis indicated that the volume of expunged material from the exposed probe area became greater with an increase in ECD discharge time. The preliminary investigation suggests that the changes in surface morphology are a function of the ECD discharge duration. This correlation of the physical evidence with the ECD discharge time can be established by using a combination of SEM and surface profilometry analyses and may become a valuable tool in forensic investigations where the time of ECD discharge is an unknown and a potentially disputed fact.

**Duration of Taser ECD Exposure, Probe Surface Morphology, Surface Profilometry**



ATLANTA 2012

# GENERAL



ATLANTA 2012

## D1 New Suicide Modalities: The Use of a Plastic Bag and Helium - First Case Report in Puerto Rico

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After this presentation, attendees will better understand the pathophysiology of oxygen displacement by helium and learn information needed in order to process a case like this, should you encounter one. This presentation will impact the forensic science community by giving the attendees the information needed in order to understand the pathophysiology and process autopsy evidence in a case of this nature. It also adds to the growing list of case reports of this type of suicide.

In statistical terms, suicide (defined as the act of voluntarily taking his/her own life) in Puerto Rico has shown a relatively stable trend during the last decade, shadowing the trend in the United States. Although the methods by which the act is performed have not varied much, new worrisome tendencies have been described in literature. Suicide by hanging is the most common method used in Puerto Rico (for both men and women) and the second most common in the United States. While the use of a plastic bag around the head as a suicide tool had been described for some time, its practice was boosted in 1991 after the publication of the book, *“Final Exit: The Practicalities of Self-Deliverance and Assisted Suicide for the Dying.”* Directed towards terminally ill patients, the book presented multiple mechanisms for a dignified death, including, in full detail, the “exit bag” mechanism. In 2002, the third edition of the book included a chapter on the utilization of inert gases as a faster way to reach their objective. Inert gases (e.g., argon, radon, helium) accelerate the death by effectively displacing or substituting the oxygen being aspirated, and depriving the body (especially the brain) of it. Only twenty suicide cases using this methodology have been reported in international scientific literature. Since helium is seldom searched for in the toxicological analysis of a forensic autopsy, it has been hypothesized that the real incidence may be exponentially higher, possibly reaching into the thousands. A case is presented of a 30-year-old male who five years previously had been diagnosed with bipolar disorder, who was found dead on the floor of a hotel room with an inflated plastic bag around his head, and closed around the neck by an elastic strap. Underneath the bag, a rubber tube entered the bag, connected by a T-fitting connecting to two helium canisters, which were empty by the time the body was found. A piece of elastic strap circled his waist through the belt loops and restrained his wrists without the use of a knot. No suicide note was found on the scene. The scene investigation revealed no sign of forced entry. The deceased had possession of the book, *“Final Exit,”* which was found at his residence. Autopsy revealed mild pulmonary congestion, eyelid petechial hemorrhages, and pressure marks around the neck, wrists, and waist. Toxicology was positive for Buspirone. Airway headspace gas sampling was not performed due to lack of equipment. After ruling out secondary involvement and taking into consideration the scene, family interview and the circumstances around the death, the cause of death was determined to be asphyxia secondary to

helium inhalation and the manner of death was determined to be suicide. This is the first suicide case seen in Puerto Rico’s Forensic Science Institute utilizing this method.

**Helium, Suicide, Puerto Rico**

## D2 Species Composition of the Maggot Mass

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After attending this presentation, attendees will learn how molecular identification techniques were used to validate the hypothesis that the species composition of the maggot mass is comprised of multiple species.

This presentation will impact the forensic science community by determining the species composition of the maggot mass to be a single or multiple species complex. A maggot mass that is composed of multiple species will bear significant impact on insect development studies since the presence of one species can slow or accelerate the developmental rate of another species. These developmental rates will have a direct effect on the postmortem interval estimation.

During initial human decomposition, it has been observed that several adult female fly species visit the body. Even closely related carrion species can differ in their growth rates, diapause response, and/or ecological habits. Therefore, accurate identification of an insect specimen is crucial. Immature stages of many forensically significant species are notoriously difficult to identify in early developmental stages (instars) and lack defining anatomical characteristics. Identifying each maggot according to distinguishing morphological characteristics can be a time-consuming task. In addition, the process can suffer from human error if performed by an untrained forensic entomologist. For these reasons, DNA-based methods of identification should be used.

Currently, the maggot mass is assumed to be of multiple species composition, but this idea has not been validated. Therefore, it is unknown if adult female flies of different species will lay their eggs in the same location on a corpse as other adult female flies. This study will test the hypothesis that a maggot mass is composed of several different species of larval flies. This project will employ the use of automated DNA sequence analysis standards and phylogenetic methods to compare “unknown” maggots to “known” adult flies. Co-Oxydase enzyme I (COI) and Co-Oxydase enzyme II (COII) gene sequences will be amplified and sequenced. COI and COII are unique markers that are highly conserved because they code for respiratory processes (electron transport); and are species-specific genes. The combination of slowly evolving regions coupled with wobble regions makes these genes a perfect marker to use in molecular species identification techniques.

Adult flies and maggots will be collected from three different cadavers during June, July, and August of 2011. Flies will be actively collected with nets for five consecutive days once the human body has been placed. The adult flies will be stored in vials of 95% alcohol until they are identified by species via use of published dichotomous keys, a reference collection, and an Olympus SZX16 stereomicroscope equipped with a DP72 color digital camera. Adults will be stored and saved to serve as positive controls for the maggot DNA sequencing. After one week, first and second instar maggots from the initial maggot mass will be collected and stored in 95% ethanol at

80°C. Fifty maggots from each body will be randomly selected and identified by molecular techniques that have been proposed and validated by forensic entomologist Jeffrey D. Wells<sup>1</sup> and the Bucheli Lab (in prep).

Gut content from frozen maggots will be cut and removed. The remaining maggot muscle tissue will be blended in a tissue homogenizer and DNA will be extracted according to a Chelex extraction protocol. Purification of the extraction will be performed with phenol chloroform protocol. DNA will be quantified by ultraviolet (UV) spectrometry. Polymerase Chain Reaction (PCR) will amplify samples on the GeneAmp PCR System 9700 thermal cycler. Promega Hot Start polymerase master mix will be used in conjunction with primers published by Wells<sup>1</sup> to generate the PCR products. The resulting amplicons will be cleaned using a QIAquick PCR purification kit from QIAGEN. An agarose gel check will be performed to ensure DNA has been amplified prior to sequencing. The sequence will be detected on the Applied Biosystems 3500 Genetic Analyzer with BigDye Terminator version 3.1. The Geneious Pro cross-platform bioinformatics software suite will be used to analyze the resulting sequences. Phylogenetic analysis will be used to perform identification of the maggots sampled from the initial maggot mass. Parsimony analysis will be used to construct a phylogeny where the unknown maggot species and known maggot species will be used. The sister relationship of unknowns to knowns will be used for identification criterion. Bootstrap and jackknife statistics will be calculated to assess clade support and the confidence of the sisters relationship.

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**Forensic Entomology, DNA, Postmortem Interval**

### **D3 One Murder Case: Advantages of a Holistic Approach in Forensic Science**

*João De Sousa\*, Judicial Police of Portugal - Criminal Investigation Department of Setúbal, Praça General Luis Domingues, n° 27, 27A e 28, Setúbal, 2910-585, PORTUGAL*

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After attending this presentation, attendees will understand the extreme importance of a multidisciplinary approach to ensure the resolution of a crime of this nature. In this case, a murder crime in which, several areas of research were required, even some that are not commonly applied in this type of investigation, and we will demonstrate clearly that this multidisciplinary approach was crucial to solve the crime.

This presentation will impact the forensic science community by highlighting the importance of a holistic approach that should be used in crime investigations, which is reflected in a multidisciplinary method. With this presentation, the forensic science community will be able to understand the extreme importance of each area of expertise that relates to criminal investigation and leads to the excellence of police work.

Usually, thanatology, biology, and dactylscopy are forensics areas most commonly requested during a murder investigation. In this case, the reported murder is an example of several disciplines, even those that usually have not been requested for this type of investigation, were crucial for the murder resolution.

On July 10, 2005, in Setúbal District, Portugal (approximately 20 kilometers from the country capital, Lisbon), after a fire extinction in a tree zone used for garbage disposal, the presence of a corpse was detected. This corpse was of a burned and unidentified-male, with no signs of tattoos or any articles that would allow a positive identification. The corpse was gagged with the hands tied behind his back, and after preliminary examination, presented signs of beating. Due to the lack of sufficient identification and interpretation elements, it was necessary to expand the forensic science investigation into areas not commonly used. The use of

thanatology, forensic anthropology, lofoscopy, and forensic chemistry for corpse identification was necessary.

About two months later, the corpse remained unidentified. In this period, a vehicle with blood stains was delivered to Judicial Police (Lisbon headquarters), and after collection of blood samples from the corpse (stored in National Institute of Legal Medicine – Lisbon headquarters) and investigation by the Department of Forensics Biology from the Laboratory of Scientific Police, a DNA profile match was obtained.

The corpse was identified as Mr. X, a 71-year-old male individual. The vehicle was purchased during an illicit sale including fake signature (sale contract). Evaluation of the contract was requested and performed at the Department of Handwriting from the Laboratory of Scientific Police.

At this time, the investigators had the victim's identity and, after studying the murderer(s)' profile(s), it was necessary to identify the perpetrators.

After collaboration with the crime scene team of Judicial Police, a home search in the residence of the main suspect, established it was the place where the murder had been committed with help of another individual. Information regarding the exact places where the victim was tortured and murdered as well as the place where the body was placed during two days, the artifacts used during beating, and relocation (covering, transportation, and destruction by fire) of the corpse was obtained. The murderers were sentenced to 18 and 25 years respectively.

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**Holistic Approach, Murder, Forensic Science**

### **D4 Fatal Wounds Sustained From Falling Bullets: Maintaining a High Index of Suspicion in a Forensic Setting**

*Amy Rapkiewicz, MD\*, Miami Dade Medical Examiner's Office, 1851 Northwest 10th Avenue, Miami, FL; and Mark J. Shuman, MD, and Kenneth D. Hutchins, MD, Miami-Dade County, Medical Examiner's Office, One Bob Hope Road, Miami, FL 33136*

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After attending this presentation, attendees will recognize the risk factors and fatal wounding potential of falling bullets.

This presentation will impact the forensic science community by showing how celebratory gunfire poses a unique challenge as the investigative and medical community must be made aware of the wounding potential of the falling bullets. We will present two cases of fatal celebratory gunfire injury on New Year's Eve in Miami-Dade, FL.

**Case 1:** A 69-year-old man collapsed at an outdoor party and was brought into the hospital in cardiac arrest. He had a history of hypertension and was prescribed lisinopril. The emergency room physician certified the death as consistent with hypertensive cardiovascular disease. During an autopsy following postmortem tissue procurement, massive left hemothorax was identified and a fully jacketed projectile was retrieved from the left ventricle. A previously missed entrance gunshot wound was identified in the posterior triangle region of the neck. The bullet lacerated the aorta and heart.

**Case 2:** A witness saw a 35-year-old man fall down and have seizure-like activity while outside on New Year's Eve. Emergency medical services were called and noted a wound to the top of the head resembling a gunshot wound. At autopsy, an entrance gunshot wound was found at the top of the head with penetrating injury to the parietal region of the brain with a depth of one inch. A copper-jacketed bullet was recovered from the brain.

Fatal celebratory gunfire injury is an uncommon reported event in the continental United States. Cases are predominately documented from the Middle East, Southeast Asia and a rare case series from Puerto Rico regions. Celebratory gunfire injury can be defined as unintentional injury from gunfire into the air. In the American literature, holidays such as New Year's Eve and Independence Day pose as a risk factor for this type of occurrence and a high index of suspicion to recognize such injury is

required during such events. Celebratory gunfire fired from both handguns and rifles can reach a minimum velocity required to penetrate skin and bone producing fatal injury. In terms of inflicting fatal wounds, the head and shoulder are most likely to be associated with deaths from celebratory gunfire; non-fatal injuries are mostly seen in the extremities. In the series from Puerto Rico, as compared to non-celebratory gunshot wounds occurring during the same time period, women and children were more likely to be injured by falling bullets. This case report illustrates that fatal gunfire injuries sustained from falling bullets may pose as a mimic to sudden natural deaths especially in patients with prior medical history.

#### **Falling Bullet, Homicide, Gunfire**

## **D5 UPLC-MS/MS for the Screening, Confirmation, and Quantification of Illegal Drugs Added to Herbal/Dietary Supplements for the Enhancement of Male Sexual Performance**

*Salman Azimi, MSc\*, Drug Quality Control Lab, Supreme Council of Health, PO Box 1919, Doha, Qatar 1919, STATE OF QATAR; and Michelle Wood, MSc, Waters Corporation, Atlas Park, Simonsway, Manchester, M22 5PP, UNITED KINGDOM*

The goals of this presentation are: (1) to provide insight into the global problem of the adulteration of herbal and dietary supplements with erectile dysfunction (ED) drugs; (2) detail their synthetic analogues; and, (3) to present a validated method for these compounds.

This presentation will impact the forensic science community by providing a new method for the simultaneous analysis of >30 ED drugs and their analogues.

The adulteration of herbal/dietary supplements with erectile dysfunction (ED) drugs and their analogues is reported worldwide and is an increasing problem.<sup>1,2</sup> The sale of so-called 100%, "all-natural" products has become a highly profitable business for online pharmacies; however, these products can pose a serious threat to consumers due to the undisclosed presence of approved/prescription drugs or the unknown safety and toxicity profile of unapproved ED drugs. Government authorities play a crucial role in the control of these products for the safety of human health. The Drug QC Laboratory in Qatar, has been involved in the testing of adulterated and counterfeit products for a number of years.<sup>3</sup> The goal of this study was to develop an analytical procedure for herbal and dietary products that are marketed to improve male sexual performance and imported to Qatar.

A simple and rapid Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) procedure for the analysis of >30 synthetic chemicals in herbals without sample cleanup is presented. A spectral library for synthetic compounds (including 28 ED drugs and their analogues) was generated from reference standards for automated routine sample screening. Full scan MS analysis was performed simultaneously in both positive and in high energy negative Electrospray Ionization modes (ESI); the latter function permitted the detection of new, unknown ED analogues by generation of common, high intensity fragment ions at  $m/z$  282, 298, and 232. In addition, a highly sensitive and selective MS/MS method was developed for confirmation and quantification using two multiple reaction monitoring (MRM) transitions for each compound. This method was validated for three different matrices (capsules/tablets/pills, honey, and herbal drink). Calibration curves (0.2ng/mL – 1000ng/mL) were prepared both in the absence and presence of matrix. The limit of quantification was 0.5ng/mL for most compounds based on a signal-to-noise ratio of  $\geq 10:1$  for both quantifier and qualifier ions and with %CV reported less than 11% for 29 compounds spiked in herbal matrices at 2ng/mL. The developed method was applied to 43 suspected dietary products that had been imported into Qatar during 2010 and 2011. The

samples were received from customs, herbal registration section and clearance section of health authorities, and analyzed by the developed procedure. A total of 18 products were found to be adulterated: 11 with sildenafil, two with thiodimethyl-sildenafil and five found to contain a combination of yohimbine, tadalafil, aminotadalafil, dimethylsildenafil, and thiosildenafil.

#### **Reference:**

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2. Hidden Risks of Erectile Dysfunction "Treatments" Sold Online, <http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048386.htm>
3. Dubai raid nets Dh70m worth of fake Viagra, <http://www.thenational.ae/news/uae-news/dubai-raid-nets-dh70m-worth-of-fake-viagra>

#### **ED Analogues, Herbal/Dietary Supplements, UPLC-MS/MS**

## **D6 Time Since Death Estimation From Gut Flora**

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After attending this presentation, attendees will understand: (1) the principles of current time since death (TSD) investigation including the strengths and limitations; (2) how specific species of gut flora change over time; (3) the relationship between postmortem gut flora change and TSD; and, (4) how to practically utilize the method of estimation.

This presentation will impact the forensic science community by facilitating criminal investigation with a universal method of estimating TSD allowing for the removal of the body from original decomposition context as well as a sampling window encompassing various stages of decomposition

The method limits to stages of decomposition where intestines are present and not mummified. Because the gut flora of an individual impact the earlier stages of decomposition, monitoring the gut flora change over time can yield a formula to estimate TSD. This preliminary study uses 10 human individuals for the basis of the relationship between the change in postmortem gut flora and TSD. None of the individuals were buried, clothed, or covered in any artificial wrap. All individuals were placed in the supine position on the ground at the Anthropological Research Facility in Knoxville, TN, which consists of sparse forest. Individuals are sampled every three days in the first two weeks of May 2011, and due to temperature increase, are sampled once a day from the last two weeks of May 2011 to present. At each time point for each individual, three different samples are taken from the same location in the cecum and stored at  $-20^{\circ}$  C until processing. After the gut flora from the cecum of the large intestine of deceased individuals is sampled, the DNA is isolated using the PowerSoil<sup>®</sup> DNA Isolation Kit, and specific, common species are amplified in real time with the use of quantitative, real time polymerase chain reaction (PCR) using primers for conserved, ribosomal genes (16S). The real time PCR bases the amplification of the selected bacterial species off a standard curve, which is developed by isolating an organism of the specific species and transforming it into a plasmid so the species chain can be sequenced or decoded, to confirm the correct organism is chosen. The confirmed organism is then run through real time PCR by serial dilutions, which creates the standard curve. The environmental samples compare to the standard curve to confirm the amplification of the correct organism based on size as well as compares to the standard curve to delineate the number of copies. The change in these species over time can be formulated with the known TSD to produce a standard relationship reproducible for popular

use. The study also includes samples taken at known time points from previously un-sampled individuals to cross-verify the determined relationship between the specific species of gut flora and the TSD. A total of six human individuals are sampled for this blind study. The results of this study show observed gut flora change over time as decomposition conditions change resulting in an increase of some species and the decline of others. Shortly after death, the number of observed microbes increases exponentially and decreases in the later stages of decomposition as the intestines no longer remain and other, soil microbes continue with decomposition, or the body and organs mummify to provide an inhospitable environment for the specific flora. The study includes two organisms specifically to observe the phenomenon of microbial competition in the process of decomposition. After publishing, the method of TSD estimation also permits acceptance as expert witness testimony in legal contexts, because the method is based from empirical tests, the error rates are known, and the method uses previously published sampling and processing practices so is generally accepted within the relevant, scientific community. The purpose of the study is to formulate a legally legitimate method for the estimation of TSD so individuals of varying technical backgrounds can understand and easily interpret results. The results obtained from this study of 16 human individuals additionally serve to provide a foundation for future work relating microbial patterns to the practice of Forensic Anthropology; this provides empirical methods off which the field can build.

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**Time Since Death, Gut Flora, PCR**

## **D7 Singular Skull Fractures Pattern in a Pedestrian**

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After attending this presentation, attendees will understand the importance and the necessity of a close collaboration between investigators and forensic pathologists in order to reach a comprehensive resolution of the case by integrating their skills.

This presentation will impact the forensic science community by detailing the importance of an accurate external examination of the corpse in order to understand events and cause of death.

The forensic pathologist along with his or her clinical trauma service colleagues, play a similar role in identifying injuries and mechanisms of death. Interpreting injury patterns can provide useful information for accident reconstruction. The injuries to the body can be the equivalent of a report from a reliable witness of the accident. Information that the pathologist provides helps law enforcement and survivors to understand what happened, provide details on how quickly they died, and give potential causes of the accident. Seldomly, forensic pathologists go to the scene of a motor vehicle fatality: however, viewing the scene can often provide vital clues about the dynamics of the accident and the source of the injuries, for example, looking for blood, tissue, or hair can reveal impact sites.

There are many factors to consider: the speed and type of the vehicle, adult versus child pedestrian (stature), position of pedestrian, etc. The causes of death in pedestrians are commonly head and neck injuries: skull fractures, epidural and subdural hemorrhages, cortical contusions, atlanto-occipital dislocation, and cervical fractures. Head injuries can be caused by the impact of the vehicle or the fall to the ground. Many autopsies are performed without information about the circumstances of death. The medical examiner must then attempt to determine the time, cause, and manner of death relying only on autopsy results.

In November 2006, a young woman was found dead on a country road. The postmortem interval was estimated to be about two to three hours prior to the recovery of the body. No witness to the murder was found. The crime scene investigation did not find traces of blood or signs of dragging on the ground near the location of where the body was found. A full autopsy was performed which identified a stab wound by a single edged knife localized in the abdomen and multiple abrasions and cuts on the upper limbs and thorax. However, these were very superficial to represent the cause of death. Also noted were a skull fracture and lacerations of the brain. The depressed circular skull fracture with fragmentation of the bone perfectly matched to each other. In the thickness of the front edge of fracture revealed a series of multiple and minute fractures. These multiple injuries were probably produced by a tool with multiple cutting edges. It was possible to assume that a screw plug located below the engine oil sump could have caused the head injury. Toxicology results were negative.

Investigators examined all the cars that had been identified near the area of crime scene. The morphological and metrical characteristics of the lesion of the head of the victim was then compared with those different engine oil sump screw plugs of those different cars, allowing investigators to identify the car model that could have possibly hit the victim.

In conclusion, it was hypothesized that the young woman was first stabbed with a single edged knife in the abdomen and then run over by a car. The car was finally identified thanks to information supplemented by the autopsy; moreover, blood traces were found on the bottom of the car, which were consistent with the blood type of the victim.

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**Pedestrian, Identification, Run Over by Car**

## **D8 Deaths Related to Epilepsy in Brazil From 2005 to 2009**

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After attending this presentation, attendees will gain basic knowledge about epilepsy deaths and the profile of victims who die of epilepsy in developing countries, as well as forensic and legal problems posed by this type of death

This presentation will impact the forensic science community because the study of deaths due to epilepsy calls attention to this kind of death, to the profile of the victims in developing countries and also in how to direct the postmortem examination to make the correct diagnosis of the problem.

The occurrence of death due to epilepsy is an issue of great importance, due to its legal and forensic aspects, and should be analyzed on all relevant matters. The identification of the main factors involved in the deaths of people with epilepsy may provide information for public adoption of public remedial action and also for solving problems related to the forensic cause of death. Sudden death in epilepsy (SUDEP) is well known and attracts scientists in the search for the identification of control mechanisms for these types of deaths in Brazil; however, there are few studies dedicated to this purpose. Hence the importance of this presentation is to alert doctors and authorities about the profile of this population in Brazil and help in prevention of this type of death. This problem occurs in various parts of the world. As stated by Leestma, "in most locales in the United States, and likely in other developed countries, such deaths are usually not attended by a physician and are without detailed historical or medical information. They will usually be brought to the attention of a medical examiner or coroner, who is responsible for determining the cause and manner of death and generate a death certificate before the remains may be interred or otherwise disposed of." The goal of this retrospective and descriptive study is to make a statistical analysis of deaths due epilepsy

in Brazil from 2005 to 2009, and to correlate age, sex, race, region of the country, and place of death. This will construct the profile of this population to assess possible factors that may impact public health policy and alert the medical examiners to at least suspect this type of cause of death. The data analyzed in this study were obtained by the Brazil Ministry of Health Department of Informatics (DATASUS), which provides information about the key health indicators, including information on vital statistic, at the national level. Deaths in Brazil for the last five years available in the DATASUS database were evaluated. All variables contained in it were incorporated into the study, including age (from zero to over 80-years-old), gender (female, male), race (Caucasian, black, brown, yellow, indigenous), region of Brazil (north, northeast, southeast, south, and Midwest) and place of death (hospital, other local health, home, street, other, or unknown). Nine thousand, three hundred, eighteen deaths due to epilepsy in Brazil between 2005 and 2009 were analyzed and represented a risk of death around 0.177 when compared with the total number of deaths in the country in the same period. The study consisted of 6,058 men, 3,219 women, and 41 whose data were not available. Approximately half of the deaths involved young adults between 20 and 49 (51.83%) of which the predominant age was between 40 and 49 (40.34%), followed by 30 and 39 years (34.8%). The Caucasian and African subjects predominated over other races at 46.51% and 36.62% respectively. The southeast region, the most populous in the country, as well as the northeast region accounted for the largest number of occurrences. There was a slightly higher prevalence of deaths in hospitals (46.6%) when compared to those which occurred at home (41.34%). The results of this study are consistent with others, especially regarding the subjects' age at the time of death and the predominance of males. Neither the exact description of the death or the characteristics of epilepsy in these subjects were available; however, the large number of events at home, age (young adults), and male predominance show that most of these deaths were due to SUDEP. Therefore, detailed analysis of deaths involving people with epilepsy is a necessity, given the large number of people affected by this disease.

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#### **Epilepsy, Death, Epidemiology**

### **D9 Investigation Techniques of a Child Death Involving a Dental Procedure**

*Sarah R. Weil, BA\*, and Martha J. Burt, MD, District 8 Medical Examiner's Office, 606 Southwest 3rd Avenue, Gainesville, FL 32601*

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After attending this presentation, attendees will recognize the importance of thorough scene investigations and continued utilization of medicolegal death investigation techniques in unusual or complicated medicolegal death investigations.

This presentation will impact the forensic science community by demonstrating the value of continued medicolegal investigation in supporting cause and manner of death.

Cases of child deaths as a result of routine dental procedures are extremely rare and require special investigative considerations. This is an unusual case of a previously healthy 5-year-old boy who went to the dentist for a routine dental procedure. The child was administered a dose of the sedative chloral hydrate. The child experienced respiratory distress during the procedure and was transported to a local hospital where the child died in the emergency room. The subsequent investigation revealed toxic levels of trichloroethanol, the metabolized drug chloral hydrate.

At the time the death report was received, the child had been pronounced deceased in the emergency department and initial information received was from an emergency room physician. Very little information was initially received, other than the child had been at a local dentist office for a routine procedure and had experienced respiratory distress and subsequent cardiac arrest. No known cause for these mechanisms of death existed as the child was reported to be healthy prior to this dental visit.

A scene investigation was initiated with local law enforcement and the local medicolegal death investigator. The dental office was thoroughly documented with photography and copies of medical records obtained from the medical file on-site. Dental equipment was thoroughly inspected for proper working order and initial interviews were conducted with office staff.

Interviews conducted with office staff provided a sequence of events that was later corroborated with testimony from the deceased child's mother. Additionally, the timetable provided by office staff was later corroborated with emergency medical service dispatch times and information from first responders.

An interview conducted with the child's mother revealed her son was previously healthy and had no known medical conditions. The child's most recent medical records were obtained as well as an accounting of the child's most recent physical activity.

Following the initial scene investigation, an autopsy was performed on the child. No apparent cause of death could be established from the gross examination. Further toxicology studies showed significantly high levels of trichloroethanol (the metabolite of chloral hydrate) indicating an overdose of chloral hydrate.

A follow up interview by the medicolegal death investigator was conducted jointly with the dentist and a law enforcement detective. During this interview, several questions were answered about the procedure for administering the chloral hydrate and the measuring device used.

Information was obtained about the chloral hydrate manufacturer and distributor to ensure there was no error in the concentration of chloral hydrate obtained, and therefore administered, by the dental office. Finally, as a result of a thorough scene investigation, the bottle of chloral hydrate collected as evidence was sent to the FBI Toxicology Laboratory for analysis and quantification of chloral hydrate concentration.

This investigation spanned over a year and required continuous involvement from the forensic pathologist, medicolegal death investigator, and law enforcement. Through the information gathered with a thorough initial scene investigation and continued extensive follow up and evaluation of the evidence in the case, many subtle and important questions were answered. The ability to take into consideration the special circumstances of a child death, especially one that occurs as a result of a routine dental procedure, allowed a thorough investigation to be conducted. This thorough investigation will hopefully provide insight into future incidences in which a seemingly benign procedure turns deadly.

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#### **Child Death, Chloral Hydrate, Dental Procedure**

### **D10 Unusual Suicides Using Common Mechanical Devices — Case Series and Literature Review**

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After attending this presentation, attendees will understand the scene investigation autopsy performance, and, reporting of unusual methods of suicide using common mechanical devices. Additionally, attendees will be better prepared to identify clues in these cases that have led to a ruling of suicide as the manner of death.

This presentation will impact the forensic science community by providing key information to help identify the manner of death in rare cases.

Suicide is the 11th leading manner of death in the United States of America. Suicides are classified by the Centers for Disease Control and Prevention in one of three ways: firearm suicides, suffocation suicides, and

poisoning suicides. The forensic community is very familiar with scene investigation and autopsy procedures for these types of suicides. More unusual methods of suicide may pose a challenge for scene investigators and forensic pathologists. By becoming familiar with some of the interesting and creative ways with which persons have committed suicide, scene investigators, coroners, and forensic pathologists will be better equipped to process and evaluate such scenarios. Additionally, this information can serve as a knowledge base which can be applied to unique but similar cases that are scarce within the academic literature.

The use of a circular saw and a power drill which yielded unfamiliar findings upon scene investigation as well as injuries rarely encountered on the autopsy table are reviewed. Interpretation of this information can yield more specific and accurate results if investigators are familiar with the type and severity of wounds that these devices inflict. It is of particular concern that these unusual methods might lead an investigator to consider homicide or accident as a manner of death when a case is actually a suicide.

Cases of suicide using common household mechanized devices are presented. These previously unpublished cases include death by circular saw, chain saw, and elastic-cord-saw apparatus.

Additionally, the existing literature to further supplement the information gleaned from the cases presented are reviewed. Although rare, reports in the literature have described suicides using electric drills, circular saws, band saws, pneumatic hammers, nail guns, and chain saws. Each scenario may have unique scene and autopsy findings important to consider when determining the manner of death.

The violent, unusual, and seemingly painful nature of these injuries may raise doubt as to whether they were truly self-inflicted, considering the numerous, well-known, and often minimally painful methods readily available. It is important to evaluate each individual case empirically, based on the history, the evidence documented at the scene, and the autopsy findings. This information must then be interpreted in the context of the relevant literature and the investigator's experience. The goal of this presentation is to assist investigators, coroners, and forensic pathologists in determining the manner of death in these rare cases.

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**Suicide, Forensic Pathology, Mechanical Devices**

## **D11 Firing Pin Aperture: Uncertainty of Measurement and Usefulness for Class Characteristics**

*Heather J. Seubert, BS, Erich D. Smith, MS\*, Theodore Chavez, BS, and Preston C. Lowe, MS, Federal Bureau of Investigation Laboratory, Firearms/Tool Marks Unit, Room 4340, 2501 Investigation Parkway, Quantico, VA 22135*

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After attending this presentation, attendees will gain a better understanding of: (1) the accuracy and reliability of firing pin aperture marks; (2) the use of discernible class characteristics during the examination process; (3) differences in the appearance of sequential test fires; and, (4) measurements obtained from test fires and casts of the actual firing pin apertures.

This presentation will impact the forensic science community by showing how discernible class characteristics for elimination can affect the outcome of the examination process.

In 1985, the Association of Firearm and Tool Mark Examiners (AFTE) Committee formalized the Criteria for Identification. Their goal was to reach consensus on articulating the three principles for the theory of identification as it related to tool marks – identification, inconclusive, and elimination. One of the examinations integral to the firearms/tool mark examination process is the evaluation of class characteristics, particularly when it comes to the measurements. Class characteristics are those features that are predetermined prior to manufacture (design features) and are restrictive to a particular group (i.e., caliber). For elimination, the AFTE criteria requires there to be a significant disagreement of discernible class

characteristics and/or individual characteristics. This study examines the threshold for what is a significant disagreement in class characteristics, through the evaluation of firing pin apertures using the same firearm with different brands of ammunition. When a cartridge or shotshell is fired, the base of the cartridge and primer is forced against the breech face under high pressures. These high pressures sometimes cause the primer to flow into the firing pin aperture creating an outline of the firing pin aperture. When a firearm is manufactured, the size and shape of a firing pin aperture is designed to an acceptable tolerance to allow for the proper passage of the firing pin. A difference in firing pin aperture dimensions between two cartridge cases would indicate a difference in class characteristics, thus allowing for elimination. This study evaluates this effect, the measurement, and the impact of uncertainty of measurement when making an elimination.

In a cartridge case comparison, where firing pin aperture marks are present, the measurement of this feature may be considered a discernible class characteristic. In this particular study, each examiner and trainee made a series of firing pin aperture measurements of test fired shotshells and cartridge cases. These measurements were recorded and the uncertainty of measurement was determined to evaluate the threshold of elimination based on measurable differences. Two types of 9mm Luger ammunition, CCI Blazer, and American Eagle (Federal), were test fired in a Cobray M-11/Nine pistol. The CCI Blazer ammunition included an aluminum cartridge case with a nickel primer, while the American Eagle ammunition consisted of a brass cartridge case with a brass primer. Additionally, two types of 12 gauge ammunition, Winchester #8 Shot and Winchester 00 Buckshot, were test fired in a Mossberg 500-A shotgun. The Winchester 12 gauge, 2 3/4, #8 shot shotshell contains a low brass, gray hull, and copper primer. The Winchester 12 gauge, 2 3/4, 00 buckshot shotshell contains a low brass, red hull, and copper primer. Casts made of the breechfaces, to include the firing pin aperture on the pistol and shotgun, were collected prior to test firing. Both the apertures present on these test fires and those present on casts of the actual firing pin apertures were measured for this study. Prior to performing any measurements, a performance check was conducted on the stage micrometer using a National Institute of Standards and Technology (NIST) traceable calibrated caliper. The measuring platform was also checked to determine if it was level. One comparison microscope was used for all measurements. There were a total of eighty samples – forty shotshells and forty cartridge cases. Each sample was marked with a permanent marker to indicate the position of the extractor mark. There were two measurements recorded for each shotshell and cartridge case – one with the extractor in the three o'clock position and one in the six o'clock position. The approximate line of symmetry for each firing pin aperture was determined using the crosshair reticule. Once the line of symmetry was found, the micrometer was zeroed and the diameter on the aperture was measured. Each participant then recorded their results of the measurements on a data sheet. It should be noted that differences were observed in the general appearance of firing pin aperture marks on the two types of cartridges that were test fired. This presentation will provide the firearms examiner with some useful data regarding the accuracy and reliability of firing pin aperture measurements and their use as a discernible class characteristic.

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**Firing Pin, Uncertainty, Elimination**

## **D12 Elder Abuse: A Simple Likert Assessment Tool for Investigators**

*Laura La Cagnina, RN, MSN\*, 1980 Kramer Way, Marietta, GA 30062*

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After attending this presentation, attendees will be able to: (1) identify the top five criteria that place elders at risk for abuse; (2) identify the top five criteria that place caregiver at risk for being abusers; and, (3) demonstrate the correct use of a Likert Scale assessment tool.

This presentation will impact the forensic science community by giving investigators a simple tool to assist in determining credible risk

factors for victims to be abused as well as risk factors for caregivers to be abusers.

Primary care medical professionals, as well as emergency department medical staff, continue to have limited face-to-face time with clients. The fact is, more elders are not being assessed for abuse until the abuse is blatantly evident. Part of this problem is that as our aging population numbers grow, there is no standardized assessment tool to determine risk factors for elders.

While there is physical abuse, there are types of elder abuse that are not as easily visible. Elder abuse can take place in the elder's home, assisted living, nursing home, or living with other family members. Financial exploitation, sexual abuse or harassment, emotional intimidation or humiliation, and healthcare fraud are not as easily witnessed or assessed. Non-accidental injuries that cause pain, misuse of drugs, restraints, and confinement may only be circumstantially evident. Intimidating, isolating, and ignoring behaviors or verbal threats that may not be witnessed. The use of an assessment tool can aid the investigator by establishing baseline criteria to assess risk.

There are spouses, adult children, other relatives and friends who find care giving of an elder to be satisfying and enriching; however, the responsibilities and demands of elder care giving, which can escalate as the elder's condition deteriorates, can take on abusive coping mechanisms. Caregivers are not immune to the stressors that everyday life, work, family, and financial responsibilities include while providing care to a loved one. Assessing caregivers, professional and non-professional, at risk to abuse is equally as elusive as assessing the risk of the elders they abuse.

Vulnerable populations include the economically disadvantaged, racial and ethnic minorities, those with severe mental illness, children, homeless, women and the elderly to name a few. The vulnerability of these individuals is enhanced by race, ethnicity, age, sex, and factors such as income, insurance coverage (or lack thereof), and/or an absence of a usual source of care. Their health and healthcare problems intersect with social factors, including housing, poverty, and inadequate education. We already understand that familial caregivers have an increased risk to abuse. When an elder entrusts caregivers who themselves may be part of a vulnerable population for assistance with regular, everyday activities of living, risk factors for both will increase and can go undetected.

While the medical community has standardized many assessment tools such as pain, wound, and coma scale, it has not agreed on any to assess risk for abuse. Dr. Miri Cohen developed an assessment tool that included both the elder and the abuser. This Expanded Indicator of Abuse (E-IOA) tool is a lengthy comprehensive assessment tool. Using the Likert scale, Dr. Cohen's tool is eight pages long and is numbered as to which questions should be asked of each. Most primary care providers (PCP) spend less than 20 minutes with patients and emergency care providers usually spend less than 25 minutes. Neither has enough time to include any additional assessment tools. Technology may have assisted in expediting certain information but any time saved was immediately replaced by another patient.

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**Elder Abuse, Assessment Tool, Risk Factors**

## **D13 The Role of the Forensic Nurse Examiner and Persons With Disabilities**

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*Janean M. Fossum, RN, BSN\*, PO Box 11053, Eugene, OR 97440*

After attending this presentation, attendees will be able to: (1) identify the severity of violence in the community of individuals with disabilities; (3) identify the importance of the history and interview, recognize the signs of abuse as it relates to individuals with disabilities; and, (4) recognize the special needs of the individual with disabilities and the role of the forensic nurse or forensic nurse examiner.

This presentation will impact the forensic science community by increasing awareness that persons with disabilities represent a population who experiences a greater incidence of abuse.

Historically, persons with disabilities represent a population who experience a greater incidence of abuse or neglect. According to experts, over ninety percent of all persons with disabilities will experience one or more abusive episodes during their lifetime. The reason for this phenomenon is multifaceted and complex. Contributing factors have been identified within the body of research, which includes decreased understanding of their rights, group home or institutional living conditions, lack of boundaries, diminished knowledge, or education level; and stereotyping portrayed by the public. Therefore, raising awareness and educating the public regarding this issue should be a public health priority. Unfortunately, abuse against persons with disabilities is significantly underreported to law enforcement and human service agencies, and even ignored by their family and/or care providers. All too often, the responsibility for identification and reporting of abuse are placed upon the medical community thus leaving the medical provider in this pivotal role.

Forensic nurses are specifically educated to provide professional nursing care; this includes holistically assessing individuals through the nursing process for abuse and neglect. This information provides investigators with valuable details that otherwise would be overlooked. One of the major roles identified within the field of forensic nursing is bridging the gap between the legal and medical arenas; therefore, forensic nurses by nature of their role are uniquely positioned to assist the disabled individual who may be experiencing abuse or neglect. Furthermore, it is this reason that the forensic nurse is such a significant element of any medical examination or in any situation where another medical person suspects abuse or neglect. The forensic nurse is educated as a registered nurse and trained in forensics. Through the application of forensic principles related to evidence collection, the forensic nurse documents injury through multiple means; thereby, assisting the investigator and exploring the possibility of abuse and neglect with the patient. Additionally, the forensic nurse provides community resources that are customized for the disabled individual based on the nurse's assessment. Moreover, safety issues, mandatory reporting, and protection for this vulnerable population is one of the primary functions that a forensic nurse provides in their plan of care.

This presentation will examine the many indicators of abuse and neglect that medical providers and investigators should be aware of regarding abuse with specific focus on the disabled population. Through case presentation, the attendee will identify through comparative analysis, accidental versus non-accidental injuries. Additionally, a statistical review will reveal disabled individuals as the prevalent vulnerable population. Moreover, it will provide the audience with a greater knowledge base and a greater understanding of the victimization within this population. The forensic nurse's role will be investigated and clear delineations will be given regarding the significant responsibility the forensic nurse holds and contributes in abuse investigations with disabled individuals. Specific information will be provided regarding the offender profile and the process of reporting and documenting information. However, the greatest focus will be the significant impact the forensic nurse can make regarding investigations concerning individuals with disabilities.

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**Abuse, Disabilities, Forensic Nurse**

## **D14 Do Forensic Nurses Have a Role in Child Maltreatment Investigations?**

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*Melodie Brooks, MSN\*, 3091 Widdock Street, Erie, MI 48133*

The goal of this presentation is to identify the role of the forensic nurse within the multidisciplinary team (MDT) as well as describe the solutions to MDT challenges in rural communities regarding the medical component.

This presentation will impact the forensic science community by case study exploration leading the attendee to realize the significant and unique role the forensic nurse assumes within the multidisciplinary team (MDT) during a child maltreatment investigation.

Child maltreatment investigations traditionally have excluded medical professionals who are specialized and trained in this emerging field, which is contradictory to experts' recommendations.<sup>1</sup> Moreover, a lack of qualified medical experts has compounded this problem, leaving investigators no alternative but exclusion of the medical component during their investigations. On the other hand, many MDT's are utilizing the expertise of a forensic nurse examiner, propelling this nurse specialty into the forefront of the interdisciplinary team based on the responsibility of linking the legal arena with healthcare, and placing the nurse within the coordinator of care role.<sup>2</sup> According to the American Association of Colleges of Nursing (AACN), one of the essential roles of a registered nurse is to be a coordinator of care, specifically engaged with an inter-professional team.<sup>3</sup> Further exploration within this role can reveal a unique set of skills, which is invaluable during child maltreatment investigations. These activities include medical records review, interpretation of chronic/acute physical findings, and education to the investigative team regarding medical issues and sexual development.<sup>2</sup> Consequently, forensic nurses who work within child maltreatment teams serve a very diversified but significant purpose.

As logical and practical as the forensic nurse role may appear, conflicts and growing pains still persist in some organizations attempting to bridge the gaps that may exist in the MDT regarding medical exams. With the current economic crisis looming in the United States and abroad, communities are faced with limited resources and personnel; however, these entities are held to the same standards and judicial outcomes by the public and the legal system's burden of proof.<sup>4</sup> Moreover, many juries have an expectation of physical evidentiary findings during trials, thus leaving the investigators and prosecutors disadvantaged if the child's testimony is the sole piece of evidence. Budgetary reductions within law enforcement and prosecutor departments have compounded the problem and many communities are forced to reexamine services to victims. With challenging economic times and limited resources, new innovative ideas must emerge to meet these deficits.<sup>4</sup> Through education, determination, and evidenced based practice principles, MDT's have the ability to flourish and provide quality outcomes for children and their families through inclusion of forensic nurses on the MDT.<sup>1</sup> As the coordinator of care, nurses provide education to patients and families; however, forensic nurses have an added educational responsibility of instructing juries on medical findings with patients entrusted to their care.<sup>5,6</sup>

This presentation will describe through case studies the challenges and solutions faced by a rural community MDT providing investigative and support services to children experiencing child maltreatment. The participant will view the significant role the forensic nurse examiner assumes within the MDT and the importance of the coordinator of care role. Evidenced based practice principles will be emphasized and the participants will leave with ideas for MDT development in rural community settings when attempting to fulfill the medical role.

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#### Forensic, Nursing, Maltreatment

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## D15 Win in Court - Analysis of a Home Environment to Make It Wheelchair Accessible

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After attending this presentation, attendees will understand how to: (1) make the case in court for or against the extent and construction costs of wheelchair-accessible home renovations; (2) determine what is needed to base the case; (3) evaluate the home, determine what is required to make it accessible and usable, and design renovations; (4) Write an effective report; and, (5) know what exhibits are needed.

This presentation will impact the forensic science community by providing a technically correct and legally solid path to make a successful claim for plaintiff or defense for defendant in court for compensation to make necessary accessibility changes to the home of a claimant injured or diseased.

**Making the case:** Determine the nature of the client's needs as determined by the data of medical reports, life-care plans of both plaintiff and defendant, existing home conditions (floor, site plans, and photographs), and local costs of construction. Life care plans are typically divergent in their evaluations of the disabled client, and thus possibly quite different in evaluating the disabled client's needs, both currently and especially in the long term or end of life evaluation. This could have significant effect on what modifications are determined to be needed in order to make the client's home accessible and usable.

**What is needed to base the case legally:** Review the data for consistency, engage expert opinion by an architect experienced in both the disability needs of the client and residential design, and write a report supporting the case in narrative and graphic (drawings & photographs) form and include defensible construction estimates consistent with local building conditions. Where significant divergence is found between plaintiff and defendant, it is important to try at that point to obtain agreement between the life care planners as to what is actually required for the disabled person. If agreement cannot be reached by the life care planners, then design and cost estimates for construction must proceed on the basis of either the plaintiff or the defendant, whoever the expert is working for. This situation will put the onus of decision on the court and will necessitate a trial by judge or jury to determine the outcome.

**Design:** It is important to engage an experienced architect whose practice demonstrates expertise in design for persons who have disabilities, and who can reliably prepare designs and construction cost estimates for the locale in which the claimant's home is situated. If engaged at an early stage, the architect may contribute valuable guidance as to what data must be acquired and may advise the attorney as to the best path of strategy to win. The architect should be able to technically defend or oppose a case made by his or her testimony in deposition or at trial. The architect should be conversant, experienced, composed, and articulate in the sometimes adversarial atmosphere of legal testimony.

**Writing the report:** The expert report in support of or to defend against the claim must consist of accurate, comprehensive, and persuasive narrative, supported by clear, descriptive graphic floor plan drawings and credible construction cost estimates predicated upon local conditions.

**Exhibits:** Exhibits need to be brief and cogent in their narrative and clear in their presentation. The narrative should be tied, issue by issue to a set of graphic documents (drawings) and to an itemized list of construction items. An example case will be presented as representative and will be included in the handout.

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#### Wheelchair, Injury, Plaintiff or Defendant

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## **D16 Post-Coital DNA Recovery Phase 2: Early Results From Participating Couples**

*Patricia M. Speck, DNSc\*, University of Tennessee Health Science Center College of Nursing, 877 Madison Avenue, Suite 653, Memphis, TN 38163; Jack Ballantyne, PhD, University Central Florida, Department of Chemistry, 4000 Central Florida Boulevard, Orlando, FL 32816-2366; Pamela D. Connor, PhD, University of Tennessee Health Science Center, Department Preventive Medicine, 600 Jefferson, Memphis, TN 38105; Marion L. Donohoe, DNP, University of Tennessee Health Science Center, College of Nursing, 877 Madison Avenue, Memphis, TN 38163; Erin K. Hanson, PhD, PO Box 162367, Orlando, FL 32816; Wendy Likes, DNSc, DNP, University of Tennessee Health Science Center, College of Nursing, 920 Madison Avenue, Memphis, TN 38163; and Ann Cashion, PhD, College of Nursing, 920 Madison Avenue, Memphis, TN 38163*

After attending this presentation, the attendees will be able to review factors that may impact DNA detection after coitus as found in Phase 1 of the Post-Coital DNA Recovery Study<sup>1</sup> and will receive the results of Phase 2. This is a feasibility study on the methods and tools used in the Post-Coital DNA Recovery research in an early sample of participants.

This presentation will impact the forensic science community by providing attendees to plausible biological environmental explanations that impact DNA recovery (Phase 1) and present the research findings from the early group of couple participants (Phase 2) enrolled in the post-coital recovery of DNA.

The current published forensic science evidence implies that recovery of DNA occurs from the posterior fornix within a 36 hour time frame and from the cervix up to 72 hours. Medical infertility literature and one forensic study found that the cervix might yield DNA up to 72 hours. One forensic science publication found DNA at five days using routine analysis. When additional Y-STR methods were applied, DNA has been found up to seven days. Phase 1 of the Post-coital DNA Recovery research project brought together subject matter experts (SMEs) in 2010 to identify the biological and environmental factors that impact the recovery of DNA.<sup>1</sup> This presentation will briefly review Phase 1, including history of the 72 hour time limit for evaluation of victims and the methods for identifying factors that influence recovery of post-coital DNA. Attendees will be introduced to the IRB approved protocols and the feasibility study (Phase 2) of the first few couples that evaluated the procedures, methods, tools, and analysis of the blinded methodologies and evidence-based tools used by the data collectors. In the feasibility study (Phase 2) and the full-scale study (Phase 3), the data collectors and the evaluator will be blinded to the activities in the forensic laboratory. Demographic data will also be blinded to the data collectors, the forensic laboratory and the evaluator in Phases 2 and 3; however, the focus of the presentation will be on Phase 2, the barriers in the protocol for collection of sample data, the strengths in the implemented methods, reliability of the tools used to evaluate the environment of the vagina and cervix, and the reliability of the results of the data analysis from the first group of monogamous couples submitting samples according to the Post-coital DNA Recovery protocol. In summary, this presentation will briefly review Phase 1 identification of the biological and environmental barriers to locating DNA post coitus and present. Phase 2 that evaluates the feasibility of the methods, tools and protocols, as well as provide an explanation about the appropriateness and difficulty of using the new tools designed to capture specific characteristics of the female at the time of collection of the post-coital sample. These results are expected to provide specific insight for the researchers about the process and outcomes from the chosen methods so they may proceed to the full-scale study (Phase 3) with up to 150 couples entitled Post-Coital DNA Recovery. The feasibility results from Phase 2 will be presented and discussed.

### **Reference:**

- <sup>1</sup> Speck, Patricia, et al. From the Bed to the Bench: Defining the Vaginal and Cervical Environment for Post-Coital DNA Recovery. *Proceedings of the 63<sup>rd</sup> Annual Scientific Meeting of the American*

*Academy of Forensic Sciences, Feb 21-16, 2011 Chicago, IL. Colorado Springs, CO: American Academy of Forensic Sciences, 2011*

## **Post-Coital DNA Recovery, DNA, Rape and Sexual Assault**

## **D17 Sexual Assaults in Geneva, Switzerland: 2006-2010**

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After attending this presentation, attendees will understand the incidence and the prevalence of the sexual assaults of female victims in Geneva in the last years, including the reasons which encourage formal complaint. Results of toxicological analyses performed on victims of sexual assaults are also presented.

This presentation will impact the forensic science community by explaining the necessity of collaboration between the gynecologist and the forensic pathologist with the utilization of a kit. Finally, this study looked at socio-demographic characteristics of the victims of sexual assaults such as age, site of offense, marital status, numbers of offenders, time of offense, and time from the offense until the examination.

The number of women who visit the Maternity of Geneva, the hospital which is a part of the department of Gynecology and Obstetrics at the Faculty of medicine of Geneva, for sexual assault cases has been increasing in recent years from about 20 cases per year in the late 1990s to about 100 cases per year in the years leading up to 2010. For this reason, a protocol was established so that all victims of sexual assault are supported in the same way, no matter when they may arrive.

In Switzerland, since April 1, 2004, rape or forceful sexual relations among spouses is considered in the same way as with strangers, and it is prosecuted automatically and not just treated as a complaint. The incurred penalty is a maximum of 10 years, if no other aggravating factors are involved, such as the use of weapons or the jeopardizing of the life of the victim. The women who consult medical professionals after a sexual assault do so primarily for therapeutic purposes, that is to say, to rapidly receive care (disinfections of wounds, etc.), to detect and treat sexually transmitted diseases, including hepatitis and the HIV virus, and to detect a possible pregnancy (emergency contraception). Victims also seek relief for psychological trauma, where they are heard and understood. If the psychic trauma is sufficiently affected, a psychiatrist sees the patient in the emergency department or room and decides whether she should be hospitalized or not. In other cases, the victim is sent for a consultation at the Interdisciplinary Medicine and Prevention of Violence unit the next day, where doctors and psychologists coordinate and take the necessary steps to care for the victim.

In Geneva (450,000 inhabitants), all female victims of sexual assaults who undergo a medical examination are examined by both a gynecologist and a forensic pathologist, even if the victim has not formally made a complaint. Indeed, different situations are imaginable. A victim can arrive with the police after they file a complaint. The victim can also depose a complaint after the medical examination. The third possibility is that the victim waives a complaint. In this instance, respect for medical confidentiality is maintained and prosecutorial action is not taken.

The role of the forensic pathologist is to perform a complete examination of all lesions observed on the body and to ensure that all necessary specimens are taken in case of a complaint, or in the case the victim changes her mind and makes a complaint in the future. A certificate, with all the findings, is systematically given to the victim ten days afterwards.

Finally, this study looked at socio-demographic characteristics of the victims of sexual assaults such as age, site of offense, marital status,

number of offenders, time of offense, and time from the offense until the examination.

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**Sexual Assaults, Geneva, Socio-Demographic Characteristics**

## **D18 Forensic Experiential Trauma Interviews – A Conversation With the Brainstem**

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The goal of this presentation is to familiarize the attendees with the impact of physical and emotional trauma on a victim's ability to accurately recall key data points needed in criminal investigations.

This presentation will impact the forensic science community by providing an understanding of how trauma victims undergo physiological changes in the brain stem during trauma which causes memory and recall to be reduced.

Trauma victims undergo a process many professionals and victims do not commonly understand. Most criminal justice professionals have been trained to believe when an individual experiences a troubling event, particularly, emotional or physical trauma, the cognitive brain records the vast majority of the event including: who, what, where, why, when, and how, as well as peripheral information. Therefore, when the criminal justice system responds to the report of a crime, most professionals are trained to obtain this type information. Sadly, collecting information about the event in this manner actually inhibits memory and the accuracy of the details provided. Trauma victims do not record the experience in the same way most non-trauma victims do. Research shows that the body and brain react to and record trauma in an entirely different way than we have been led to believe. When trauma occurs, the cognitive brain frequently shuts down leaving the brainstem to experience and record the event. The brainstem does a great job recording experiential and sensory information but does not record traditional investigative information very well. Most of our interview techniques have been developed to interview the cognitive brain and obtain cognitive information such as the color of shirt, description of the suspect, time frame, and other important information. Some victims are capable of providing this information in a limited fashion. However, most trauma victims are not only unable to accurately provide this type of information, but when asked to do so often inadvertently provide inaccurate information and details which causes the fact finder to become suspicious of the information provided. The vast majority of our training and experience has caused us to focus on the cognitive brain. Research clearly shows the cognitive brain is not generally involved in recording the experience when a victim is experiencing trauma, whether physical (i.e., a sexual assault) or emotional (i.e., witnessing a horrific death). We must develop and implement proven methods to properly interview the brain stem. This innovative and revolutionary interview technique is a way to interview the brain stem in a manner that not only reduces the inaccuracy of the information provided but greatly enhances understanding of the experience, increasing the likelihood of a better understanding of the event and ultimately, the collection of better investigative information. This interview technique revolutionizes the manner in which forensic physiological evidence is identified and collected. The Forensic Experiential Trauma Interview has already been proven to be a game changer in the investigation and prosecution of many forms of violence including child abuse, adult sexual abuse, and domestic violence. This technique enhances crime scene investigation by enabling trauma victims to recall critical information in a more thorough manner than traditional interview techniques.

This interview technique draws on the best practices of child forensic interviews, critical incident stress management, and motivational interview

techniques combining them into a simple three pronged approach to unlock the trauma experience in a way we can better understand.

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**Trauma, Memory, Physiological Evidence**

## **D19 Profiling as a Utility to Criminal Interrogations**

*Alan A. Price, MA\*, University of Northern Colorado, PO Box 336433, Greeley, CO 80633*

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After attending this presentation, attendees will understand the significance of incorporating seven basic functions of profiling when conducting interrogations.

This presentation will impact the forensic science community by informing investigators of seven profiling components that should be integrated into a criminal interrogation.

Criminal profiling consists of seven integrated components: (1) the investigator's knowledge and experience; (2) the crime scene; (3) victimology; (4) offender behavior before and after the crime; (5) analysis of direct evidence; (6) crime analysis of the *modus operandi*; and, (7) knowledge of the "case controls" used to validate information being provided by the suspect. This presentation will show how and why all seven of these profiling components should be thoroughly assessed prior to initiating an interrogation.

As a prelude to this discussion, it must be noted that interrogating a suspect is not the same as interviewing one. These two investigatory functions are distinctly different and defined by law as having two separate legal standards. As such, the terms cannot be used interchangeably. For this presentation, the focus is on interrogation and specifically, how criminal profiling is used during the interrogation process to manipulate a suspect into confessing and providing details of their participation in a crime.

A skilled interrogator must be experienced and have a good knowledge of human behavior. Additionally, the interrogator must be knowledgeable in a suspect's neurolinguistics and kinetics. Being able to recognize and interpret a suspect's body language during interrogation is crucial.

The interrogator must have a comprehensive knowledge of the four different types of crime scenes (organized, disorganized, confined and extended) and what each one contributes to revealing the personality of the offender. Frequently, the crime scene may exemplify the mental culpability of the suspect during the commission of a crime. Was there premeditation or did the offender supply his own instrumentalities for the commission of the crime? This alone might provide the offender with an alibi of self-defense versus premeditation.

A strong understanding of victimology is imperative for constructing questions to present to the suspect at the time of interrogation. Interpreting what happened to the victim is essential. Whether the victim is alive or deceased, the investigator must be able to decipher the interaction that transpired between the victim and the suspect. Examining and understanding whether the victim is associated with a high, medium, or low lifestyle risk category is very important.

It is vital for the interrogator to have a working knowledge of psychology and basic principles of psychiatry in order to determine the possible motivation for the crime. For example, understanding the motivation of the arsonist compared with that of the pedophile are entirely different and the interrogator must understand and deploy these distinctions against a suspect during the interrogation. These issues may lead to questions of insanity or mental incompetence by the defense.

The interrogator must have a strong knowledge of the direct or physical evidence in the case being investigated. Sometimes this is the most challenging of all seven components. Evidence collected from the victim, the crime scene, or the suspect requires time to have it analyzed in a forensic laboratory. Most frequently the more violent a crime, the more

physical evidence is available. Being able to confront a suspect with forensic findings can frequently weaken an individual's alibi or denial.

Crime analysis of the *modus operandi* is a crucial analytical process that contributes significantly to assessing possible patterns of serial crimes. Patterned behaviors of the suspect before, during, and post-crime activities become very beneficial in assessing serial patterns. Knowing these patterns prior to the interrogation, an investigator can prepare questions that only the suspect is going to be able to validate. Any suspect's signature left at a series of crime scenes can eliminate "copy cat crimes" or crimes with a similar *modus operandi*.

The interrogator should have knowledge of existing "case controls," since those "case controls" are a means of validating information being disclosed by a suspect, and they can contribute to the various interrogation strategies. Only case investigators should have knowledge of this information. A skilled interrogator must have the ability to elicit specific information from a specific crime without disclosing the "case controls."

By incorporating these seven components of profiling into an interrogation, the investigator can deploy the appropriate strategies and props for guiding the suspect into providing valid confession.

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#### **Criminal Profiling, Interrogation, Case Controls**

## **D20 Forensic Polygraphy in a Global Environment: Comments on the Worldwide Growth of an "American Obsession"**

*Frank Horvath, PhD, National Academy for Credibility Assessment, 7540 Pickens Avenue, Fort Jackson, SC 29207-6804; and Thomas P. Mauriello, MFS\*, 8775 Teresa Lane, Laurel, MD 20723*

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After attending this presentation, attendees will be educated on the early history of "lie detection" in the United States and how it influenced the public perception of the role of forensic sciences in the resolution of criminal investigations. More to the point of the theme of this AAFS meeting, the audience will also learn about the global growth of "lie detection," a component of forensic Credibility Assessment, as it has been adopted in countries outside of the United States.

This presentation will impact the forensic science community by revealing a rationale for global growth in the field of forensic polygraphy and demonstrating how U.S. based developmental features in that field specifically and the forensic sciences generally, good and bad, have been influential elsewhere.

In his recent book, *"The Lie Detectors,"* historian Ken Alder, focused on the personalities credited with the early development of the "lie detector." Leonarde Keeler, Dr. William Moulton Marston, and Fred E. Inbau are prominently featured. However, Alder's perspective, generally, is antagonistic. "The "lie detector," he says, is a peculiarly American device. Americans, and Americans alone have been obsessed with the "lie detector."" "Why, despite the avalanche of scientific denunciations, does the United States—and only the United States—continue to make significant use of the lie detector?"

Alder answers his own question by stating that: "The lie detector has thrived in America because the instrument played into one of the great projects of the twentieth century: the effort to transform the central moral question of our collective life—how to fashion a just society—into a legal problem." "In the end, though, we believe in the lie detector because—no matter what respectable science says—we are tempted." The "lie detector," more appropriately, the polygraph instrument, was indeed fashioned in the United States. The use of that instrument, in its early history, was given extraordinary media attention. And, of course, the device captured the public imagination as it played a key role in many of the most heinous and media-driven criminal investigations of the time, such as the Lindbergh kidnapping. The "lie detector" focused attention on the forensic sciences in the way that DNA has today. Much of the attention was driven by the expectation that the "lie detector" would forever alter social relations;

criminal activity, because it could no longer be hidden from the authorities, would be dramatically reduced. Some of this thinking was the result of promotion by early practitioners. Dr. William Moulton Marston, for example, was especially prominent. He promoted in many ways, one still popular today: his comic book character Wonder Woman, who with her magic lasso could ensnare the most pathological liar and learn the truth.

Aside from the media attention and the sensationalized publicity "lie detection" received, there was also a more serious side. The "lie detector" became one of the mainstays in the nation's first Crime Laboratory in Chicago, eventually to be headed by one of the early Presidents of the American Academy of Forensic Sciences, Fred E. Inbau. He deserves credit for taking "lie detection" with a polygraph seriously. He brought to forensic polygraphy a sense of professionalism and a belief that it deserved a proper place among the various forensic techniques.

History shows that the polygraph was used in Europe since at least the 1950's, possibly earlier. An astute observer of polygraphy today, though, would surely see that the field has been and is continuing to expand dramatically, more so outside of the United States than within. This is not because American gimmickry is easy to pass on to naive audiences. Nor is because other countries wish to be foolhardy, to defy the ostensible wisdom of American criminal courts and scientific opinion as Alder argues. The record shows that there is clearly something more going on here. It is undeniable that in spite of its many flaws and limitations, the field of forensic polygraphy is growing dramatically around the world. Why is this so?

In this presentation the growth in forensic polygraphy will be assessed using a number of primary sources of evidence: membership rosters in professional associations, the development of training schools catering to those with an interest in polygraphy, the interest shown in U. S. government-sponsored attendance at international conferences on polygraphy, published descriptions of the development of polygraphy in a number of countries, and internet-based searches of popular media focused on polygraphy-related articles of interest.

In addition to discussing the growth of polygraphy on a global basis, in this presentation there also will be commentary devoted to polygraph testing as it can be seen in contrast to other forensic techniques. Some of this will be revealing of a rationale for global growth. In reinforcement of that rationale, the presentation will conclude with a discussion of empirical data collected to uncover reasons for the growth in polygraphy as seen by those outside of the U.S. who have experienced it firsthand.

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#### **Forensic Polygraphy, Worldwide Lie Detection, Forensic Credibility Assessment**

## **D21 Privileged Access: Trained Listening to Serial Killers Yields Insights**

*Katherine Ramsland, PhD\*, DeSales University, 2755 Station Avenue, Center Valley, PA 18034*

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After attending this presentation, attendees will learn about historical efforts in psychology and psychiatry to identify factors in the development of extreme offenders (serial and mass murderers), and how these efforts became the foundation for today's neuroscience of violence.

This presentation will impact the forensic science community by illustrating how intensive professional interviews may have drawbacks and benefits of clinical associations with extreme offenders.

Serial killer Ted Bundy once said that keeping him alive to study would provide valuable insights about the type of extreme violence he committed. Others have echoed him. Over the past century, some mental health experts took this idea seriously. They ventured beyond the typical evaluation period and devices to thoroughly explore a specific criminal's mind. These singular in-depth studies, starting with nineteenth-century "criminal autobiographies," offer tools and techniques for spending productive clinical time with violent offenders. Although some theories have been discarded as psychiatric fashions evolved, interviewers continue

to seek clarity about motives, criminal behavior, and viable therapeutic interventions. We have more than a dozen case examples of extended professional interviews with serial and mass murderers from the past century from which to learn.

Thanks to clinical training, coupled with privileged access, these professionals have provided ideas about what makes the most perverse serial or mass murderers tick. No one is better positioned to offer intimate details than experienced professionals who know how to examine an abnormal mind.

Ever since the earliest days of psychiatry, “alienists” have tried to understand the violent acts of the criminally insane. At first, they believed that anyone who acted contrary to rational sense must be psychotic, but then a certain type of rational criminal stood out. In 1809, Philippe Pinel became the first to acknowledge the disturbing behavior of psychopathy. Following him, other wardens of insane asylums studied “moral insanity” to learn how the faculty for socially appropriate behavior could become corrupted.

French pathologist and jack-of-all trades Andre Lacassagne first urged offenders to tell their stories in full, instigating what he called criminal autobiographies. He encouraged a number of prisoners to write about themselves, and each week he checked their notebooks, correcting and guiding them toward insight. He learned that their family histories were full of violence, tension, and disease, so he developed a theory about social criminality.

During the 1930s and 40s, Dr. Fredric Wertham examined a demented deviant named Albert Fish, who cannibalized a child, and Karl Berg interviewed sexual sadist Peter Kürten. Two decades later, Marvin Ziporyn befriended mass murderer Richard Speck, who’d killed eight nurses in Chicago in a single night. While Ziporyn thought Speck’s upbringing was overly protective, he also considered the unique new idea of a brain disorder. As serial murder became more prevalent, more professionals have taken time with these offenders to gain greater comprehension. Today, neuroscientists are putting criminal psychopaths through MRI machines.

Collectively, psychologists and psychiatrists who have used their training and skills to probe the minds of these extreme criminals have retrieved important information about motives, pre- and post-crime behavior, fatal fantasies, mental rehearsal, compartmentalized personalities, and the role of mental disorders. Thus, it has become possible to isolate recurring conditions and factors. From the first person who believed that criminals had self-insight to today’s advanced technological approach, much has been learned from extended, engaged professional listening about how and why some people commit shocking acts of violence.

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**Criminal Autobiography, Violence, Serial Killer**

## **D22 The Evolution of Polygraph in the People’s Republic of China**

*John J. Palmatier, PhD\*, Dawn Associates, LLC, 8600 Northwest 53 Terrace, Suite 121, Miami, FL 33166*

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After attending this presentation, attendees will understand the legal system that has facilitated the growth and application of the polygraph process in the People’s Republic of China (PRC), as well as significant contributors and the probable future for polygraph in China.

This presentation will impact the forensic science community by providing a glimpse of how an applied forensic science began in, and is used by, a country comprising approximately one fourth of the world’s population.

In 1947, United States Army asked the Michigan Department of State Police to assist in the training of military officers from the forces of the Kuomintang, the Nationalist Chinese party who were at war with the communist following World War II. In 1990, Dr. Palmatier, then a PhD

candidate at Michigan State University and a Detective Sergeant with the Michigan State Police, was the first foreigner to be asked by the PRC to present information regarding the assessment of credibility using the polygraph process.

In 1991, Dr. Palmatier traveled to China where he gave several seminars and demonstrated the use of an analog polygraph instrument to members of the Ministry of Public Security, the armed police and members of the military. The relationships formed continue today and provide a unique insight into the entire history of China’s quest to procure, develop, and apply the polygraph process for the purposes of assessing credibility.

This presentation begins with a short discussion of the Chinese legal system, which is substantive in nature, and why it is conducive to the use of polygraph as compared to Western legal systems, which are procedural in nature and questionably focused on the truth. This is followed by a discussion of the actual history, which began in 1947, but was used by the Kuomintang to root out communists during the 1947 – 1949 Civil War, which created a great deal of skepticism regarding the validity of the polygraph by the communist (i.e., new China or the PRC).

Following the Civil War, the People’s Republic of China first explored the possibility of polygraph testing in the 1960s by purchasing an instrument from the West, but their efforts were cut short by the Cultural Revolution and the idea of polygraph died until 1980. In 1989, China began a program focused on the development of a viable computer-based polygraph instrument and the following year invited Dr. Palmatier to speak in China.

Although it started out slow, over the next 21 years use of the polygraph process and its related methodologies have continued to grow. Some police universities in China offer graduate programs focused on the polygraph process and its use in many contexts. All levels of government, law enforcement, procurator’s offices, courts, military, and the Ministry of State Security have varying levels of polygraph ability. Estimates suggest that today, the People’s Republic of China administers somewhere between a low of 30,000 and a high of 60,000 examinations each year; and the use continues to grow exponentially.

Surprisingly, even though the United States government does not prohibit the teaching of any subject related to the assessment of credibility using the polygraph process, the United States Department of Commerce still prohibits the exportation of any American polygraph instrument to that country. This has only spurred China’s development of its own instruments and purchase of instruments from countries other than the United States.

**China, Polygraph, Credibility**

## **D23 Objective Determination of Eyewitness Identification Accuracy Employing Ocular Measures**

*Frank M. Marchak, PhD\*, Veridical Research and Design Corporation, 211 West Main Lower Level, PO Box 6503, Bozeman, MT 59771*

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After attending this presentation, attendees will understand the potential application of a novel methodology to objectively determine the accuracy of eyewitness identification using cognitive and physiological measures that do not solely rely on traditional verbal decision making processes.

This presentation will impact the forensic science community by providing an additional tool that can be combined with current techniques used in eyewitness identification to add an objective measure to perpetrator recognition and supplement current computer-based lineup approaches.

Mistaken identifications are a leading factor in wrongful convictions in the United States and have contributed to over 75% of wrongful convictions overturned by post-conviction DNA evidence. In addition, witness subjective confidence is a poor indicator of memory accuracy.

Compilation of mug shots by federal and local law enforcement has provided a corpus of data that can be drawn upon to construct lineups.

Recent efforts have focused on developing computer systems that facilitate administration of eyewitness identification tasks. These systems have been shown to be effective in both laboratory situations (MacLin, Zimmerman, and Malpass, 2005) as well as with local law enforcement (MacLin and Phelin, 2007).

Such advances in the ability to create computer-based lineups have been complemented by advances in non-contact, easy to use eye tracking technology. Current systems can be incorporated straightforwardly into video monitors and permit simultaneous collection of gaze, pupil diameter, and blink rate data unobtrusively while individuals view presentations of text and images.

Previous work has shown that individuals examine images of previously seen faces differently from novel faces, exhibiting fewer eye fixations, less overall viewing, and less statistical constraint in their viewing patterns (Althoff and Cohen, 1999). Further studies have shown that other ocular parameters, including changes in pupil diameter and blink rate, also reliably differentiate between novel and familiar faces (Marchak, et al., 2007; 2010). The goal of this effort was to determine if these measures could be used to objectively measure the performance of an eyewitness determining the presence of a perpetrator in a lineup.

Participants watched a video that involved a secretary entering an office, putting down a purse, and leaving the room to get coffee. A perpetrator passed by the open door, rifled through the purse and took objects, fleeing when the secretary returned. At the end of the video, the participants engaged in a word search distracter task for five minutes to prevent rehearsal and then completed a questionnaire describing the suspect and the crime. Lastly, half of the participants viewed a simultaneous lineup and half a sequential lineup of six faces while eye movements, pupil diameter, blink rate, electrodermal activity, and judgment confidence ratings were measured. In addition, for each lineup type, half did not contain the perpetrator.

The findings showed that eye movement measures can differentiate between the perpetrator and foils, in particular the first return fixation to a previously viewed region, the greatest fixation duration, the proportion of fixations, and the proportion of gaze time. Further, differences in maximum pupil diameter between the perpetrator and foils were also highly diagnostic. Blink rate data were mixed and a less reliable predictor. Of special note, these ocular measures were indicative of the perpetrator even in some instances when the participant made an incorrect response and selected a foil as the perpetrator.

A detailed overview of the paradigm and an analysis of the contribution of the multiple ocular measures will be presented, as well as the differences in effectiveness of the approach when applied to simultaneous and sequential lineups. The implication of the findings and recommendations for field application and evaluation will be discussed, along with plans for future research.

**Eyewitness Identification, Memory, Methodology**

## **D24 Computer Tomography (CT) Superimposition of Unidentified Skull to Postmortem Photograph of James-Younger Gang Member**

*James A. Bailey, PhD\*, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC 28403; and B.G. Brogdon, MD\*, and Brandon Nichols, MD\*, University of South Alabama Medical Center, 2451 Fillingim Street, Mobile, AL 36617*

After attending this presentation, the participant will understand: (1) serendipitous discovery of a possible James-Younger gang member's skeleton, (2) evaluation of gunshot wound locations on postmortem photographs of gang members; and, (3) the process of craniofacial superimposition using computer tomography (CT) images of unidentified

skull with postmortem photographs of Clelland D. "Clell" Miller for personal identification.

This presentation will impact the forensic science community by familiarizing forensic scientists with a new superimposition technique using CT images in an historic case. Moreover, forensic scientists will be able to adapt this technique in contemporary cases.

On September 7, 1876, the James-Younger gang robbed the First National Bank of Northfield in Minnesota. Joseph Heywood, bank clerk, was mortally wounded and a citizen, Nicolas Gustavson, was shot on the street and died three days later. Henry Mason Wheeler, a Northfield resident and University of Michigan medical student, shot and killed Clell Miller with a .50 caliber Smith Carbine. Anselm R. Manning, a Northfield merchant, shot and killed William "Bill" Chadwell also known as William "Bill" Stiles. The robbers fled Northfield with a posse in pursuit. However, before departing with the posse, Wheeler directed two classmates to disinter Miller and Chadwell's bodies from the Northfield Cemetery. Since the medical school was closed, to temporarily preserve the bodies, they stored them in barrels and submersed them in Chub Creek north of Northfield. In due course, Miller and Chadwell's bodies were shipped in barrels to the medical school to be used by students in anatomy classes.

The posse killed Charlie Pitts, also known as Samuel Wells, near Madelia, Minnesota, on September 21, 1876. Dr. John H. Murphy, Surgeon General of Minnesota, took possession of Pitts' body and purportedly gave it to an unidentified medical student to be used as an anatomical model. The Younger Brothers were captured and the James Brothers escaped eliminating their remains for analysis.

Controversy exists regarding disposition of Miller's remains. When Miller's family learned Miller's body was at the medical school, they sent Edward, Miller's brother, and Samuel Hardwicke, a lawyer, to retrieve Miller's body. They received a corpse and shipped it in a barrel filled with preservatives to Missouri. The corpse's head was damaged in transit and the alleged remains were buried in Muddy Fork Cemetery, Kearney, Missouri.

In 1880, after graduation from medical school, Wheeler practiced medicine in Northfield. He relocated in 1881 to Grand Forks, North Dakota where he remained until he retired in 1923. Although Miller's alleged remains were buried in Muddy Fork Cemetery, Wheeler claimed a skeleton he displayed in his office belonged to Miller. The Grand Forks Herald reported the skeleton was destroyed when Wheeler's office burned November 22, 1884; however, in 1985 Wheeler's son, Henry M. Wheeler, Jr., and Peter Nickle, who assisted with disposal of Wheeler's estate, refuted that claim.

When Wheeler retired, he donated a skeleton purported to belong to the man he killed in Northfield to the Odd Fellows in Grand Forks. In the mid 1980s, an auction was conducted liquidating Odd Fellows property. During the auction, a citizen searched the building, discovered a skeleton and arranged to acquire the skeleton. In 2010 while searching for the current owner of the Smith Carbine that Wheeler used to kill Miller, a skeleton allegedly belonging to Wheeler was accidentally discovered. It was the same skeleton acquired from the Odd Fellows in the mid 1980s.

Visual examination disclosed no bullet injury evidence to the skeleton in Grand Forks. However, postmortem photographs of gang members show bullet wounds to the chest. In his memoir, Coleman "Cole" Younger, a James-Younger gang member, described both Chadwell and later, Pitts as being "shot through the heart." Miller, he described as shot by Wheeler "lay dying in the street." Using anatomical surface markings to overlay proportional drawings of the bony thorax and pectoral girdle on the postmortem photographs, it became obvious only in Miller's case that there was likelihood the bullet could have left no postcranial skeletal stigmata.

The skull and associated mandible from the skeleton were scanned by Altru Hospital Imaging Center, Grand Forks, North Dakota, using thin sectional (2mm) CT slices permitting high resolution 3D reconstruction of the skull capable of 360° spatial manipulation. This allowed superimposition of the skull upon Miller's photograph, with remarkable concordance of craniometric and cephalometric landmarks between the

skull and photograph. A demonstration of the new superimposition technique is presented elsewhere in the program.

In conclusion, the identity of the alleged James-Younger gang member's skull could not be excluded from Miller's postmortem photograph based on a craniofacial superimposition. However, numerous factors must be evaluated in cases to establish personal identification. Although the craniofacial superimposition of Miller's postmortem photograph could have eliminated the skull as a possible match to Miller, the craniofacial superimposition suggests the skull could be that of Miller.

#### **Craniofacial Superimposition, Computer Tomography, James-Younger Gang**

## **D25 Ashamed Orgasm: A Rogerian Approach**

*João De Sousa\*, Judicial Police of Portugal - Criminal Investigation Department of Setúbal, Praça General Luis Domingues, n° 27, 27A e 28, Setúbal, 2910-585, PORTUGAL*

After attending this presentation, attendees will understand the importance of a Rogerian approach as an alternative solution for sexual abuse cases. After this presentation, the importance of this method of approach in the collection of testimonies in children's sexual abuse cases will be shown, either as a witness or the onlooker's first step of the therapeutic intervention.

This presentation will impact the forensic science community by detailing the application of a new approach method, the Rogerian approach, to collect testimonies in cases of the sexual abuse of minors where another method based in a cognitive interview is commonly used.

In 2010, the Criminal Investigation Department of the Judicial Police of Setúbal (Portugal) was asked to investigate another case of the sexual abuse of the two minors. The police were presented with the parents of two minor girls, one 13-years-old, and the other 8-years-old. A male subject, 45-years-old, reported it as sexual abuse that had the confidence of the family. Once the investigation began, one of the capital moments came in investigations of this nature: the questioning of minors.

The Judicial Police officers who work in this area have training that allows them to work through the complexity of a sexual abuse victim's complaint. The training is based on the Cognitive Interview (C.I.).

Although the exposed above, in this case, the C.I. was not used, the reason is presented in this "case study." In this research, the choice relapsed on the Rogerian method - an approach based on the individual.

It was flagged the fact that the 13-year-old girl presented a testimony that was not truthful in its entirety, showing gaps in the information she could provide, being evident a notable incongruence between the "self" and the traumatic experiences, as well as a defensive distortion and denial of the facts. During the interaction, the minor had a defensive maintenance of the "self", a condition that was overtaken only when the interaction was supported by an unconditional positive regard and empathetic understanding by the interviewer about what had happened to the victim.

The blockage in the testimony had its genesis in the fact the minor may have experienced pleasure during the various abuses that she was subjected to by the individual, creating a latent incongruence between what she thought should be the experience and what she felt on this occasion. The use of the Rogerian method, emphasizing the importance of self-consciousness and congruence between the "self" and the experience allowed the young abused to realize that what she felt at the time was not reprehensible, but the result of mechanical stimulation of her genitals, that can promote pleasure and feelings, without being an acceptance of rape and active participation in the act.

We believe we also showed that the Rogerian method could be used in the collection of the children's testimonies in sexual abuse cases, either as a witness' onlooker or as a first therapeutic step of the intervention.

#### **Rogerian Method, Cognitive Interview, Sexual Abuse**

## **D26 Identifying Potential Molecular Chronometers in Fingerprints Using C60+ Secondary Ion Mass Spectrometry**

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After attending this presentation, attendees will be familiar with some of the possible uses of Dynamic C60+ Secondary Ion Mass Spectrometry (SIMS) for the chemical analysis of fingerprints. The potential abilities of this technique to image a fingerprint and monitor the changes in composition with time will be emphasized.

This presentation will impact the forensic science community by providing an evaluation of a new technique to analyze fingerprints.

C60+ SIMS involves the bombardment of a focused ion beam on a sample to generate secondary ions characteristic of the chemical composition of the sample. The benefit of C60+ SIMS over the traditional techniques is that C60+ SIMS could provide spatially resolved analysis and also has the potential to probe the composition of a fingerprint as a function of depth. With respect to mass spectrometry techniques, the C60+ is a soft ionization technique, which allows for a less fragmented molecular profile of the sample. In addition, using this technique also does not completely destroy the fingerprint, making it possible for comparisons to be made after being analyzed by C60+ SIMS.

Fingerprint development and imaging is a well-established and well-researched area in forensic science; however, the ability to both image and chemically analyze a fingerprint has been less commonly studied. The research focuses on the changes in the composition of fingerprints with time when exposed to a variety of environmental conditions (i.e., heat, humidity, and ultraviolet radiation). By doing so, potential molecular chronometers can be identified in an attempt to determine a timeframe of deposition. This could be useful evidentiary information as it may allow investigators to place a suspect at a scene within a certain timeframe or, similarly, rule out the presence of a suspect during the time of a crime based on the timeframe. To better understand changes which occur due to environmental factors, a chemically relevant artificial fingerprint material was developed to mimic both the eccrine and sebaceous secretions found in a normal fingerprint. The artificial fingerprint material provides more consistent and repeatable results than using actual fingerprints.

In this study, synthetic fingerprint material was either drop coated or printed, using a high viscosity polymer printer, onto silicon disks which were analyzed using the SIMS technique. A control disk was studied simultaneously with all environmentally exposed disks to note any changes in sample due to the vacuum. Experimentally exposed disks were subjected to a variety of conditions and mass spectra of the disks were collected at various time points throughout the study. The spectra were then compared to determine what, if any, chemical changes occurred which could be used as molecular chronometers for measuring time since deposition. Once completed using the artificial fingerprint material, the studies were replicated with actual fingerprints to see if the same chronometers were found to produce similar results. The imaging capabilities of actual fingerprints using C60+ SIMS were also studied.

#### **Fingerprints, C60+ SIMS, Chemical Analysis**

## **D27 Conducting Laboratory Analysis of Gunshot Residue (GSR) on Clothing to Identify a Shooting Suspect**

*Alan A. Price, MA\*, University of Northern Colorado, PO Box 336433, Greeley, CO 80633*

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After attending this presentation, attendees will understand the significance of why laboratory analysis is not routinely conducted on clothing.

This presentation will impact the forensic science community by showing how television dramas misrepresent the forensic laboratory analysis capabilities when analyzing gunshot residue (GSR) on clothing.

Fictitious annals of television crime dramas depict criminalists in crime laboratories being able to determine which individual discharged a firearm by examining their clothing. From this misrepresentation of laboratory capabilities, attorneys and law enforcement personnel frequently have incorrect perceptions of the analysis process for gunshot residue. Actually, most crime laboratories do not routinely examine clothing for GSR. Considerable research has been conducted to determine muzzle to target distance using GSR and stippling; however, studies are absent in assessing whether a specific item of clothing worn by a shooting suspect contains GSR. This research focused upon the utility of examining fabric for GSR in an attempt to identify a specific individual's clothing subsequent to a shooting incident, using a scanning electron microscope (SEM). Three common pieces of fabric were used as samples for collecting GSR and were then examined. Researchers used cotton, polyester, and denim sleeves for collection of residue samples. The revolver used in this project was a .357 magnum, with a 2.5 inch barrel. The three basic elements of gunshot residue are barium (Ba), antimony (Sb), and lead (Pb). Carbon-coated aluminum stubs were used to collect GSR particles from each individual fabric sample.

Each of the three fabric samples were washed with liquid detergent to remove any potential contaminants. The samples were collected on an outdoor firing range to avoid cross-contamination from any extraneous indoor particulates. Six bullets were fired for each collection sample. To prevent contamination researchers wore latex gloves while handling each sample. Immediately after the fabric was exposed to the weapon's discharge, they were placed and stored in individual clean paper "sleeves." All three-fabric samples had a 10 mm square marked onto the top of each sleeve. This is the area directly behind the "web" of the thumb and index finger and mostly exposed when holding and discharging a handgun. The primer control was discharged without gun powder or a bullet being present in the shell casing. Particulates from the primer were collected from stubs mounted on paper.

In the laboratory, the sample sleeves were pressed with the prepared stubs ten times. The samples were collected from within each 10 mm square previously marked on each sleeve. The two detectors used were backscatter and energy-dispersive spectrometry (EDS). After a GSR particle was located, it was then analyzed for five minutes at one location for EDS confirmation. Each fabric sample was manually scanned for one hour to search for GSR particles. In order to limit selection of possible particles on the samples, auto-brightness and contrast were performed on the primer sample. With this setting the sample stubs were examined, which prevented extraneous particles from showing up. The findings are as follows: GSR was readily detected from the primer standard; GSR was detected on the cotton fabric; GSR was detected on the polyester sample; and no GSR was detected on the denim fabric sample. Although not all elements of GSR were detected on the cotton and polyester samples, sufficient elements were present to conclude that GSR existed.

The analysis of clothing for GSR can be very inconclusive. Multiple extraneous variables will influence GSR analysis results. Movement or shaking of the fabric sample may dislodge particulates from the fabric. Rubbing or contact with another object may also result in dislodging GSR particulates. Air currents around the weapon and the fabric may have a

significant influence of GSR adhering to a fabric. Without having the scanning software capabilities for an SEM/EDS, searching for and identifying GSR is very difficult and time consuming. This study illustrated that GSR on fabric cannot by itself be initially identified merely by shape, size and brightness under the SEM/EDS. Even though no GSR particles were found on the denim fabric, it cannot be concluded that a specific type of fabric has any more or less propensity of retaining GSR particles. As previously stated, mythical television entertainment is incorrect in representing forensic laboratory capabilities.

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**GSR Particle Identification, Misperception of GSR Analysis Capabilities, Television Drama Misrepresentation**

## **D28 Forensic Art: Another Piece of the Identification Puzzle — A Case of Homicide in Mammoth Lakes, California**

*Sandra R. Enslow, BA\*, Los Angeles County Sheriff's Department, 4700 Ramona Boulevard, Room LL40, Monterey Park, CA 91754*

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After attending this presentation, attendees will understand two different aspects of forensic art, the forensic composite and three dimensional skull reconstruction as well as their role in the identification process of an actual criminal investigation. The presentation will show how forensic art, along with mtDNA and isotope analysis, was used to gain information about the victim.

This presentation will impact the forensic science community by raising awareness about forensic art and the role it plays in the identification process.

Popular culture often refers to composite drawings as images of criminals, which is another aspect of forensic art; however, this aspect of forensic art delivers the image of the victim. This presentation includes a timeline for this case and shows the detective's integration of other forensic science disciplines in order to get this victim identified – the first step to solving this crime. By reaching out and tapping into all of these forensic resources, the detective's due diligence paid off.

Composite sketches are done when a forensic artist sits with a witness to the event and creates a drawing of the witness' memory. In this case, the witnesses were employees at the Mammoth Lakes Visitor's Center and had contact with the victim before her death, the previous autumn. Once discovery of the skeleton was made, in the early spring and the case distributed to the local media, these witnesses came forward with the incident they remembered. The Los Angeles County Sheriff's Department forensic artist was contacted to do the composite drawing. She drove up to Mammoth Lakes from Southern California. These witnesses met with her and described the woman they recalled. While each witness met separately with the artist through the process, one final drawing of the victim was completed.

Betty Pat Gatliff, of the Skullpture Lab, was contacted and asked to do the three-dimensional skull reconstruction for this case. The victim's skull was sent to her office in Norman, Oklahoma.

The practice of restoring facial features from a human skull was first used over a hundred years ago. At that time, its purpose was to identify remains of famous historical figures. Betty Pat Gatliff, a medical illustrator, developed forensic facial reconstruction in the United States of America in the 1960s.

Three dimensional reconstruction is done by marking tissue depths, according to a tissue thickness measurement chart, onto a skull or a cast of the skull and then fleshing the face out with clay. There are also formulas for the size and shape of the lips and length of the nose. The European method of facial reconstruction is by creating the musculature of the face before fleshing it out, while still matching to tissue depth markers.

This case example shows the consistency of the image of the unidentified woman from the two forensic art techniques. These techniques, created by the two different artists at two different times, in

different parts of the country, demonstrate the strength of forensic art as a discipline. Through the forensic composite, the witnesses speak and give their description. With the three dimensional skull reconstruction, the skull speaks and corroborates that description.

Forensic art, isotope analysis, and mDNA made it possible to get this victim's physical features, country and town of origin, and finally a name, Barbara. While this case is still an open investigation, the detective, through forensic science, developed more information about his victim than other traditional and earlier methods of investigation could have given him.

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**Forensic Art, Skull Reconstruction, Criminal Investigation**

## **D29 Analysis of the Organic Composition of Gunshot Residue**

*Amanda Roth, BA\*, 188 Sheridan Avenue, Apartment 1, Pittsburgh, PA 15202; and Stephanie J. Wetzel, PhD, Duquesne University, Department of Chemistry and Biochemistry, 600 Forbes Avenue, Pittsburgh, PA 15282*

The goal of this presentation is to demonstrate the analysis of the organic composition of gunshot residues by LC-MS/MS and to determine if the residue produced by particular ammunition is unique to its manufacturer.

This presentation will impact the forensic science community by providing an analysis method for the organic composition of gunshot residues and by exhibiting a comparison of the organic compositions to be used as a tool by forensic investigators to discriminate between manufacturers of ammunitions.

Gunshot residue can be defined as the particles expelled from a firearm upon its use and can be deposited on the skin or clothing of both the shooter and the victim. The residues are generally composed of inorganic particulates from the primers and organic particulates from the propellant powders used in the firearm's ammunition. Traditional gunshot residue analysis entails the determination of the presence of particles containing lead, barium, and antimony using SEM/EDX. Due to safety concerns, however, many manufacturers are no longer using lead in the production of their ammunitions. Previous methods analyzing the inorganic composition of gunshot residues are therefore no longer as valuable in forensic investigations.

Analysis of gunshot residues by liquid chromatography tandem mass spectrometry provided both qualitative and quantitative data on the organic compositions of the residues. This research is presented as an alternative to the traditional inorganic methods previously used and as a means of comparing residue compositions. Certain organic components of propellant powders, such as nitrocellulose and nitroglycerine, are commonly used in the manufacturing of other products, and as such were not analyzed. The seven organic compounds primarily studied in this research are explicitly indicative of gunshot residue: ethyl centralite, methyl centralite, akardite II, diphenylamine, N-nitrosodiphenylamine, 4-nitrodiphenylamine, and 2-nitrodiphenylamine.

Initially, standards of each of these compounds were analyzed, both separately and as a mixture, to optimize the liquid chromatography and mass spectrometer parameters. Gunshot residues produced from several different ammunitions were then collected on fabrics and extracted before being analyzed under these optimal conditions. A solid-phase extraction technique was utilized with C-18 cartridges to concentrate the residue samples. A multiple reaction monitoring (MRM) method was used to discriminate between the parent and daughter ions of each compound for increased sensitivity. All samples and standards were prepared and processed with a combination of acetonitrile, methanol, and water, and were ionized by the mass spectrometer via positive electrospray ionization. Gunshot residue samples were also subjected to Quadrupole Time-of-Flight mass spectrometry to qualitatively identify any additional significant organic compounds.

Various gunshot residues were analyzed to determine if this method is reliable for use in forensic investigations to positively identify a substance as gunshot residue. The organic composition of each residue was evaluated to establish variations between manufacturers of ammunitions. Any significant deviations in composition would allow investigators to identify the manufacturer of an ammunition that produced a particular sample of gunshot residue. Following Locard's principle, the ability to identify the manufacturer of ammunition from gunshot residues can further assist investigators by linking possible suspects to the scene or by eliminating potential suspects.

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**Gunshot Residue, Organic, Mass Spectrometry**

## **D30 Analysis of Footprints and its Parts for Stature Estimation in Indian Population**

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After attending this presentation, the attendees will be able to recognize that stature can be estimated from footprints and its parts with a reasonable accuracy in Indian population.

This presentation will impact the forensic science community by recognizing that stature can be estimated from footprints with a reasonable accuracy in males and females. The findings may prove to be useful in identification of unknown prints. It can give vital evidence in identification of the perpetrator of the crime in cases where the footprints are left behind at a crime scene.

Footprints are often encountered at crime scenes especially in cases of murders and sexual assaults. Estimation of stature from the footprints found at the crime scene can be crucial in the identification of the perpetrator in forensic examinations. A positive correlation exists between stature and foot size and hence, analysis of footprints can be useful in estimation of stature. Although a few researchers have attempted to estimate stature from footprints, the recent studies on the subject are confined to males only. The present research is aimed to estimate stature based on detailed analysis of length measurements of footprints in Indian population using statistical considerations.

The present research was conducted on 100 young adults (50 males and 50 females) at the Department of Forensic Medicine, Kasturba Medical College, Mangalore, India. Healthy individuals aged between 20 – 25 years were included in this study after giving informed consent. The subjects with any disease/deformity of the foot/spine were excluded from the study. The stature of each subject was measured in centimeters using standard techniques. Each subject included in the study was asked to wash their feet with soap and water. A clean plain glass plate was uniformly smeared with black duplicating ink with the help of a roller. The subjects were asked to step onto the smeared plate and then transfer them onto a white paper. Regular pressure was applied on the foot area to obtain the footprints. Five measurements were taken in centimeters on right and left footprints obtained from each subject. The measurements were taken from the mid-rear heel point to the most anterior point of each toe on right and left sides and designated as T1, T2, T3, T4, and T5 for the measurements of heel to toe 1, 2, 3, 4, and 5 respectively. Male-female differences in stature and foot measurements were analyzed using Student's t-test. Asymmetry between sides in the foot measurements was calculated and tested using paired t-test. Pearson's correlation coefficients were calculated between stature and various measurements of the foot. The stature was estimated from foot and its various measurements by using linear and multiple regression analysis. Statistical significance was defined at the standard 0.05 level.

Mean stature was significantly higher in males than females. Statistically significant sex differences were observed in the various measurements on the footprints between males and females in right and left feet. Right-left differences were observed in footprint measurements among males and females. Statistically significant correlation coefficients were observed for correlation between stature and various footprint measurements. Thus, the stature is found to be positively and strongly related to various foot measurements in males and females. In males, various foot measurements show relatively higher values of correlation coefficients than in females. The linear regression models were derived for stature estimation from each measurement as "S (stature) = a + b x" + SEE, where, "a" is constant and "b" is the regression coefficient of independent variable (i.e., individual foot measurement) and "x" is an individual variable/foot measurement. Multiple regression models were derived as S (stature) = a (constant) + b1 (1: regression coefficient of the variable) x X1 (1: variable) + b2 (2: regression coefficient of the variable) x X2 (2: variable) + . . . bn (n: regression coefficient of the variable) x Xn (n: variable) + SEE. Multiple regression models show a higher accuracy than linear regression models in stature estimation.

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**Forensic Science, Stature Estimation, Footprints**

### **D31 Forensic Devices for Maximizing Crime Scene Sample Procurement**

*Luciano Garofano, PhD\*, General of Carabinieri, Via G. D'Annunzio N.9, Parma, ITALY; and Pasquale Linariello, BSc, and Luca Salvaderi, BSc, NGB Genetics, Via Ruggero Grieco 5, Bologna, ITALY*

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After attending this presentation, attendees will understand the importance of innovative collection devices for recovering and preserving DNA traces for crime scenes investigation.

This presentation will impact the forensic community by introducing innovative and more reliable devices for collecting and preserving small amounts of DNA for profiling. It will show how cotton, polyester, rayon, or paper swabs are widely used for evidence collection from crime scenes; however, swabs can affect the evidence obtained and subsequently analyzed. FLOQSwabs™ (FFS) are specifically designed to facilitate and maximize crime scene sample collection, and neutralize microbial contaminants while preserving nucleic acids (NA) integrity without the need for drying the swab prior to transport or storage. Each forensic collection kit consists of specially designed regular, flat, round/rims, or nails flocked swabs associated to a Nucleic Acid Optimizer (NAO), a semi-permeable basket that allows efficient release of all sample collected from small traces during a crime scene investigation.

The objectives of this study were to: (1) compare the 4N6 FLOQSwabs™ collection kits (FFS+NAO) to traditional forensic collection devices for procurement and preservation of nucleic acids for forensic investigation; (2) to validate the FFS ability to preserve nucleic acids in samples with a heavy load of bacterial flora; (3) to validate the quality of nucleic acid for profiling; and, (4) to validate the ease of use of the special designed FFS for sample collection.

In this study crime scene traces (n=13) were simulated in the laboratory and included: Seven dry blood traces spotted on different types of surfaces including two with strong bacterial contamination; four sweat traces on different types of surface; one saliva trace on bottlenecks; and one human skin trace under fingernails. Six replicates were prepared for each trace and duplicate samples were collected with FFSs, and traditional rayon forensic (RFS) (Sarstedt), and absorbent filter paper forensic swabs (PFS) (Whatman). Each swab was pre-wetted with 50ul of sterile distilled water. All samples collected were stored 10 days at room temperature and then tested for DNA quantity and quality for profiling. The FFSs were used in association with the NAOs during the purification procedure in order to completely drain the swabs after the lysing step. Nucleic acid was extracted with DNA blood mini elute, quantified by Real Time PCR, and profiled with a amplification kit.

In all the samples tested, the Copan FFS recovered from blood, saliva, sweat, and skin under nail traces 0.20ng/ul, 0.83ng/ul, 0.15ng/ul, and of 0.3ng/ul of human DNA, respectively, compared to 0.15ng/ul, 0.07ng/ul, 0.05ng/ul, and 0.06ng/ul of human DNA for the RFS and 0.0097ng/ul, 0.01ng/ul, 0.0033ng/ul, and 0.0044ng/ul of human DNA for the PFS, respectively. When comparing the total qPCR results, Copan FFSs recovered an average of 0.24ng/ul of human DNA compared to an average of 0.106ng/ul for the RFS and of 0.0072ng/ul for the PFS. From two heavily contaminated blood traces, FFS detected 0.61ng/ul versus 0.014ng/ul for RFS and 0.0ng/ul for PFS. Copan FFS collected 2.26 times more DNA than RFS and 33.3 times more DNA than the PFS.

When analyzing the STR profiles, Copan FFSs recovered from blood, saliva, sweat and skin under nail traces respectively 85.90%, 100%, 57.40% and 93.80% of the total alleles amplified, compared to 72.10%, 93.80%, 21.10% and 26.60% recovered by RFS and 24.30%, 31.30%, 0.40% and 4.90% recovered by PFS.

The 4N6 FLOQSwabs™ easily collected all samples from all collection sites due to the specially designed geometries. The traditional swabs performed fine but were limited due to a single geometry, shredded during collection, and did not have a transport tube.

Copan forensic collection kits are increasing and preserving DNA collection from 2.2 to 33 times and increasing the percentage of recovered alleles in STR analysis. The FFS can be used for sample collection in a heavily contaminated environment even after 10 days storage at room temperature without the need of drying. The FFS were easy to handle especially for the hard surfaces, bottleneck, and under nail collection.

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**FLOQSwabs, Forensic, Collection**

### **D32 A Comparison of Cyanoacrylate, Ninhydrin, and Gel Lifters for the Development of Latent Prints on Latex Gloves**

*Stephanie A. Johnson, BA\*, Marshall University, 1401 Forensic Science Drive, Huntington, WV 25701; Jay M. Peterson, BS, Michigan State Police Marquette Forensic Laboratory, 1924 Industrial Parkway, Marquette, MI 49855; Catherine G. Rushton, MS, Marshall University Forensic Science Graduate Program, 1401 Forensic Science Drive, Huntington, WV 25701; and Pamela J. Staton, PhD, Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701*

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After attending this presentation, attendees will gain a better understanding of the effectiveness of ninhydrin, cyanoacrylate, and gel lifters when developing latent prints on latex gloves.

This presentation will impact the forensic science community by providing latent print examiners with added insight into the most effective technique for developing latent prints on latex gloves as well as whether or not time is a factor in the efficacy of these techniques.

Latex gloves are notorious for being difficult substrates for the development of latent fingerprints. Varying degrees of texture and fit, along with a multitude of other, uncontrollable variables make developing prints a very difficult task. Some of the most commonly used techniques in latent print development are cyanoacrylate fuming, ninhydrin, and gel lifters. These three techniques are relatively inexpensive and have been used in the past to develop prints on a variety of substrates, including latex gloves. Time is also important when processing evidence and can affect how well a print is developed or how well a technique will work. Fingerprints can potentially last for years depending on the substrate and conditions; however, it is still important to understand the effects of time on evidence and the quality of the results.

The experiment compared cyanoacrylate fuming with magnetic powder, ninhydrin, and black gel lifters to determine which produced the highest quality results when developing latent prints on latex gloves and whether or not time affected the quality of these results. Multiple pairs of size large, powder-free latex gloves were worn by eight different

participants for 15 minutes at a time and were then divided into batches which were processed after being stored for varying amounts of time, from one day to six weeks. Latent prints were developed both on the inside of the fingers of the gloves as well as in the palm area, where a print was placed by the participant after removing the glove. Gloves from each batch were further divided into three different sub-groups which were processed by the different techniques. After processing, any results were given a rating of 0-3, with 0 being no results or only a fingerprint present, 1 being small amounts of ridge detail present with or without the overall pattern visible, 2 being sufficient ridge detail to make an identification but without either or both 1st and 2nd level detail, and 3 being an identifiable print which has all three levels of detail. Results with a score of one or higher were photographed and reviewed a second time. The percentage of identifiable results produced was calculated for each technique along with the average rating over the course of the experiment.

The results of the experiment indicate that the cyanoacrylate with magnetic powder and the black gel lifter techniques produce comparable results, with the black gel lifter producing a slightly higher percentage of identifiable prints. The ninhydrin technique produced no identifiable prints in either of the finger and palm areas and consistently yielded much lower amounts of ridge detail than the other methods. Additionally, the amount of time the gloves were allowed to sit did not appear to affect the quality of the results, as prints with a score of three were developed in the six week group. The most important factors when it came to the quality of prints developed seemed to be the fit of the glove, with tighter fitting gloves producing better results.

**Latent Prints, Latex Gloves, Fingerprint Analysis**

### **D33 Development of Rapid Semi-Quantitative Colorimetric Test for Trinitrotoluene (TNT)**

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After attending this presentation, attendees will understand the adaptation of a simple colorimetric chemical technique for the identification of TNT in power and extracted from soil samples to provide semi-quantitative information.

This presentation will impact the forensic science community by providing useful insight into the modification and development of an existing colorimetric test to facilitate the semi quantitative analysis in the field.

Explosive compounds are increasingly used around the world by terrorist groups and individuals; as a result, the rapid identification, detection, and quantification of explosives has become an important topic in forensic science and antiterrorist activities. Often explosives have also been used for military purposes, mining, and other industries. They can contaminate soil and water due to military activities (e.g., manufacturing, testing and training, demilitarization, open burning/open detonation, buried land mines, and industrial activities). This can cause significant environmental pollution and there is a need for a rapid, low-cost method for semi-quantitative or quantitative trace explosives detection for hazardous waste site characterization and land mine detection.

One important method for explosive detection involves the use of colorimetric techniques. This method could be used both off-site as the field test and as an on-site laboratory screen. Such techniques have the advantages of speed and low facilitating efficient sample analysis per unit time. This method has been used to analyze a large number of samples in mobile laboratory units as well as a number of commercial chemical kits

have been developed; however, these can only produce qualitative analysis. Spectrophotometric detection has been reported for quantification of the product from colorimetric techniques; however, these require access to appropriate instruments. Therefore, there is a gap in current provision for field operators for a rapid, reliable, and low cost quantitative or semi-quantitative detection system for explosive trace and residue samples, both pre- and post-blast.

In this work, a rapid semi-quantitative colorimetric test for trinitrotoluene (TNT) was developed using digital image analysis. TNT was reacted with Nessler's reagent providing a purple colored product, which was recorded using digital images. The colored images were analyzed using a graphics editing software program to obtain analytical data in the form of Red Green Blue (RGB) values. The relationship between the individual RGB intensity and absorbance and the concentration of the target species were presented as calibration curves. A wide linear range and low detection limit was obtained in each case (1-50 mgL<sup>-1</sup> for the color intensity and absorbance for red and blue as well as absorbance for green, and 1-25 mg L<sup>-1</sup> for the color intensity of green, limit of detection of 0.73±0.01 mg L<sup>-1</sup> to 1.75±0.07 mg L<sup>-1</sup>). The known concentration of TNT solution and TNT spiked in soil were tested for the accuracy of the method and satisfactory results were obtained. The results show great potential to continue the development for use as a semi-quantitative field test for TNT.

**Colorimetric Test for TNT, Semi Quantitative, Digital Images**

### **D34 A Case Study of DNA Typing From Human Feces**

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After attending this presentation, attendees will have a better understanding on the subject of STR as genetic analysis.

This presentation will impact the forensic science community by highlighting STR as genetic analysis.

It is possible to have an individual identification through DNA analysis from body secretions such as hair, saliva, sperm, and an epithelial cell which are found in a crime scene. Short Tandem Repeat (STR) markers which are effective genetic markers that use a common forensic DNA typing. STR is studied in many fields such as a genetic map, a disease diagnosis, evolutionary biology, and forensic medicine, and it uses DNA profiling with amplified STR points by PCR using many kinds of STR kits.

Feces are composed of complex compounds such as a digested microorganism, food scrap, mucus, and a cell from the intestine's wall. It is also used in the field of disease diagnosis. mtDNA is extracted successfully and analyzed from bear feces in a study by Hoss, et. al., in 1992. DNA analysis from human feces is also studied by the field of medical diagnosis.

Feces, which is one of the body's excretions, provides useful information on the physical condition of the colon through a microorganism in the colon and a desquamated epithelial cell. It is also provides the source of mtDNA for sequencing and nDNA for genotyping. From the same individual, sequences of samples of feces and blood are equal, but it is observed 4.88 average sequences per 400bp, between the ranges of 1-10 nucleotide.

In March 2009, victim A (a 50-year-old female) and victim B (A's daughter, a 23-year-old female) were murdered by stabbing in CheonAn-si, ChungNam, South Korea. Victim A was found in her master bedroom and the victim B was found in a garden next to her home. During the investigation, feces believed to be the suspect's and a glove with blood were found, and upon the analysis of evidence, DNA was found and the suspect C was arrested.

In this case, extracted DNA from human's feces, the glove with blood, and the part of wrist in glove were used to STR point's analysis after STR DNA profiling. There were three conclusions leading to the arrest of the suspect: (1) the man's DNA was found from feces, and it was the same as DNA from the suspect's oral swab received from the CheonAn SeoBuk police station; (2) the blood on the glove was identified the victims' blood, when analyzing to wipe the wrist part of glove's inside, complex DNA linking victims and the suspect were found; and, (3) when analyzing Y-STR which is the male DNA marker, it matched the DNA of suspect. Therefore, the suspect was found to be male and the DNA of the suspect was analyzed in more detail from the wrist part of glove.

In many crime scenes, criminals leave feces because of psychological pressure, and as a result it is evidence equally important as blood and saliva.

This case shows the DNA collected from the feces was the same as the suspect's DNA, shown through DNA typing. The compound of feces is very complex and the occurrence of microorganism ever changing; however, it can be analyzed perfectly with DNA. Therefore, it can be one of the most important evidence retrieved in crime scenes and it needs various studies.

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**DNA, Human Feces, STR**

### **D35 Analysis of Triacylglycerols in Fingerprint Samples as a Dating Technique by Laser Desorption/Ionization Time-of-Flight Mass Spectrometry**

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After attending this presentation, attendees will understand: (1) how TAGs in fingerprint samples can be detected; (2) the mechanism and duration of their degradation; (3) and examples of decomposition over different experimental conditions.

This presentation will impact the forensic science community by providing a reliable way to detect the degradation products of TAGs in fingerprint samples. This detection could serve as a potential dating method for fingerprints. At the present time, no established method is accepted for dating fingerprints or fingerprints.

The chemical composition of fingerprints could potentially be important in the investigation of criminal activity. This includes application of a possible dating method to place an individual identified through a fingerprint at the time of a crime. Determination of the age of a fingerprint, in particular, would be useful to confirm or dispute a suspect's alibi. For example, an individual could assert that a fingerprint recovered from a crime scene was present for weeks before the crime was committed. Determining that the print was left hours before the offense could be critical in establishing a time-line of events and a subsequent conviction.

Fingerprints are the recovered traces of material transferred to other surfaces upon contact. The material transferred is a complex mixture of chemicals, mostly lipids including triacylglycerols (TAGs). In this study, fingerprint samples containing TAGs were analyzed using laser desorption/ionization (LDI) time-of-flight mass spectrometry (TOF MS). Only LDI appeared to be useful for this application while conventional matrix-assisted laser desorption/ionization (MALDI) TOF MS was not. Analysis of fingerprint samples exposed to light conditions on a stainless steel target indicated the formation of TAG degradation products. As the sample degraded, additional peaks were observed in the mass spectrum particularly in the  $m/z$  range of 650-750. The decrease in relative intensities for some TAGs occurred after exposure to light for less than 12

hours with almost complete degradation taking place after 72 hours. These products were not detected in samples exposed to dark conditions. TAGs that decreased in intensity corresponded to those previously identified as having at least one unsaturated fatty acid. Other TAGs that were still present after 72 hours were identified as containing all saturated fatty acids. Monitoring these changes in the TAGs over time and under different conditions could establish a consistent pattern for dating applications.

Analysis of an unsaturated TAG standard (triolein, C57:3) was used for comparison. After exposure of this sample to the same conditions as fingerprints, decomposition was observed under light conditions. Tandem mass spectrometry was used to identify/confirm selected TAG degradation products. Formation of bound C8:0 and C9:0 aldehydes and carboxylic acids were observed. These products are consistent with those found in fingerprint degradation products.

Fingerprint samples were also allowed to undergo degradation on alternative sample surfaces to determine if the process is rate dependent upon the sample medium. Comparison of different surfaces, as would be the case for fingerprints collected at crime scenes, is thus important for determining degradation rates. Samples were allowed to degrade on surfaces of glass, plastic, and wood under light and dark conditions. These samples were then analyzed in 12 hour increments for 60 hours by LDI-TOF MS for comparison to the stainless steel target samples.

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**Fingerprints, Triacylglycerols, LDI-TOF MS**

### **D36 Electronic News Media Reports of Potential Bioterrorism-Related Incidents Involving Unknown White Powder**

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After attending this presentation, attendees will understand how bioterrorism (after the 2001 anthrax attacks in the United States) is a potential threat in the United States and around the world. Incidents related to potential anthrax-related unknown white powder/suspicious packages occur in practically every state in the United States, and responses to these incidents could entail significant amount of human and financial resources.

This presentation will impact the forensic science community by analyzing the submitted specimens. Furthermore, this presentation will help the attendees to understand that potential bioterrorism-related incidents continue to exist and pose a threat to national or local security, and to help to allocate limited resources efficiently.

Potential anthrax-related incidents involving unknown white powders that were reported online by news media from June 2009 through May 2011 were reviewed and compared them with incidents reported to the U.S. Centers for Disease Control and Prevention, Division of Preparedness and Emerging Infections, Emergency Preparedness and Surveillance Branch (CDC DPEI/EPRB). Geographic distribution and location of these powder-related incidents, the identity of the unknown white powders, and responders involved in these incidents will be presented.

Internet searches for "unknown white powder" using various search engines were performed. Incidents reported to and responded by the CDC DPEI/EPRB were also reviewed. The following types of information were collected: report date, state of incidence, specific location of incidence, identification of the unknown white powders, emergency responders involved, and FBI involvement. Using Microsoft Excel® 2003, an electronic spreadsheet was constructed, and a descriptive statistical analysis was done using SPSS 17.0.

There were 267 news media reports from 43 states and the District of Columbia included in this study. One incident might be reported in two or more electronic news media; however, this event was counted only as one incident for purposes of this study. In addition, there were reported

incidents from eight U.S. Embassies in Senegal, Turkey, Israel, Canada, Norway, England, Malta, and Cyprus which were not included in this analysis. In comparison, there were only ten white powder incidents from various states and 14 white powder incidents from U.S. Embassies around the world that were reported to and responded by CDC DPEI/EPRB.

Most of the media-reported U.S. incidents came from California (n=29, 10.9%), Florida (n=26, 9.7%), Texas (n=21, 7.9%), New York (=19, 7.1%), and Alabama (n=11, 4.1%). More than half of these reported incidents (n=158, 59.2%) occurred in government facilities (including law enforcement offices and courthouses (n=96, 36.0%)). The remainder occurred in private business establishments (n=32, 12.0%); schools at all levels (n=19, 7.1%); private homes (n=11, 4.1%) and other sites (n=109, 40.8%) including banks, hospitals, churches, airplanes, airports, TV stations, newspaper mail rooms, a school bus, and theaters. Although the majority of news reports (n=207, 77.5%) did not mention the final identity of the suspicious powder, many of the white powders were identified as flour, baking powder, talcum powder, baby powder, cornstarch, or sugar. None of the articles were identified as a harmful substance.

Even though the suspicious white powder was harmless in all of these reported incidents, public health emergency responders, and forensic investigators responded to the incidents. Responders included fire department, police department, bomb squad, FBI, U.S. Postal Inspection Service, public health laboratories, State Department of Environmental Protection, National Guard, U.S. Marshal Service, and/or regional transit authorities. In some cases, the media reported the incidents led to evacuations and precautionary hospitalizations. Joint public health emergency responder and FBI investigations were mentioned in only a few reported incidents (n=35, 13.1%).

Because of the highly sensitive forensic nature of some cases; however, some white powder incidents were investigated by the FBI and were not covered by news media. Thus, it is likely that powder incidents in this study were underreported. Furthermore, emergency responses to unknown white powder incidents are generally managed at the local level and therefore, they may or may not be reported to CDC DPEI/EPRB for emergency response assistance.

Results of this study showed that unknown white powder incidents continue to occur. In addition, these incidents require integrated medical, public health, preparedness, and response activities from various emergency responders and cause substantial concern (as evidenced by media reports).

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**Bioterrorism, Unknown White Powder, Anthrax**

### **D37 Shooting Euthanized Pig Heads to Determine Penetration Capabilities of Frangible Bullets and the Impact on a Forensic Investigation**

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After attending this presentation, attendees will better understand weights, velocities, and kinetic energies of bullets frequently encountered at criminal shooting incidents. The attendee will learn the differences between common lead-based bullets and frangible bullets, their intended uses, the potential loss of bullet evidence at shootings involving these bullets, and the destructive effects of shooting frangible bullets into euthanized pig heads.

This presentation will impact the forensic science community by providing a definition of frangible bullets, their uses in tactical and close quarter combat/shooting events, and their ability to penetrate tissue and bone. The presentation will also explain the complexities for the

investigator and forensic firearms examiner in evaluating the frangible bullet or remnants involved in the shooting incident.

**Frangible Bullet Penetration Study:** What is a frangible bullet, what are they utilized for, what are their wounding capabilities on tissue and bone, and how does the use of frangible bullets affect a forensic investigation?

Many students of forensic science, forensic science practitioners, law enforcement officers, and other justice related personnel are not familiar with frangible bullets, their availability on the U.S. market, and the potentially negative evidentiary effects of a frangible bullet when involved in a criminal forensic investigation. In the United States, the majority of homicides are committed by use of a firearm. During systematic crime scene searches and postmortem examinations of victims, it is common to recover bullets or fragments of bullets. Most bullets are made of lead and normally contain a metal jacket to provide strength. These bullets and/or fragments can be examined in the forensic laboratory for class and possibly individual characteristics. These bullet characteristics may make it possible to further the investigation by linking the physical bullet evidence to a suspected shooter, a firearm in his control, a box/lot of cartridges, or a previously known firearm common to the suspect. This presentation will include the basics of bullet composition, a variety of common firearm calibers, weights of bullets, velocity of these common bullets, and the differences in kinetic energy frequently found in firearm bullets observed in shooting incidents in the United States. Frangible bullets typically do not contain lead and therefore are considered to be more environmentally friendly. From an environmental standpoint, these bullets are preferred to lead-based bullets which may pose a more significant negative impact on the environment. This presentation is the result of a graduate school study set forth to answer the question of what the impact would be of a frangible bullet on tissue and bone, and their ability to be examined after striking a target or victim. Frangible bullets are commonly used with the intent of not over-penetrating solids, such as wooden and metal doorways. This study set out to answer whether or not frangible bullets were capable of penetrating tissue and bone and whether or not they could do so without breaking apart. The study also sought to answer the question of whether or not the frangible bullet would remain intact sufficiently to establish class or individual characteristics. This presentation will help answer these questions, as well as, provide insight to the attendee on the impact on homicide or death investigations involving the frangible bullet.

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**Frangible Bullet, Firearms, Shooting**

### **D38 Pilot Comparison of Conducted Electrical Weapon Effectiveness: Old vs. New Technology**

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The goal of this presentation is to discuss and view new generation Conducted Electrical Weapons (CEW) and their effectiveness in the field by comparing them to older generation technology. The audience will understand how effective each of these technologies is when compared to each other.

This presentation will impact the forensic science community by aiding in event reconstruction and in understanding the capabilities of these devices. Event understanding and reconstruction of events involving

newer technology will remain accurate by comparing effectiveness to familiar and known (older) technology effectiveness.

The CEW is a popular law enforcement tool used in the control and restraint of potentially violent persons. The TASER X26 CEW, in service since 2003, is the most widely used CEW in society, and is considered older generation technology. The TASER X2 CEW has been recently released for service, is expected to replace the X26 over the next several years, and is considered new generation CEW technology. In general, the X2 is less familiar to most forensic personnel.

The X2 is different than the X26. In addition to a multi-shot capability, it has redesigned electrical waveform and output characteristics when compared to the X26. This pilot study describes an initial head-to-head comparison of the effectiveness of these devices in stopping a motivated person when compared to each other. This information may be important forensically when evaluating or reconstructing a situation where new generation CEW technology has been used.

This study presentation should provide attendees with an understanding of how effective each of these CEWs is in controlling/restraining a motivated person. It will also help attendees to understand how effective the CEWs are when compared to each other. This information should prove to be helpful to the forensic, legal, and investigative community when reconstruction of events must be made.

Four human volunteers were recruited and had metal TASER XP probe pairs manually placed to a depth of 13 mm. Each volunteer had two pairs of probes emplaced (one pair on the right and one pair on the left of the abdominal/inguinal region). Superior probe placement was at the costal margin, five inches lateral of midline (as guided by the umbilicus). The Inferior probe was placed vertically inferior at predetermined spread distances. These distances were 6, 9, 12, and 16 inches apart (15.2-40.6 cm). Each volunteer was given the goal of holding a rubber knife and slashing a suspended dummy 10 feet (3.05 meters) away during the CEW exposure. As a means of motivation, they were told that the CEW exposure would continue until they reached their goal (in reality, the CEW exposure was terminated when the operator determined that no further forward progress was being made). Each volunteer received two CEW exposures, one each from an X26 and an X2 factory standard CEW. The order of the exposures was randomized. There was an approximate two-minute rest period between the two exposures. All exposures were recorded by a high-speed, high-resolution video camera. The videos were later reviewed and scored for effectiveness by a panel of physician and law enforcement experts.

No subjects were excluded and all completed the testing protocol for a total of eight exposures (four pairs) for evaluation. The review experts evaluated each exposure for degree of upper extremity effect, lower extremity effect, total body incapacitation, and whether or not they were able to reach their goal. The exposure reviews were then descriptively compared independently for probe spread distances and then compared between devices.

In this pilot study, results show that there was no discernible, descriptive difference in effectiveness between the TASER X26 and the X2 CEWs when compared in head-to-head fashion. Based on this, end-users and forensic investigators should expect similar performance characteristics during use and in event reconstruction. As new generation CEW technology becomes more popular, it may be important to increase the scope of this research for validation.

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**Conducted Electrical Weapon, Electronic Control Device, TASER**

### **D39 Shooting Incident Analysis: Critical Case Studies**

*Alexander Jason, BA\*, ANITE Group, PO Box 375, Pinole, CA 94564*

After attending this presentation, attendees will better understand the elements within an analysis and reconstruction of shooting incidents.

This presentation will impact the forensic science community by providing new data from original research on shooting incident analysis and reconstruction.

A central element in any analysis of a shooting incident is the realization that all shootings involve time and motion: from visual perceptions, decision process, neural transmission, to muscle movement during the “squeeze” of the trigger, bullet travel, and gross defensive or offensive movements of the shooter and person being shot. Along with this understanding, the analysis and reconstruction of shooting incidents often requires consideration of several forensic and human performance components including wound ballistics, psycho-neurological factors, bullet flight dynamics, terminal ballistics, gunshot residue characteristics, firearms operation, and other associated areas of knowledge such as bloodstain interpretation. The integration of the data from these areas can be extremely useful in any forensic examination of a shooting incident – particularly when multiple shots are involved.

Of these dynamic elements pertaining to shooting incidents, the movement of persons involved in the shooting incident is often of extreme significance in the analysis of the incident and the legal determination (homicide, self-defense, etc) that is always produced after a shooting incident. The location of entry wounds and the associated wound paths within the body are often utilized as indicators of a victim’s body orientation and stance when the bullet(s) struck. While this data is highly useful, its value can only be validated when the data from these elements are integrated into the dynamics of the shooting incident.

Over several years, this study covers numerous research projects to address these issues. The results of the research and the significance of the results when applied to the analysis of shooting incidents are presented within this presentation. The research presented in this presentation is the result of forensic analyses and reconstruction of shooting incidents performed for criminal and civil litigation. Many of the findings presented have been used during court testimony and found to be relevant to the understanding of significant issues relating to the position, orientation, and location of persons involved in shooting incidents.

Case reviews of actual shooting incidents have established that in many shooting incident analyses, the movements of shooters – and particularly – the person(s) being shot are inappropriately ignored. Research on the actual movements of participants in shooting incidents was performed and the results demonstrate that a consideration of body movements during the incident can provide significant data directly useful in the analysis, reconstruction of shooting incidents, and in the legal determination (adjudication) of the incident.

Shooting cases reviewed and presented in this presentation include one in which a (police officer) shooter’s description of the incident was described as “inconsistent with the physical evidence” by an expert witness biomechanical engineer. This opinion was based on the expert’s attempt to analyze the incident without consideration for the movement of the shooter and person being shot. In another shooting case, a key element was the integration of dynamic movement of a suspect and the repositioning that occurred with the suspect’s clothing during movement.

Additional examples of shooting victims are presented on video with detailed analyses of the various movements that occur and their potential significance in shooting incident analysis.

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**Shooting Analysis, Shooting Reconstruction, Shooting Incidents**

### **D40 Term and Definitional Analysis of Sex-Related Homicide**

*Tanisha V. Henson, MFS\*, Richmond Office of the Chief Medical Examiner; Central District, 400 East Jackson Street, Richmond, VA 23219*

After attending this presentation, attendees will: (1) understand the difficulty in conducting proper research when no single term or definition exists; (2) understand the difficulty in maintaining statistics when no single

term or definition exists; and, (3) understand the importance of having a single term and definition both for law enforcement and clinicians.

This presentation will impact the forensic science community by better informing attendees of the importance and need of a single term and definition in the recognition, investigation, and classification of sex-related homicide.

Sex-related homicide is a heinous crime. It is also a crime that grips the community in which the crime occurs. What is not well known about this particular crime is what the appropriate, correct, or even most descriptive term or definition is. What is also not known about sex-related homicide is what the true statistics are. Researchers vary in their opinion as to whether or not sex-related homicide is rare or rampant. With 18 terms and 48 definitions, it is easy to see how it would be difficult to decide if it is rare or rampant. These terms range from erotophonophilia to lust psychotic killing to sexual murder. Several of the terms have multiple definitions and some terms have no definition. Given the broad spectrum of terms and definitions, it is easy to see how it would be difficult to determine the statistics. The Federal Bureau of Investigation (FBI) uses information voluntarily submitted by law enforcement agencies all over the United States to prepare the Uniformed Crime Report (UCR) and the Supplemental Homicide Report (SHR). From the information submitted, they use motive to classify the homicide. A cursory glance of UCR and the SHR would lead one to believe that sex-related homicide is rare if they were to only consider rape, other-sex offenses and possibly prostitution & criminalized vice. Sex-related homicide is much more than rape, other-sex offenses and prostitution & criminalized vice and should be recognized as such.

This research explored a wide range of terms and definitions, as well as unpublished data from the FBI. The resources utilized were from peer reviewed articles, educational textbooks, and the National Clearinghouse for Science, Technology, and the Law (NCSTL). The unpublished data revealed the statistics prepared by the FBI for the UCR and SHR for homicides with known or obvious sexual relations range from 0%-2%. These particular homicide statistics only include rape, other-sex offenses, and prostitution & criminalized vice. There is also a large homicide category which is labeled as unknown. The unknown category averages 29% of the annual homicides. A brief overview of the total number of homicides in comparison to the unknown motive category shows: the total number of homicides from 1980 through 1989 was: 190,020. Of these homicides, 42,363 or 22.29% were placed into the unknown motives category. The total number of homicides from 1990 through 1999 was: 190,906. Of these homicides, 56,409 or 29.55% of these were placed into the unknown motives category. The total number of homicides from 2000 through 2008 was: 129,559. Of these homicides, 48,732 or 37.61% were placed into the unknown motives category. From the 1980's to the 1990's there was an increase of 7.25% in homicides placed into the unknown motives category. From the 1990's to the 2000's there was an increase of 8.07% in homicides placed into the unknown motives category. Overall, from the 1980's to the 2000's there was a 15.32% increase in homicides placed into the unknown motives category. With the number of unknown motive homicides increasing, the need for better categorization becomes more apparent. Several researchers acknowledge the unknown category as a veritable dumping ground for homicides with no known motive and this category has a high likelihood of being the category where the majority of sex-related homicides are placed.

There are a wide variety of terms and definitions to choose from when researching sex-related homicide. These can contribute to research weakness, inaccurate statistics, and subpar investigations. To remedy this problem, there should be one universal term and definition which both law enforcement and clinicians can use. In addition to the use of one term and definition, the statistics for sex-related homicide would be better maintained with the use of the four categories as ascribed by Vernon J. Geberth rather than on the basis of motive as is currently used by the FBI. At the conclusion of this study, the following facts remain: researchers and

law enforcement cannot agree on a universal term or definition, they cannot agree on whether sex-related homicide is rare or rampant, and the available statistics are not entirely accurate.

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#### **Sex-Related Homicide, Lust Murder, Sexual Killing**

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### **D41 Detection of Gasoline From Lung Tissue and Heart Blood for Use in Determining Victim Status at the Time of a Fire**

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The goal of this presentation is to understand how gasoline can be detected from the lungs and heart blood of a deceased victim post fire for use in determining the victim's status at the time of the fire.

This presentation will impact the forensic science community by demonstrating the importance of analyzing a victim's tissues for ignitable liquid residues post fire as it can allow for the determination of a the victim's status.

In Ontario, fire investigators from the Office of the Fire Marshal (OFM) are responsible for determining the origin and cause of fires and explosions within the province and in particular those of suspicious nature. As part of the fire investigation, debris samples are collected from the scene and analyzed by the Center of Forensic Sciences. The standard practice is to collect items that are porous, highly absorbent, or adsorbent with high surface areas as they allow for better retention of the ignitable liquids. The items collected most often are carpets, cardboards, soils, cloths, and other items that have not been impinged by flame such as material under baseboards. These samples are analyzed for the presence of any ignitable liquid residues which may be evidence of an accelerant used in the fire. This information will aid in determining if the fire was intentionally set.

The purpose of this study was to determine the feasibility of identifying whether a victim was alive or deceased at the time of a fire started with accelerants by detecting gasoline residues within their lungs and heart blood post-fire. It was hypothesized that only when a victim was alive and performing respiration would sufficient gasoline for detection be present in the lungs and heart blood post-fire. This experiment involved anesthetizing a live pig, exposing the pig to gasoline vapors for 10 minutes, and then euthanizing it. The carcass was clothed with a cotton t-shirt and placed in a house where additional gasoline was poured onto it and the house set ablaze. The house also contained two pig carcasses, one with gasoline poured directly onto it (positive control) and the other with no gasoline exposure (negative control). Thermocouples were placed under each carcass and in the center of each room at ceiling and floor levels to measure the temperature. After the fire had reached flashover and was suppressed, the carcasses were collected and their lungs and heart blood excised at a necropsy. The lungs and heart blood were then placed into glass jars as per OFM protocol. The headspace from the samples was analyzed by thermal desorption-gas chromatography-mass spectroscopy for the presence of a gasoline signature.

Preliminary results showed that only the lungs and heart blood from the live pig that inhaled gasoline contained gasoline residues. This indicates that it is possible to determine a victim's status at the time of the fire based on the detection of gasoline in the lungs and/or heart blood. The thermal data showed that the bodies act as an insulator and protects the underside, as the temperatures under the carcasses did not exceed 30°C while the room was over 900°C. This protective feature of the body was also demonstrated when portions of the t-shirt were found intact underneath

the carcasses after the fire. To validate these findings an additional three house fires and four vehicle fires will be conducted.

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**Fire, Accelerants, Chemistry**

## **D42 Hyperspectral Remote Sensing of Individual Gravesites**

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After attending this presentation, attendees will better understand airborne hyperspectral remote sensing as it relates to locating clandestine gravesites including known limitations and areas that need future exploration.

This presentation will impact the forensic science community by outlining a potential new tool for detection of individual gravesites using airborne platforms, enabling coverage of a wider area than is possible using ground based detection methods such as Ground Penetrating Radar and Cadaver dogs.

The use of hyperspectral remote sensing for the detection of clandestine graves is emerging as a potential alternative tool in forensic investigations. Previous studies have demonstrated it is possible to use hyperspectral remote sensing techniques in detection of mass graves. With this study, however, the goal is to demonstrate the feasibility to utilize this same technology for the detection of individual burial sites under specific conditions.

Detection of clandestine burials is of interest to police and first responders, with cases arising from victims of crime as well as situations where no foul play is suspected, such as missing hunters and hikers. Airborne hyperspectral remote sensing enables coverage of a wider area than is possible using ground based detection methods, such as ground penetrating radar and cadaver dogs. However, as with all detection technologies, it has its limitations and a fundamental aspect of using this technology for single grave detection is to understand what these limitations are.

Detection is based on of the alteration of the environment by the body through decomposition; it essentially being a form of environmental contamination which can affect both the soil and vegetation. It is known that a decomposing body alters the surrounding environment and that the changes in the soil matrix can alter plant chemistry. The degree to which this alteration takes place is highly dependent on season, geographical location, vegetation type and the state of the body when it is buried, as well as characteristics of the body such as its weight. Due to the chemical changes in the soil, plants undergo a stress response, changing the levels of plant pigments. These changes in plant pigments have been shown to be detectable by hyperspectral sensors with mass graves. However, the case of a single grave poses a far more difficult detection problem, primarily because the body mass is much smaller than with mass graves. Furthermore, the simple question of how burial depth affects detectability and persistence of the spectral changes is unknown.

For this study, 20 pig (*Sus scrofa*) carcasses were utilized as proxies for human cadavers. The effects of three burial scenarios – surface were examined, and 30cm and 90cm soil cover (all with and without the bodies being wrapped in garbage bags) - on the detectability of single bodies (150-200 lbs each) from an airborne sensor, as well as from laboratory analyses of the spectral signatures of the soil and subsurface methane concentration and surface methane flux.

A Twin Otter with hyperspectral sensors covering the visible to shortwave infrared range sensors flew over the site and collected imagery as time and weather permitted from July - October. The two airborne hyperspectral sensors were used coincidentally to cover the 450 to 2450 nm range of the electromagnetic spectrum. In addition to the airborne sensor, a portable spectroradiometer was used to collect plant spectra in field and soil spectra in the lab.

This study compares and contrast the signatures of graves at different burial depths with and without garbage bags and discuss the influence of these two factors on the detectability of single graves using this technology. Situations in which these tools may be feasible for the location of single bodies as well as a description of the detailed calibration procedures that are required in order to adequately use such data will be discussed.

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**Hyperspectral Remote Sensing, Clandestine Graves, Cadaver Detection**

## **D43 County Burial: A Look at the Final Resting Place of Unclaimed and Unidentified Remains**

*Sharon M. Derrick, PhD, and Patricia C. Smith, BA\*, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

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The goal of this presentation is to identify the process for referring unclaimed and unidentified remains to county burial. The steps will include how the remains are handled, the period prior to the county burial referral and how county burials are handled after family has been located and notified. This presentation will also examine other county and state programs and compare those processes with Harris County. Statistics regarding the number of remains that have been unclaimed and the number of remains that remain unidentified will be included. Statistics on the number of remains where family was located after the referral for county burial was completed will also be provided. Lastly, case studies for a 2007 homeless veteran, two 1973-murder cases that have been identified, and a 1980's death of a Marine will be provided.

This presentation will impact the forensic science community by serving as an educational tool for family members of those deceased and can help other jurisdictions augment or implement new procedures for handling unclaimed or unidentified remains. A county burial program will continue the respect and support for those remains that are unclaimed and unidentified. A county burial program will allow time for family members or friends to come forward to pay their respects and claim or acknowledge the deceased and their life.

The state of the economy has created difficulty with locating family members of deceased persons. The unemployment rate is on the rise and job prospects are fewer and farther between. Society has to travel farther and in some instances, move out-of-the-state in order to obtain employment. This makes it more challenging for families to keep up with loved ones and maintain close relationships. This also creates difficulty for HCIFS when trying to locate next-of-kin (NOK).

This presentation will provide information to the attendees regarding the timeline, process, and requirements needed for county burial referrals. The Investigation Division utilizes a checklist of contacts to look for the legal next of kin. After the checklist is exhausted, then the case is referred to the ID Unit for county burial referral. Part of the checklist is various internet searches, like public records, ancestry, and county clerk databases. Information from criminal and civil histories, bankruptcy court records, child support payments, life insurance, property ownership, and licenses can help in locating family. A news router or consulate letter is sent when NOK cannot be located, in the hope of someone reading the article and recognizing the description or picture provided. An abandonment letter is sent to the NOK 15 days after the NOK was notified. Fifteen days after that mailing, county burial will be referred, unless the NOK responds with their

choice of a funeral home. Families may apply for county burial assistance through the Harris County Community Services Department Bereavement Services program. The status of the families and the decedent's estate will need to meet financial criteria before assistance will be approved. Upon approval, the referral is usually immediate.

Fetuses and infants have a separate criteria regarding burial. Fetuses and infants must meet live birth or gestation/weight requirements of >500g/22 weeks and have been abandoned. The HC Burial Services will not provide burial assistance for an unclaimed fetus of <20 weeks and/or 500 grams; a surgical specimen disposal form is completed and processed by the morgue as a surgical specimen. HCBS will provide burial assistance for an unclaimed neonate, regardless of age or weight, if neonate took a breath or showed signs of life at birth.

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#### **County Burial, Unidentified/Unclaimed Remains, County Burial Program**

### **D44 Animal Attack-Related Deaths in Florida**

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After attending this presentation, attendees will have an appreciation of the nature of the varied types of deaths resulting from the interactions between humans and indigenous and exotic animals and reptiles in Florida, and the species involved.

This presentation will impact the forensic science community by providing a unique insight into Florida's varied fatal human/animal encounters and by illustrating the importance of the multidisciplinary medicolegal death investigation in these cases.

Florida has been the setting of several high profile deaths caused by captive animals in its amusement parks; the most recent being the death of a trainer killed by an orca whale, as well as two previously reported deaths caused by elephants. Nationally Florida also has the third highest rate of deaths due to attacks by canines, the most common offending species being the pit bull. Additionally, the state has also been the location of a number of deaths caused by indigenous species, as well non-native mammals and reptiles, which are frequently kept as pets in the state. Florida's unique climate provides an environment suitable for many potentially hazardous native and exotic species, including reptiles. This has resulted in a multitude of unforeseen fatal encounters between these animals and the ever expanding human population.

Files from the District 21 Medical Examiner's Office, which serves three counties in southwest Florida, and the District 5 MEO, encompassing five counties located in the central part of the state, in addition to previously tabulated records of deaths resulting from animal attacks/encounters, to elucidate the types of deaths and the species involved were reviewed. The most frequently reported deaths have resulted from shark attacks (25) on swimmers visiting Florida's many beaches and alligator attacks (18), most commonly attributed to alligators that have lost their innate fear of humans because they have been fed by individuals who have ignored the widespread warnings against such feeding. In addition to deaths caused by horses and dogs, Florida jurisdictions have seen five alligator-related deaths and one death caused by the mauling by a Siberian tiger kept in a private sanctuary. In addition, two deaths caused by snakes, including the first death due to the envenomization of a coral snake reported since anti-venom was developed 40 years ago, and in spite of the fact that non-lethal coral snake bites are frequent in Florida. The other snake-related death was that of a 2-year-old child who was the victim of mechanical asphyxiation by an improperly housed albino Burmese python that was kept as a pet. In spite of Florida's large bear population, there has never been a reported death in the state caused a bear. The immediate causes of death in these cases varied nearly as much as the species involved, and included exsanguination, drowning, sepsis, asphyxia, and blunt force trauma. The determination of

the cause and manner of deaths in these cases requires a multidisciplinary investigation and necessitates the cooperation of the investigating law enforcement and wildlife conservation agencies, the medicolegal death investigator and the forensic pathologist. Whether foreseen or unforeseen, the underlying cause of death is always a result of the human/animal interaction.

While the literature and knowledge pool for more routine deaths (motor vehicle collisions, gunshot wounds and overdoses) is abundant, that of animal-related deaths is far less widespread. The deaths reported in this presentation provide a unique insight into Florida's varied fatal animal encounters.

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#### **Animal Attack, Death, Florida**

### **D45 Animal Nutrition Investigative Techniques Essential to Obtaining Investigative Forensic Information From Multiple Sites**

Gary M. Pusillo, PhD\*, INTI Service Corporation, 2017 230th Street, Marshalltown, IA 50158

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After attending this presentation, attendees will understand how careful interpretation of alleged animal nutrition related deaths and abnormalities in the presence of concomitant examination of all husbandry and environmental factors, is critical to scientifically valid conclusions.

This presentation will impact the forensic science community by showing how to use valid scientific principles to help differentiate the consequences of feed and nutritional inadequacies from husbandry practices, improper veterinary care, idiosyncratic drug induced complications, intentional abuse, accidents, and genetic disorders

Contaminated, distressed, or adulterated animal nutrition inputs can result in feed associated dysfunctions (FAD) within an animal population or in a specific individual. Nutrient deficiencies, excesses, and imbalances can result in nutrition associated dysfunction (NAD). NAD is associated with nutritional profile and ingredient mistakes and oversights in the manufacture, preparation, formulation, or the presentation of daily dietary components.

An essential component in the evaluation of animal feed and nutrition related claims is a scientifically valid set of investigative techniques required to accurately evaluate FAD and NAD in animals. As an example of the practical application of these techniques, a case study will be presented involving multiple Alpaca's at multiple sites, and a commercial Alpaca feed alleged to be contaminated with an alleged toxic substance, salinomycin.

Alleged toxic substance exposure from a commercial Alpaca feed requires the professional investigator to consider all other factors, management, environment, infectious agents, additional feeds, nutrition, and accidents; that may influence the appearance of abnormalities in a typical alpaca operation. Checklists and techniques will be provided to attendees, which along with firm scientific reasoning can be applied in the examination of an animal or population, the feed related components, and the facilities that existed at the time prior to, during and after exposure to an alleged toxic substance in commercially prepared feed.

Forensic nutrition requires a specific application of highly specialized and exacting disciplines that require the application of a unique set of interdisciplinary scientific skills and practical experience in animal husbandry and feeds and feeding. Defining the exact nature of the problems reported by alpaca caretakers, owners, trainers, and veterinarians requires the forensic professional to identify those dysfunctions within either the animal's environment or the daily ration, which may have attributed to the problems and financial losses relating to its performance, quality of life, and health.

Standards of what is acceptable evidence for causation in FAD- and or NAD-related illnesses in domestic animals do not currently exist. As a

result, cases of commercial animal feed alleged to be contaminated, distressed or adulterated may result in the perpetuation of a misdiagnosed abnormality and the proliferation of erroneous methods of domestic animal FAD and NAD evaluation.

Modern animal forensic investigations cannot separate valid scientific observations from the complexity of the environmental realities that act singly, together, and in a holocoenotic manner. At the same time, it is recognized that the animal or targeted population, in turn, reacts upon their environment, often producing marked modifications. Cases of NAD and FAD are often improperly investigated, documented, and analyzed. This presentation will provide actual details on how to successfully perform feed and nutrition investigations with proven techniques.

**Feed Contaminants, Nutrition, Animals**

## **D46 Using Thermal Imaging to Detect Trauma and Disease Processes in Animal Cruelty Cases**

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The goal of this presentation is to understand how thermal imaging works and how it can be used to help diagnose injuries in animal victims.

This presentation will impact the forensic science community by exploring a method of identifying recent and healed traumas, as well as infections, in victims of animal cruelty. Due to limitations set by animal fur, and the sometimes-fractionous nature of injured animals, a method of examination that requires minimal handling is beneficial.

Based on the electromagnetic spectrum, infrared light is a wavelength of light that is not visible to the human eye. Within the spectrum, visible light consists of seven colors with a range of wavelengths. Violet has the shortest wavelengths and red has the longest. The prefix “infra-” is derived from the Latin word *infra*, meaning “below or further on”. Therefore, the word “infrared” means “below red” indicating its position in the electromagnetic spectrum, with wavelengths longer than the color red.

According to the International Commission on Illumination, infrared light can be broken down into three bands:

- IR-A: 700 nm – 1400 nm (0.7  $\mu\text{m}$  – 1.4  $\mu\text{m}$ )
- IR-B: 1400 nm – 3000 nm (1.4  $\mu\text{m}$  – 3  $\mu\text{m}$ )
- IR-C: 3000 nm – 1mm (3  $\mu\text{m}$  – 1000  $\mu\text{m}$ )

This division structure is commonly broken down into five sub-divisions:

- Near-infrared: 750 nm – 1400 nm (0.75  $\mu\text{m}$  – 1.4  $\mu\text{m}$ )
- Short-wavelength infrared: 1400 nm – 3000 nm (1.4  $\mu\text{m}$  – 3  $\mu\text{m}$ )
- Mid-wavelength infrared: 3000 nm – 8000 nm (3  $\mu\text{m}$  – 8  $\mu\text{m}$ )
- Long-wavelength infrared: 8000 nm – 15000 nm (8  $\mu\text{m}$  – 15  $\mu\text{m}$ )
- Far infrared: 15000 nm – 1,000,000 nm (15  $\mu\text{m}$  – 1000  $\mu\text{m}$ )

Near infrared and short-wavelength infrared are considered “reflective” infrared. It is within these two ranges that forensic science applications typically fall. Both ranges are effective based on the amount of water absorbed by the surface being examined and its contrast to the evidence on it (i.e. fingerprints, bite marks, bloodstains, etc.). The near and short-wave infrared ranges are also used to identify chemicals based on the substance’s ability to absorb the wavelengths.

The term “thermal infrared” includes the mid- and long-wavelengths of infrared light. Unlike the near and short-wavelengths of infrared light which are reflected off of surfaces, thermal infrared is emitted by an object in the form of heat (radiation). These ranges are used only minimally in forensic sciences. The long-wavelength range is considered the “thermal imaging” range and is typically used for the investigation of mechanical and structural systems.

In the practice of human medicine, thermal imaging is being researched as a possible detection tool. It is currently used as an alternative, non-invasive diagnostic procedure for detecting breast cancer, as well as for monitoring conditions such as back injuries, vascular disease, and stroke

screening. The principle behind using infrared imaging for the detection of cancerous growths is that tumors have an increased amount of blood vessels, which are necessary to sustain the high metabolic rate of cellular growth and replication. This increase in blood flow results in an increase in temperature. Similarly, nerve compression and soft tissue inflammation that may be a cause of pain can be visualized through thermal imaging when it may not be apparent on x-ray or MRI.

Thermal imaging has been researched and used within veterinary medicine for over 40 years. While the technology has been used on a variety of animals (including livestock and zoo animals), the majority of research has centered on injury detection and diagnosis in racing horses. Thermal infrared has been used to diagnose joint inflammation, tissue injury, muscle atrophy, and general diagnostics. The same principles will apply, to the detection of contusions as to that of cancerous growths. If there is an area of increased blood, this should show as an area of increased heat. Areas of infection should also show up as hot spots. In contrast, scars should show up as cold spots due to minimized blood flow.

Case studies will be conducted using a Forward Looking Infrared (FLIR) camera, model i60. This camera has a spectral range of 7,500 to 13,000nm (7.5 to 13 $\mu\text{m}$ ) and a temperature range of -4 to 662°F. Information will be gathered from canine and feline patients of the University of Florida College of Veterinary Medicine. Case studies will include patients with confirmed traumatic injuries, suspected trauma, no traumatic and surgical procedures performed. When possible, animals will be scanned multiple times in an attempt to record the thermal image differences between recent and healing injuries. Trauma data, as well as thermal images will be documented and analyzed.

The use of thermal imaging in veterinary medicine has primarily focused on large animals. In dealing with animal cruelty cases, the question has arisen as to how thermal imaging can be used as a non-invasive way of diagnosing trauma and infection in animal victims. Because animals are typically covered by fur, the identification of contusions and scars can be difficult and sometimes impossible. It is hypothesized that these injuries will be detectable with the thermal infrared camera, and possibly a general stage of healing/time since injury can be established. Because close contact is not required to use this device, veterinarians and technicians will be safer in dealing with fractious animals. The data acquired is also in real time, allowing for quick diagnoses.

**Animal Cruelty, Thermal Imaging, Infrared Photography**

## **D47 The Scientific Working Group on Disaster Victim Identification**

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After attending this presentation, participants will understand the operating structure, purpose, and objectives of the Scientific Working Group on Disaster Victim Identification (SWGdVI).

This presentation will impact the forensic science community by raising awareness of SWGDVI efforts to assemble organizations and individuals to exchange ideas, develop, disseminate, and advance consensus guidelines, best practices, and other recommendations and/or findings relevant to disaster victim identification.

Disaster victim identification (DVI) refers to the component of mass fatality management that involves the scientific identification of human remains. Based on DVI experiences both here and abroad, forensic practitioners in the U.S. voiced the need to assemble professionals from the medicolegal and forensic communities in a collaborative effort to identify and promulgate DVI guidelines and best practices. In response, the National

Institute of Justice provided funding to the Federal Bureau of Investigation (FBI) to develop the SWGDVI.

The twenty-five member SWGDVI Board is composed of permanent member agencies/organizations and individual members. Permanent member agencies/organizations are entitled to appoint representatives with subject matter expertise in the broad DVI discipline. Individual members are selected based on individual qualification, not by agency affiliation. The SWGDVI Board consists of fifteen individual members and nine permanent member organizations, including: FBI, National Transportation Safety Board, Department of Health and Human Services/Assistant Secretary of Preparedness and Response, Department of Defense/Armed Forces Medical Examiner System, National Association of Medical Examiners, International Association of Coroners and Medical Examiners, INTERPOL DVI Steering Group, International Commission on Missing Persons, and the International Committee of the Red Cross.

The purpose of SWGDVI is to advance the scientific basis for disaster victim identification by assembling professionals from the DVI community, including international participants, in a collaborative effort to exchange ideas regarding scientific analysis methods, protocols, training, and research related to DVI. The SWGDVI will develop, disseminate, and advance consensus guidelines and best practices, studies, and other recommendations and/or findings for DVI, with an emphasis on scientific evidence-based methods with appropriate quality assurance and quality control processes. The SWGDVI shall also encourage and evaluate research and/or innovative technology related to DVI.

To achieve these objectives, the SWGDVI has created committees, which are populated by U.S. and international professionals from the DVI community to examine targeted issues for the purpose of identifying and codifying existing guidelines, or, where clear guidelines do not exist, to formulate guidelines and best practices and disseminate them to the broader forensic community. Currently, the SWGDVI has created the following committees, chaired by Board members and populated by advisory members: Search and Recovery, Pathology, Anthropology, Odontology, Friction Ridge Analysis, Molecular Biology/DNA, Data Management, Identification Data Synthesis and Quality Control, Victim Information Center/Family Assistance Center, and DVI Management.

The SWGDVI is not, by its chartering instrument, a regulatory body with any formal enforcement authority. The SWGDVI bylaws specifically state that the SWGDVI promulgates best practices and guidelines, but not standards, because of the real or perceived notion that standards are enforceable. Essential to the successful promulgation of guidelines and best practices is general acceptance (i.e., consensus, by the international DVI community). To foster community input, committees are comprised of advisory members selected for specific knowledge and experience in the relevant subject matter areas. Advisory members are encouraged to participate in drafting proposals/products for review by the SWGDVI Board. Committees are also encouraged to solicit input and receive commentary from members of the DVI community that are not directly involved with SWGDVI. All SWGDVI draft products are posted on the SWGDVI website ([www.swgdvi.org](http://www.swgdvi.org)) for public comment before formal adoption. The public is encouraged to submit comments and propose changes to SWGDVI products at any time, either through direct contact with the SWGDVI Board, relevant committees, or through the feedback forum on the SWGDVI website. Additionally, the SWGDVI will forward proposed documents to relevant professional organizations for input.

This session will provide a general overview of SWGDVI and will present summaries of draft guidelines to the forensic community for review and discussion.

**Disaster Victim Identification, Mass Fatality, Scientific Working Group**

## **D48 The Crossroads of Forensic Science and Cultural Traditions at the Base of Mount Everest: The Crash of Agni Air Flight 101**

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The goal of this presentation is to provide an outline for the attendees on the sequence of events that occurred after the crash of Agni Air Flight 101 near Kathmandu, Nepal. After this presentation, attendees will: (1) gain a better understanding of the complex role of the forensic anthropologist in mass disasters and victim identification; and, (2) gain a greater awareness of the religious and cultural pressures often involved in multi-national aircraft crashes.

This presentation will impact the forensic science community by providing an example of how international cooperation, sensitivity, and understanding of specific cultural and religious practices led to successful identifications of victims of a multi-national aviation disaster. By addressing these potential issues, this presentation will provide the forensic community with additional approaches and insights that can be applied to a variety of mass disasters in a multi-national and multi-religious setting. With a global trend of increasing interaction between disparate societies, this disaster provides the forensic community with the opportunity to be better prepared when catastrophes strike again in the future.

On August 24, 2010, Agni Air Flight 101, a Dornier 228 turboprop aircraft with fourteen individuals onboard, was traveling from Kathmandu to Lukla, Nepal. The Tenzing-Hillary Airport in Lukla is the usual starting point for the Everest Base Camp trekking route. Shortly after take-off, the aircrew requested immediate return to Tribhuvan International Airport in Kathmandu due to increasingly poor weather at Lukla. Several minutes later, the captain reported technical difficulties. Approximately 20 minutes after takeoff, the crew made their last radio contact, reporting they were inbound to Kathmandu. The aircraft never arrived.

Law enforcement was notified of an aircraft crash in Shikharpur, a village approximately 50 miles south of Kathmandu. Due to the remote location and heavy rains, first responders arrived by foot two hours after the incident. Nepalese police and army personnel located a crash crater approximately three meters deep with a surrounding debris field over 100 meters in diameter. All fourteen on board the aircraft were killed including eight Nepalese citizens, four Americans, one British citizen, and one Japanese citizen. Recovery efforts were severely hampered by continued heavy rainfall, flooding, and landslides. The Nepalese army conducted extensive searches of the surrounding area collecting various portions of human remains in plastic bags. Due to the high-speed impact of the crash, fragmentation and disruption of the human remains were extensive. Numerous portions of human remains were commingled in bags by the recovery personnel to facilitate helicopter transportation to the Tribhuvan Teaching Hospital in Kathmandu.

Forensic pathologists of the Tribhuvan Teaching Hospital performed an initial inventory of seventeen bags of human remains detailing over 800 fragments. Due to extreme pressure from the local community to perform immediate religious mortuary rites, the team presumptively identified portions of five Nepalese individuals and released them to their next-of-kin for cremation. The condition of the human remains from this crash differed significantly from previous small (15-20 passenger-sized) aircraft and helicopter crashes in Nepal in that the remains from the Agni Air Flight 101 were severely fragmented. The Tribhuvan pathologists were accustomed to aircraft fatalities that were intact and did not present with such severe disruption. As a result, the Nepalese team contacted the U.S. Embassy in Kathmandu for assistance in the identification process.

Forensic anthropologists from the U.S. Department of Defense's Joint POW/MIA Accounting Command's Central Identification Laboratory (JPAC-CIL) traveled to Kathmandu, Nepal and arrived on August 27, 2010.

While the CIL anthropologists were enroute, the Tribhuvan pathologists presumptively identified a partial torso as that of the Japanese victim and intended to release the remains to the next-of-kin the following day. To prevent delay of this release, the CIL anthropologists, upon immediate arrival to Nepal, examined the presumptively identified portion and retained a bone sample for DNA analysis to aid in the identification process of additional remains.

The CIL anthropologists took operational control of the remains processing and with the assistance of the Tribhuvan pathologists, conducted a complete inventory of the remains and documented primary and secondary identifiers including, dentition, tattoos, piercings, and other bodymarks. Due to severe disruption and fragmentation, as well as moderate decomposition, much of the separated soft tissues was unrecognizable and could only be assigned to body region when identifiable skeletal elements were adhered. A total of 130 DNA samples were taken (mostly from bony elements) and immediately delivered to the U.S. Department of Defense's Armed Forces DNA Identification Laboratory (AFDIL) for sequencing and comparison with reference samples obtained from the next-of-kin.

Once DNA results were obtained, each sample sequence and subsequent sample identification was matched to the parent material retained at the Tribhuvan Teaching Hospital in Kathmandu. All six foreign nationals (four American females, one British male, and one Japanese male) were positively identified through nuclear DNA analysis. The remaining samples sequenced at AFDIL were individualized into seven distinct sequences (four males and three females). All next of kin of the Nepalese victims declined to submit a family reference sample and declined individual identification of any remaining body parts through DNA analysis. To this end, the parent material of these sequences was combined with the human remains that could not be sampled and designated as group remains by the Tribhuvan pathology team. It should be noted that the successful cooperation of all forensic teams, as well as the support of multiple agencies and international offices, demonstrated how efficiency and exceptional professionalism can facilitate a family's mourning and subsequent healing in their time of extreme personal loss.

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**Mass Disasters, Aircraft Crash, Forensic Anthropology**

## **D49 Mass Fatality Incident Planning and Preparation in Montgomery County, Ohio**

*Kenneth M. Betz, MS\*, Robert L. Hunkeler III, MFS\*, and Kent E. Harshbarger, MD, JD, Montgomery County Coroner's Office, 361 West 3rd Street, Dayton, OH 45402*

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After attending this presentation, attendees will understand how to prepare for a mass fatality incident by conducting exercises, procuring equipment and supplies, and preparing a written plan.

This presentation will impact the forensic science community by providing the necessary tools for a coroner/medical examiner office to process remains and property in a mass fatality incident.

In the past decade, mass fatality incidents came to the forefront through a series of terrorist-related incidents and natural disasters, where deaths ranged from the dozens to the thousands. The definition of a mass fatality incident is an incident where the number of fatalities overwhelms local resources. Some jurisdictions would be overwhelmed with five bodies; whereas others may be able to handle 50 or more with current resources. It is absolutely imperative for coroner/medical examiner offices to be as prepared as possible to handle a mass fatality incident.

Montgomery County Coroner's Office in Dayton, OH investigates over 4,500 deaths per year and conducts approximately 1,300 autopsies per year. In addition to investigating deaths in Montgomery County proper, Montgomery County Coroner's Office provides contract autopsy support for 28 additional counties, making up approximately one-third of the state of Ohio. Many of the counties supported are rural in nature and have relatively few resources should a mass fatality incident occur in their

jurisdiction. Supported counties have clearly stated that should a mass fatality incident occur in their county, the Montgomery County Coroner's Office would be called to assist. Ohio has a Disaster Mortuary Operational Response Team (DMORT) which has a significant response capability; however, if there is a multi-focal event as was seen with the 9/11 Terrorist Attack, resources to assist lesser-affected areas may be harder to obtain. In light of this, the Montgomery County Coroner's Office designed an incidence response team which would be able to stand alone for a short period of time, or be attached to a larger DMORT response should the need for additional resources arise.

The most important component to handling a mass fatality incident is a well-written plan. The Montgomery County Coroner's Office Mass Fatality Incident Plan took over two years to complete. The plan is 48 pages in length and has 31 references, 23 of which are hot-linked directly to the plan for easy access. The references include county, state, and federal documents. The plan was coordinated locally with organizations to include the Montgomery County Office of Emergency Management, the American Red Cross, and the Greater Dayton Area Hospital Association. The plan was also coordinated at state-level. The plan includes sections on roles and responsibilities, processing capacities, incident management, scene investigation, temporary morgue/examination centers, identification of remains, personal property, mass burial, communications, media interaction, Family Assistance Centers, Weapons of Mass Destruction, pandemic events, transportation, storage of remains, and local hospital and university body storage capacities. This plan was provided to all counties supported as well as other counties upon request. The plan currently serves as a baseline for much of Ohio, with each jurisdiction changing location-specific guidance. This allows all entities to be working off of the same general concept.

In addition, Montgomery County Coroner's Office has participated in and helped design multiple exercises within Montgomery County as well as with two neighboring counties. Equipment is essential in processing a mass fatality incident. Montgomery County Coroner's Office has two 18-foot refrigerated morgue trailers, each capable of refrigerating 18 sets of remains. A third trailer is a 26-foot command trailer consisting of supplies, global positioning systems, and radio communications equipment. In addition, a 22-foot mass fatality trailer has been procured, containing a 20-kilowatt generator, two 3.5 ton HVAC systems, one 13' x 21' Small Fatality Management Center, one 13' x 33' Medium Fatality Management Center, lighting, and flooring. Other equipment includes portable digital x-ray technology, automated fingerprint identification system, portable dental x-ray equipment, and portable lighting capable of lighting up areas as large as two football fields.

This equipment is available for use upon request in Ohio or in neighboring states. It is vital to ensure this type of equipment and supplies are made available to those jurisdictions needing additional resources which may be required to handle a large mass fatality incident.

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**Mass Fatality, MFI, Disaster**

## **D50 Two Portable Infrared Detectors: A New Method for Crime Scene Examinations**

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After attending this presentation, the attendees will: (1) learn the results of experiments; (2) view a live demonstration with the two devices used; (3) be provided a detailed introduction of the digital viewer in terms of the four light sources, micro digital measurements, and nine geometrical formats; (4) understand the portable feature with a laptop via a USB and with a projector via a VGA cable; and, (5) see magnification choices.

This presentation will impact the forensic science community by showing how the digital viewer may promote current optical technology to

be more reliable and valid instruments for both qualitative evaluations and quantitative measurements.

The majority of current forensic examinations rely heavily on chemical, biological or physical methods to detect, develop, and visualize latent evidence. One of the disadvantages of the three methods is their destructive effects during the examination process. In recent years, optical methods have gradually gained attention as the first or recommended selection, especially with a limited amount of evidence. Among the common optical sources, portable UV, polarization, laser, X-ray, or multi-light sources devices (technology) have been developed and employed for forensic examinations. From years in the field and research experiences, four observations on optical equipment can be summarized. First, due to certain technical obstacles, portable infrared devices have been missing from the market. Second, except for IR cameras, most IR devices are not capable of taking digital images for storage or transmission. Third, available optical devices rarely provide any quantified measurements during an examination. Finally, certain cameras with limited IR functions are very expensive, making it unrealistic equipment to purchase and difficult to operate. The four observations clearly suggest our technical challenges for optical equipment: portability, digitability, and quantifiability. These three premises are also part of criticisms or challenges from the 2009 National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward.*"

To address the challenges, a joint research project was initiated between the U.S. and China in early 2011: Examinations with Portable Infrared Detection: A Comparative Approach. Two types of IR devices are utilized: An IR flashlight (850 nm) and a digital viewer (near IR). Three test designs (before-and-after) are employed: (1) detection of bloodstains on four types of fabric surfaces (red cotton sweater, red polyester socks, red polyester carpet, and black cotton socks) by different time intervals; (2) detection of bloodstains on a kitchen knife at different time intervals; and, (3) detection of ink writings (five different pens) for potential handwriting traces in four situations (added writing without crossing, added writing with crossing, indented writing, and charred writing).

It is argued that the digital viewer has met three unique challenges for optical equipment: being portable, digital, and measurable. The device has four portable light sources of infrared, black/white, UV, and polarized lights. It also can provide nine different geometrical measurements simultaneously: (1) line; (2) continuous line; (3) polygon; (4) radius circle; (5) diameter circle; (6) three points circle; (7) three points arch; (8) three points angle; and, (9) four points angle.

Testing its further applications in a police debriefing room, in the lab for a supplemental/verification examination, or in the DA's office is strongly encouraged to colleagues. In particular, this study suggests a possible utilization during an expert testimony in court. This palm-size device can provide an effective live demonstration in court to the jury with straightforward digital images and geometric measurements of known and unknown samples. Finally, this new device should be considered to be a great tool in teaching forensic science in classrooms.

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**Digital Viewer, Infrared Flashlight, Micro Digital Measurements**

## **D51 Testing the Ability of Birds to Detect Forensic Odorants: Comparison With Canine Abilities and Instruments**

*Paola A. Prada, PhD\*, Florida International University, 3301 Arena Boulevard, Apartment 94, Sacramento, CA 95834; Gabrielle Nevitt, PhD, University of California Davis, One Shields Avenue, 196 Briggs Hall, Davis, CA 95616; and Kenneth G. Furton, PhD, Florida International University, International Forensic Research Institute, University Park, Miami, FL 33199*

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After attending this presentation, attendees will learn some basic objectives in the area of avian olfaction and the potential uses in forensic applications. As this is a new area of study, this presentation will provide

initial insights into the uses of an alternative biological detector for target chemical odorants of importance to the forensic community.

This presentation will impact the forensic science community by presenting novel techniques in biological detection for target forensic odorants using birds as a potential tool in this context.

Historically, birds have been thought to be functionally anosmic (smell-blind). However, recent work has shown that some species use olfaction in behaviors ranging from foraging, navigation to kin, and nest recognition and that their sense of smell rivals that of dogs. Because this work is relatively recent, little is known in avian species on the evaluation of olfactory sensitivity to specific odorants and direct comparisons to the olfactory tracking power of canines have not been made. To date, most work in this area has been focused on Procellariiform seabirds, which have among the largest olfactory bulbs of any bird. This study builds on recent advances in bird olfaction, and combines these approaches with studies of explosive volatile organic compounds (VOCs) and canine odorants for the evaluation of explosive analog odors in a forensic application evaluating birds as a possible biological detector.

In this study, the primary model being used is Leach's storm petrel (*Oceanodroma leucorhoa*). This species has one of the largest olfactory bulbs of any bird, and Nevitt's group has developed methods for testing olfactory abilities with respect to burrow recognition. Leach's storm petrels nest on offshore islands and are able to relocate their burrow among hundreds by olfaction. Our study co-opts this nest-recognition behavior to investigate sensitivity to explosive analogue compounds. For example, 2,4-dinitrotoluene and 2-ethyl-1-hexanol are important volatile compounds used by canines to identify cast and polymer based explosives. We are adapting similar odor delivery methods currently employed for canine explosive detection and training to scent artificial burrows. This allows us to screen biological detection ability at different concentrations with known permeation rates under field conditions. Controlled odor mimic permeation systems (COMPS) were chosen as an optimized method of delivering known concentrations of an odor mimic. COMPS devices are polymeric devices providing differing quantities of volatile compounds in a field setting. Previous studies have shown the efficient use of COMPS devices in determining olfaction thresholds using canines as experimental models as well as for determining instrumental detection limits. COMPS devices have never been used on birds even though recent studies show that olfaction plays a much more important behavioral role in some avian species than previously thought. Because these studies are relatively recent, not much is known of the olfactory sensitivity of these birds to a wide range of odors.

Initial experiments entailed an instrumental evaluation of various nest materials to identify naturally occurring VOCs as a foundation to understand the types of VOCs that are innate to their environment prior to introducing target odorants for detection. Using a forensic analytical approach, preliminary data corroborate behavioral results suggesting that most burrows have an individual chemical profile. Behavioral experimental assays were also conducted using established Y-maze and choice test approaches using target explosive odorants such as 2-ethyl-1-hexanol with both adult and chick Leach's storm petrels. These odorants were presented at varying concentrations to monitor behavioral thresholds. Data from behavioral field assays suggest that the olfactory threshold for 2-ethyl-1-hexanol is comparable (and even lower) than that of canines. These results indicate that birds are capable of detecting forensic odorants, such as the explosive odorants tested here, at levels of forensic importance.

#### **Birds, Forensic Odorants, Olfaction**

## **D52 An Interdisciplinary Study About the Role of the First Responder at the Crime Scene in Colombia**

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After attending this presentation, attendees will know the procedure of the first responder at the crime scene within the Colombian Criminal System context from an interdisciplinary (psychological, forensic, and legal) point-of-view including the relationship between mass media and crime scene protection.

This presentation will impact the forensic science community by presenting an interdisciplinary approach about the first responder work in Colombia and how the Colombian Police face several factors (mass media, stress, criminals, and victims among others) when they arrive at a crime scene and apply every preliminary procedure.

Crime is an issue in every nation of the world and especially in large and congested cities like Bogotá. With the city expansion and the population increase, the crime scenes handled by the police become more complex and the development of new technology and procedures is necessary. The nature of every crime as well as the social context in which it occurs makes the first responder's work increasingly methodological, scientific, and complex.

Bogotá, D.C. is the Colombia capital with a population of 7,500,000 inhabitants divided in 20 localities. During 2010, the Bogotá Metropolitan Police were first responders in more than 1,700 homicides, 44,000 interpersonal violence cases, and 20,000 cases of robbery, and other crimes. Although the Colombian police have the appropriate training for the procedures as first responder at the scene of crime, the high numbers of cases as well as the presence of mass media covering crimes in Bogotá have had legal, psychological, forensic, social and criminal investigation consequences directly affecting the Justice administration and the Adversarial Criminal System.

This presentation is the result of the project named "Support to management in the security and coexistence for citizens within the framework of the twenty integral plans for local security," with financial support from the Bogotá Mayor's Office, through the Surveillance and Security Fund Secretary. The goal of the project was to support and strengthen the Metropolitan Police of Bogotá in its arduous surveillance work, giving the appropriate procedures as first responders in different scenes of crime in a practical and readable way, and empowering its capabilities to write and present reports as well as to give effective testimonies in the courtroom.

This project emphasized to the relationship between the police officers and many types of people at the crime scene, showing an appropriate approach to witnesses, relatives, suspects, and mass media from psychological and criminalistics points-of-view. It also gave a special consideration to the proper management of evidence in uncommon and complex situations that requires basic forensic knowledge (trace evidence, human remains, etc).

Through the presentation of videos and three specific cases, the AFFIC foundation team will show how the Bogotá Police has been improving its procedures as first responders and how they are approaching people at the crime scene and assuring evidence is handled in a proper way. At the same time, the project also helped Bogotá Police members to avoid psychological

diseases by teaching self control techniques to prevent and manage anxiety disorders.

**Crime Scene, First Responder, Colombia**

## **D53 Pre- and Post-Maturation Growth of External Ear: It's Application in Personal Identification**

*Ruma Purkait, PhD\*, Gour Central University, Department of Anthropology, Sagar, Madhya Pradesh 470003, INDIA*

After attending this presentation, attendees will be aware of the fact that the external ear, which is emerging as one of the leading biometric traits, does not have a permanent form throughout life. The ear can be used as a biometric trait after it attains maturity of form and ceases to be useful when it loses its elasticity and starts elongating.

This presentation will impact the forensic science community by cautioning against indiscriminate use of the external ear for establishing identity throughout life. The study has for the first time furnished data for age of maturation for a central Indian population and also sets the ceiling age of 60 years (from previous study) for the external ear's use as biometric trait.

Establishing identity from the dead or living is a routine job for a forensic scientist and law enforcement agencies. When a person is alive, identification is established by comparing him with his image or identification symbol. In the present electronic age with increased networking in communication, mobility, and advances in technology the conventional methods of identification (i.e.: identification card, magnetic card, password, etc.) are replaced by a faster and more sophisticated method of identification, that is biometrics. Biometrics is the method of identification from physiological & behavioral traits of an individual. The traits can be facial features, fingerprint, hand dimensions, voice, signatures, etc. It is a machine-based vision system in which a database of the traits are stored. During authentication the image/data of the individual taken on the spot is automatically compared with the image/data already stored in the machine. In this context the external ear is emerging as a convenient and dependable biometric trait. One of the essential properties of a biometric trait is constancy of shape and size over time. If the ear undergoes rapid change in its form, there are chances of rejection of an authentic person at the time of verification, in other words the false rejection rate will be too high. Hence, it is of utmost importance for forensic scientists to identify the period during which the proportion of external ear remains unchanged.

An extensive study was undertaken with the goal of determining the age of maturation of the external ear. An earlier study has already determined the age after which the external ear starts elongating. The data was collected from hospitals, schools, day care centers, and residence of the subjects of central India. The subjects were normal and did not suffer from any craniofacial abnormality, malnutrition, or endocrine dysfunction. The sample size of 1,960 consisted of boys (1053) and girls (907) ranging in age from birth to 18 years. To determine the age of maturation of the ear, the age groups were categorized on a yearly basis up to 18 years. In the present study, dimensions have been determined by physically measuring the ear with a sliding caliper (Dial Caliper; Mitutoyo Corporation, Kawasaki, Japan, accuracy 0.01 mm). Consent was sought from the subjects or their parents before taking necessary measurements. The measured dimensions included maximum length, width of external ear, and length and width of lobule. All the measurements except lobular width were taken following the Knußmann method. Lobular width was defined by the author for the current study. The age of maturation was determined by the method suggested by Farkas & Posnick (1992).

The maturation of different dimensions of the external ear in females is found to range from 8 to 12 years, and 11 to 14 years in males. After attaining maturity the proportion of the external ear has been found to remain relatively unchanged until the age of 60 years, after which it starts

elongating rapidly. Most of the elongation was attributed to the lobule being made of elastic fiber which is more susceptible to the pull of gravity. Hence, it was inferred that the external ear can be used as a biometric tool for establishing identity from 14 to 60 years among males and from the age of 12 years among females.

The information of age of maturity of the external ear will be of great use to law enforcement agencies for using the ear as a biometric tool.

**Personal Identification, Biometrics, External Ear**

## **D54 Safeguarding Prisoner Patients and Forensic Staff Orientation at Hospitals**

*Anthony N. Potter, MS\*, Forsyth Medical Center, 3333 Silas Creek Parkway, Winston-Salem, NC 27103*

After attending this presentation, attendees will be familiar with the medical and legal issues surrounding the treatment of prisoner patients and the training of forensic staff (defined by The Joint Commission as law enforcement and corrections officers, and contract guards). Specific attention will be given to the prevention of escapes, and compliance with The Joint Commission standards and Centers for Medicare and Medicaid Services (CMS) Conditions of Participation.

This presentation will impact the forensic science community by focusing attention on the risks of treating prisoner patients in a general hospital environment, the need for adequate training of both forensic staff and hospital clinical and security personnel, and the consequences of failure to do so.

In recent years, the number of attempted and successful prisoner escapes from general hospitals has increased significantly. This is due to the closure of many correctional medical facilities due to budgetary reductions, and the increasing number of prisoners receiving external medical care. The presence of prisoners in a general hospital environment presents significant risks to the hospital's patients, visitors, physicians, and employees. These risks can be minimized, and the opportunity for escape limited, by the effective training of both forensic staff and hospital clinical and security personnel.

Hospitals, other than correctional medical facilities, are not configured or staffed to provide medical care for persons in custody. While the responsibility for ensuring their custody resides with the criminal justice agency having jurisdiction, a prisoner's presence and treatment present many standards-compliance issues that must be addressed. Both The Joint Commission and the Centers for Medicare and Medicaid Services (CMS) require that hospitals provide the same standards of care for the treatment of prisoners that are required for all other patients. The use of law enforcement or correctional restraints (handcuffs, shackles, etc.), patient privacy issues (including the presence of law enforcement or corrections officers during treatment), the configuration of patients' rooms used by prisoners, and compliance with hospital policies (visitation, telephone usage, etc.) are all potential conflicts that must be addressed. Failure to do so may result in sanctions when a prisoner's hospital stay is evaluated using tracer methodology by The Joint Commission, CMS, or state regulatory agency.

Because of the wide diversity of local, state, and federal agencies bringing prisoners to hospitals for treatment, each hospital must develop a detailed policy for the training of forensic staff (defined by The Joint Commission as external law enforcement and corrections officers, or contract security personnel) that includes subjects mandated by The Joint Commission. This policy should be reviewed and approved in advance by the heads of all local, state, and federal agencies involved. In addition, the hospital should prepare a handout for forensic staff detailing this policy, and obtain each officer's signature as documentary evidence that they have read and will comply with its provisions. Such evidence is frequently required during a Joint Commission survey, or CMS or state audit. One of the most effective ways to train law enforcement and corrections officers in a

hospital's prisoner patient policy is to have it included in the curriculum of the local training academy, or incorporated into local agencies' in-service training programs.

While responsibility for the prisoner rests with the agency having custody, hospital clinical staff and especially security officers must be thoroughly familiar with prisoner patient procedures so that they do not inadvertently compromise the prisoner's security. In addition to briefing law enforcement or corrections officers on hospital policies and procedures, security officers should monitor the prisoner and forensic staff while in the hospital, and immediately report any policy violations or other problems so they can be immediately addressed.

This presentation will include distribution of a template for a hospital policy on prisoner patients and the orientation of forensic staff, as well as a manual for distribution to law enforcement and corrections officers and a shorter handout for use when an officer brings a person in custody to the emergency department for treatment.

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**Prisoner Patient, Forensic Staff, Hospital**

## **D55 The Case for a Professional Doctorate in Forensic Science**

*Michael D. Cole, PhD\*, Anglia Ruskin University, East Road, Cambridge, CB6 2UD, UNITED KINGDOM*

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After attending this presentation, attendees will understand the process of obtaining a Professional Doctorate (Prof. Doc.) in Forensic Science. They will understand the similarities and differences between the Prof. Doc. route and traditional doctoral studies. The value of the Prof. Doc. to forensic science employers will be understood. The commitment required from candidates and employers will be detailed.

This presentation will impact the forensic science community by allowing candidates and forensic science employers to make informed decisions about the route to take for staff development at the doctoral level.

Over the last decade, forensic science has seen an unprecedented growth in the number of undergraduate programs in the United Kingdom. This growth has been mirrored, in part, by similar growth in Europe and other parts of the world. Destinations for graduates from such programs for further education and development in the subject area include traditional MSc, MPhil, and PhD programs. These can be studied as full-time degrees, part-time degrees, or as distance learning programs. Additionally, there is the opportunity to achieve PhD by publication although within the context of forensic science such doctoral candidates are rare.

Within the United Kingdom the validity of the traditional PhD as a degree training program for anything other than an academic career has come under scrutiny. There is now a movement towards doctoral programs which have a wider applicability, where doctoral candidates engage to a greater extent with their communities, the end users of the outcomes of their programs, and have developed a wider skills base including the transferable skills than has previously been the case.

One doctoral degree pathway which may be considered better suited to the needs of industry, commerce, and in our case the forensic science community is the professional doctorate. The award achieved is of exactly the same academic rigor and standard as a traditional PhD, but the award is achieved by the candidate via a different route.

The professional doctorate is undertaken by experienced colleagues who have a number of years in experience and who have, traditionally, honors level or masters level qualifications but little or no research experience. It has the advantage that it does not take them away from the organization in which they are working in the same way that a lab based doctoral program might. It has the advantage that it can be offered equally in management and administrative functions as well as laboratory based functions that are found in forensic science labs. The starting point is that an identifiable problem/challenge needs to be found in the workplace. This means that at the end of the study period – typically three to six years – a solution has been found to the problem that can be applied in the workplace

with beneficial impact demonstrable from the outset.

Professional doctorate candidates achieve their award in two stages. Stage one involves the production of three- seven thousand word documents in which the candidate develops research skills at the doctoral level. In the first of these papers, there is reflection on the practice in the workplace as it currently stands. In paper two, the candidate reflects on the material already published in the area around practice. In paper three the research design aimed at addressing the challenge identified in the workplace, is described. The three papers themselves form a substantive body of work of 21,000 words. In stage two of the program, the candidate undertakes the research itself which addresses the issues identified. The candidate produces a thesis and in time undertakes a viva voce examination in the same way that a traditional PhD is examined. Identical outcomes can be achieved.

This presentation makes the case for development of the Professional Doctorate in Forensic Science given the very great number of forensic science systems that are encountered and the large number of challenges that this can present. The similarities, differences, advantages and disadvantages between this and traditional programs are highlighted. At the end of the presentation attendees will be better placed to determine whether they would best undertake doctoral level study in forensic science via a traditional route or via a professional doctorate.

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**Doctoral Study, CPD, Professional Practice**

## **D56 Forensic Awareness for the First Responder: A Sample Training Curriculum**

*Jennifer R. Schindell, BSN\*, Linn/Benton County, 1115 Jackson Street Southeast, Albany, OR 97322*

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After attending this presentation, attendees will understand some of the key concepts used to successfully teach first responders the significance of their actions and observations during an injury or death investigation.

This presentation will impact the forensic science community by offering a sample curriculum for use in future trainings of emergency medical service providers and other first responders.

Fire personnel, emergency medical providers (EMS), and/or law enforcement personnel are routinely the first to arrive at the scene of an injury, illness, or death; and therefore, have the potential to dramatically alter the course of an investigation for better or worse. Information gathered during initial interactions can be of great value, not only to any subsequent investigation, but also to any subsequent medical care. The same information used to determine cause and manner of death can be used to determine the most appropriate medical response. Situations involving environmental hazards, evidence of drug use, and evidence of traumatic events are among the scenarios in which first responder observations can be pivotal. First responder actions and observations are particularly important in incidents involving death, injury, or Apparent Life-Threatening Events (ALTEs) in children.

First responders may be the only professional to speak with a victim before they become unconscious, intubated, or otherwise unable to communicate. They may also be the only professionals to view a scene as is, before it is altered by resuscitation attempts, suspects, or well-meaning helpers. First responders may overhear statements or may be given an account of an event that is not later repeated for investigators. Also, in their attempts to render aid, it is expected that first responders will alter scene and body to some extent.

The first step to improving forensic awareness is to provide training about the importance of initial scene findings, statements, and the potential ramifications of alteration to the scene. Patient care must never be compromised in an effort to protect evidence, but first responders can be trained to maintain forensic awareness while providing life-saving care. If the patient is declared deceased, the goal must change from "life-saving" to "information-saving." Like nurses and other medical providers, EMS and fire professionals commonly participate in forensic situations whether they are aware of it or not. Although their primary obligation is to the health and

safety of their patients, the patient and the community as a whole is better served when forensic awareness is maintained. The goal of this training is to elevate participants from a level of unconscious participation/obstruction to at least a level of basic awareness.

For this training to be effective, professional first responders must receive training that they interpret as relevant and realistic. Instructors who have knowledge regarding care and treatment of the sick and injured as well as experience in death investigation and basic crime scene procedures are best suited to provide this training. Case reviews are essential to this curriculum as they allow first responders to fully appreciate and personalize the rationale for minimizing their impact on a scene while documenting or reporting any observations or actions which may have altered the scene.

In summary, improving forensic awareness in first responders can enhance patient care as well as the efficiency and accuracy in investigation of injury or death. Cases in which paramedics and other first responders recognized, documented, and volunteered key information in a death investigation following attendance at a training based on these principals will be reviewed.

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**Evidence, EMS, Training**

## **D57 Advancing Forensics Through Training: Police Transition Teams**

*Ashraf Mozayani, PhD, PharmD, and Jason L. Schroeder, MS, MBA\*, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

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The goal of this presentation is to explore the advancement of forensic applications within Iraq and specifically the Iraqi Police.

This presentation will impact the forensic science community by highlighting the advancement of forensics in challenging and often austere environments.

Military operations increasingly occur outside traditional warfare, including counter insurgency (COIN) environments. These types of conflicts often include several types of transition teams. One type of transition team is the Police Transition Team (PTT). The mission of a PTT is to coach, train, and mentor host nation (HN) policemen in all aspects of basic and advanced law enforcement. Host nation military forces are often the primary contact agencies with a civilian populace in a COIN environment. However, police transition teams and host nation leadership seek to create and support a professional, educated police force in an effort to provide legitimacy to an effective police force. The objective of the transition team is to create an Iraqi Police Force capable of safeguarding and gaining the trust of the local populace, ultimately serving as the primary contact agency with Iraqi citizens.

A well-constructed and effective transition team is ideally an interagency and multinational operation. Police transition teams generally include military, military police units, International Police Advisors (IPA's), Law Enforcement Professional's (LEP's), and other individuals or agencies with skill sets suited to training common police skills. Additional individuals with specific skill sets may assist the PTT by conducting specialized training. This training may include detentions, interrogation, communications, traffic or riot control, or police administration. These skill sets also include knowledge of crime scene forensics involving the identification, collection, and preservation of items of evidentiary value. The challenge is establishing these skills and gaining acceptance within the Iraqi Police Department. These challenges include establishing and enhancing the capability of forensic applications as well as developing an understanding of how to effectively incorporate forensics into the judicial process, often not conducive to dramatic changes in existing processes.

Several independent but supporting divisions exist within the Iraqi National Police. One such agency is the Criminal Investigation Division (CID). This agency acts as the lead investigative agency and maintains interdependence between a code of law and a judicial system (further

supported by judicial transition teams). Accordingly, the CID served as the optimal division to introduce crime scene forensics into the Iraqi judicial process.

The application of forensics by Iraqi Police (IP's) is similar to traditional law enforcement operations with the added responsibility of a significant tempo of counterterrorism and intelligence gathering operations necessary in a COIN environment. This wide ranging relevance and broad application provide ample opportunities to introduce forensic training into PTT operations. These include both classroom and practical scenarios. Finally, the training given can be applied immediately in real world situations.

In conclusion, this presentation will provide a brief history of forensics in Iraq as well as the current efforts and state of forensics within the Iraqi National Police Agency. Additionally, the potential for future forensic applications as well as pitfalls and challenges will be presented. This information impacts the forensic community by highlighting the advancement of forensics in challenging and often austere environments.

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**Police Transition Team (PTT), Training, Iraq**

## **D58 Reinforcing the Value of Continuing Education in Forensic Science**

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The goal of this presentation is to provide the forensic science community with information regarding the value of continuing education in forensic science for practitioners.

This presentation will impact the forensic science community by providing information regarding the value of continuing education in the field of forensic science.

With a variety of disciplines, forensic scientists require a complex combination of skills, knowledge, and experience in order to carry out their role effectively. It is widely recognized that maintenance of skills and knowledge over time plays an important part in ensuring that standards of practice are current and that competence in the position is promoted. Continuing education for the forensic scientist is structured educational activities designed or intended to support their continuing development and to maintain and enhance their competence. This training can be either internal or external to the forensic science laboratory. Some commonly used approaches to continuing education are instructor led, professional conferences/seminars, distributed learning, apprenticeship, residency, internship, teaching, and presentations by trainee/employee, workshops, short courses, web-based instruction, and independent classes.

Forensic science is a continuously developing field, yet continuing education is not a requirement to the field as a whole. Certifying bodies within the forensic science community may require continuing education for certification and recertification. The quality of continuing education for the forensic scientist should follow specified minimum requirements and be consistent with recognized, peer-defined standards that are set by specific accrediting bodies or forensic disciplines (e.g., ASCLD/Laboratory Accreditation Board, Scientific Working Groups, IAI, and ABC).

Training and continuing education was identified as a significant area of need within the forensic science community in several studies including National Institute of Justice's (NIJ) *Forensic Sciences: Review of Status and Needs* (1999), *TWGED's Education and Training in Forensic Science: A Guide for Forensic Science Laboratories, Educational Institutions, and Students* (2004), and the 180-day Study Report: *Status and Needs of United States Crime Laboratories* (2004). The release of the 2009 National Academy of Sciences Report (NAS), *Strengthening Forensic Science in the United States: A Path Forward*, also addressed the issue of continuing education for all working forensic scientists. The NAS report states that continuing education is "critical for all personnel working in crime laboratories as well as those in other forensic science disciplines..." The

report also addresses that the quality of continuing education is an issue and regardless of the discipline, there are core elements that need to be followed. These elements include the following: standards of conduct (ethics training); safety (biological, chemical, physical hazards); policy (SOP's, quality assurance, accreditation); legal (expert testimony, rules of evidence, court procedures); evidence handling (recognition, collection, and preservation of evidence); and communication (written, verbal, and nonverbal communication skills). The discipline-specific elements should include history of discipline, relevant literature, methodologies, instrumentation, statistics, and testimony; plus an assessment of knowledge is needed to measure the performance of the practitioner.

Many opportunities for quality continuing education are available but the lack of financial support from the agency or crime laboratory can hinder the forensic scientist from taking advantage of this training. Funding for training is an issue with most agencies' diminishing budgets. The recommended budget for training is 1-3% of the total laboratory budget, which on average is \$1.3 million for publicly funded crime labs. Some labs have allotted \$1,000-\$1,500 per year for each person for training or continuing education. According to the Bureau of Justice Statistics (BJS), *Census of Publicly Funded Forensic Crime Laboratories* (2002), the budget for training is less than 1% of the overall laboratory budgets. Even when laboratories have the funding for training, they lack the personnel to cover the person who is away for training. A few agencies see training as a reward to the scientist and not a need to continue his or her professional development. Worse, some view training as an opportunity for the employee to travel and have fun, not to improve their skills. However, continuing education for practitioners strengthens the agency and the field as a whole.

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**Continuing Education, Training, NAS Report**

## **D59 A National View of Forensic Art: Current Forensic Art Units and the Services They Provide**

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After attending this presentation, attendees will understand the scope of forensic art and the extensive use of this discipline across the United States.

This presentation will impact the forensic science community by introducing the law enforcement departments and locations of the forensic art units and practitioners across the nation. It will also educate the forensic community about the different aspects of forensic art.

Forensic art is any art that is used in the court of law. This includes composite sketches, facial reconstruction, post-mortem images, age progressions, and image clarifications. Forensic art has evolved through the decades and now has updated tools and research to draw from. Not only are forensic artists using the traditional skills of drawing, many have also incorporated graphic software skills to enhance images for criminal investigations. Forensic art is a valuable investigative tool in multiple situations for detectives and forensic scientists alike.

The United States has approximately 28 full-time forensic art units identified around the country, with about 45 full-time artists. There are also at least 80 part-time forensic artists who work for law enforcement agencies or universities. The presentation will inform forensic scientists of the available resources in their area and around the country.

Forensic art is not, and does not claim to be, a positive identification technique. Forensic art is an information-generating tool, primarily used to create leads in a case by stimulating the memory of the public. It is used to

assist with the identification of unknown decedents as well as unknown criminal suspects. The National Institute of Justice (NIJ) funded program called NamUS is an online database where details about unidentified decedents and information of missing individuals can be matched up. This site estimates that there are approximately 40,000 unidentified human remains in the medical examiner's offices around the country. Also in a typical year, medical examiners and coroners handle approximately 4,400 unidentified human decedent cases, 1,000 of which remain unidentified after one year ([www.namus.gov](http://www.namus.gov)). In 2003, there was also a DNA Initiative launched by the Office of Justice Programs (OJP) and the NIJ that provides DNA analysis for all unidentified and missing persons across the nation for free, provided by the University of North Texas. Currently there are approximately 7,923 unidentified persons in NamUS and 7,432 missing persons in NamUS. Professional, quality forensic art is a key tool in this initiative, providing postmortem images and facial reconstructions that give the investigators examining the cases a clear image of the unidentified decedent that might foster a lead to the identification of a missing person. The presentation informs the viewers of the resources available around the country to utilize forensic art skills and to make them aware of the possible cases that forensic art may be used for.

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**Forensic Art, Unidentified, Identification**

## **D60 Detection and Identification of Volatile Organic Compounds in Dried Human Blood Samples by Instrumental Analysis and Canines**

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After attending this presentation, attendees will have a better understanding of the volatile organic components in blood collected using different extraction methods.

This presentation will impact the forensic science community by being among the first to identify such volatile organic compounds (VOCs) in dried blood samples and show that a properly trained detector canine is capable of locating extremely small quantities of human blood.

Detector canines are commonly used by law enforcement, military, and private organizations to locate a diverse collection of targets, including explosives and drugs, as well as bedbugs and agricultural products. Canines are often trained to locate living and deceased humans and human blood. It is generally accepted by the canine community that detector canines locate the targets of interest using one or a combination of volatile organic compounds (VOCs) unique to that target odor. Several studies have been performed on the VOC odor profiles of specific targets of interest to the law enforcement community, including explosives, drugs, and humans (living and deceased); however, such a study has never been conducted to determine the components of blood odor. Furthermore, in all instances involving the detection of trace human blood via canines, dried human blood is used for training, which makes it difficult to establish detection thresholds using actual blood samples, and to eliminate other non-human sources of blood from consideration while on an investigation. For this reason, the use of human blood VOC mimics to assist handlers or canine

specialists for training purposes to effectively locate dried blood associated with a crime is new and desirable.

In this research, VOCs from the dried human blood samples, similar to what may be found associated with a crime were analyzed. The VOCs were collected from the headspace of the blood samples using two different sampling methods: air sampling with traditional thermal desorption techniques and solid phase microextraction (SPME). All samples were analyzed by gas chromatography/mass spectrometry (GC/MS). The protocols used for the determination of VOCs for blood were developed for both sampling methods, using blood from a single human donor. Following method optimization, blood was collected from a small sample population in which the blood was dried under controlled conditions, and then the headspace was sampled and analyzed. The VOC content of each sample was compared and similar compounds were considered to be possible key compounds for canine detection. The VOCs produced by human blood during aging of the dried blood under oxygen-deprived and oxygen-rich environments were also compared. Further research included canine trials using dried blood from the sample population, as well as mixtures of such key compounds.

Based on the VOCs identified using both extraction methods it was observed that the two techniques do not necessarily yield similar results, yet instead can be considered complimentary extraction methods. Using both sampling methods a number of VOCs were identified and were shown to be consistent across the sample population. These compounds will likely be crucial to identifying the unique combination of VOCs utilized by blood-specific canines. Additionally, the trained blood canine was able to successfully locate small amounts of blood from different subjects. This study will be among the first to identify such VOCs in dried blood samples, and will be the first to scientifically show that a properly trained detector canine is capable of locating extremely small quantities of human blood as well as the VOCs responsible for the odor profile.

#### **Canine Detection, Blood, Volatile Organic Compounds**

## **D61 The Hiring Preferences of Crime Scene Evidence Technician Applicants: A Survey**

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After attending this presentation, attendees will understand the importance of determining whether crime scene investigation degree programs meet the needs of law enforcement agencies by evaluating their views of forensic science degree programs, and relevant research on the subject. Data from a survey of law enforcement agencies will also be presented.

The presentation will impact the forensic science community by serving as a reference for future research in determining the value of crime scene investigation degree programs for law enforcement agencies and crime scene evidence technician applicants. This research study will also benefit crime scene evidence technician applicants who decide to pursue a degree, and apply for positions within law enforcement agencies.

There appears to be a lack of research about law enforcement agencies' views of crime scene evidence technician applicants with degrees in crime scene investigation. Is education an asset for a future career as a crime scene technician in a law enforcement agency? Previous research has focused on the current issues and status of forensic science education and degrees, the qualifications of educators teaching forensic science courses, and the development of college-level degree programs that prepare students for careers as crime scene evidence technicians. Recent research has also focused on how law enforcement agencies view forensic science and the forensic science knowledge they expect their new recruits to possess. While previous studies can provide an understanding of crime scene investigation

degree programs offered in colleges and universities, research has not addressed law enforcement agencies' views of these degree programs.

To measure law enforcement agencies' views of crime scene evidence technician applicants, a survey was designed and administered to 102 various law enforcement agencies in the state of Maryland. The survey was adapted from a similar survey utilized by Lambert in a study titled, *The Forensic Science Needs of Law Enforcement Applicants and Recruits: A Survey of Michigan Law Enforcement Agencies*.<sup>1</sup> The new survey consisted of 17 questions, divided into three parts (general background, work experience and education). The questions were a combination of fill-in-the-blank, short answer, rankings, and multiple choice. After collecting all returned surveys from the respondents, answers for each question and sections were converted to numerical data for entry into a spreadsheet. Afterwards, percentages and means were calculated based on the response rates. The following statistical tests were used:

- A two-proportion z-test was used to determine if law enforcement agencies prefer crime scene investigator applicants with work experience over applicants with education in crime scene investigation.
- To determine whether crime scene investigator applicants' education is weighted less than work experience, a two sample t-test that does not assume equal variances was used.
- A two-sample t-test was used to determine whether forensic science education areas are viewed the same as the qualifications for a crime scene investigator position.

A total of 40 surveys were returned, resulting in a 39.3% response rate. Characteristics of the responding law enforcement agencies are as follows: 17 (44.7%) rural, 15 (39.4%) suburban, and 6 (15.7%) urban. Twenty-four (60%) agencies are either local or municipal, and 13 (32.5 %) are county. The mean number of sworn officers was 151.9 (SD 394.2), and the mean number of civilian employees per agency was 37.9 (SD 84.7). Additionally, 75.7% reported that they did not have an officer or a unit specifically dedicated to crime scene evidence collection; 26.3% indicated that they had their own crime/forensic lab; 24.3% hire civilians to assist in the collection of forensic evidence. When comparing work experience to education, 84.6% of the respondents indicated that they prefer work experience, and 80.7% of the respondents indicated that they prefer education. There was not enough statistical evidence to suggest that law enforcement agencies prefer crime scene evidence technician applicants with work experience over those with a degree in forensic science or crime scene investigation ( $p > .05$ ), and there was not sufficient evidence to suggest that the agencies give less weight to education than work experience ( $p > .05$ ).

Although, the number of crime scene investigation degree programs has grown tremendously in the past ten years, there is no research suggesting that law enforcement agencies prefer crime scene evidence technician applicants with these degrees. On the surface, it appears that forensic science degrees fulfill the requirements of crime scene evidence technician positions within law enforcement agencies.

#### **Reference:**

- <sup>1</sup> Lambert, E., et al. Differences in forensic science views and needs of law enforcement: A survey of Michigan law enforcement agencies. *Police Practice and Research: An International Journal* 2007; 8(5): 415-430.

#### **Crime Scene Evidence Technician Applicant, Hiring Preference, Crime Scene Investigation Degree Programs**

## **D62 Differentiation of Human Subjects Based on Scent Profile**

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After attending this presentation, attendees will gain knowledge of the research being performed to provide scientific support to the use of canines in scent discrimination, suspect trailing, and missing person searches. The areas specifically addressed by this research include method development, headspace analysis of human scent samples, and differentiation between individuals based on those scent profiles.

This presentation will impact the forensic science community by educating the public about the current status and challenges of forensic canine use in investigations. This research will provide a scientific foundation for canine validity and unbiased analysis of scent profiles that will bolster the acceptance and legitimacy of canines in both the field and in court.

Canines have been used for decades to identify individuals based upon their unique scent profile. Most commonly this involves either trailing a suspect in a crime or searching for a missing person. Although the exact mechanism by which a canine is able to identify an individual from among a group is not clearly understood, it is clear that canines are able to perform that task. However, such feats are still heavily scrutinized because there is an apparent lack of scientific evidence that supports them. In recent years, there have been several studies published aimed at identifying the essence of human scent in order to provide some of the necessary scientific evidence to support canine responses. These studies have used relatively small subject pools, strict washing procedures, and inherently selective sampling methods such as solid phase micro-extraction (SPME).

In criminal cases where canines may be used, suspects and victims will not undergo procedures to “standardize” their scent profile for the dogs, so the dogs must use these complex scent profiles to identify their target. To mimic these real-life situations, human subjects for this study were not required to perform any specific washing or cleansing procedures, but instead were asked to maintain their normal routines. The lotions, soaps, and other products that each person uses contribute to his or her volatile profile and can be used to help analytically differentiate between individuals.

A large subject group, at least several hundred, will be asked to sample their own forearms by using gauze pads in a procedure similar to those used by law enforcement canine handlers. In this study, a headspace sampling protocol will be used in an effort to eliminate the selectivity of previous methods and provide a more complete picture of human volatile emissions. These gauze pads will be sampled with a cryogenic pre-concentrator and analyzed via gas chromatography with mass spectrometry detection (GC/MS). The resulting scent profiles will be analyzed and compared to one another to determine if they can be used to differentiate between individuals or groups of individuals. Ethnicity, gender, and age will be considered in comparisons, and significant differences between groups will be explored. Ultimately, if analytical differentiation is possible, this research will provide a means for canine handlers to sample scented items given to dogs as well as sample individuals or items located or identified as the result of a canine search. These samples could be sent to a laboratory for analysis and comparison and the data could be used to support canine responses. If “markers” for groups of individuals can be identified, this

information could also be used to provide investigative leads by narrowing down a field of suspects or helping to tie an item to a specific victim.

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**Human Scent, Individualization, Canine**

## **D63 The Associated Evidence Analysis and its Impact in Human Rights Violations Cases in Chile**

*Dina A. Jimenez, DDS, and Isabel A. Martínez, BA\*, Servicio Médico Legal, Av. La Paz 1012, Independencia, Santiago, CHILE*

After attending this presentation, attendees will understand the value of the associated evidence analysis as support to establish the cause and circumstances of death, which is of inestimable emotional significance for the family in human rights violations cases.

This presentation will impact the forensic science community by showing how the analysis applied produced relevant results after 38 years of the events. On the other hand, the recovery and reconstruction of the clothing and personal belongings of the victims is of inestimable emotional significance for the family.

The analysis of associated evidence is a process that was standardized after a 2007 finding in Litueche, Chile. More than 500 associated pieces of evidence were recovered, amongst which were small fragments of textile of different types, footwear, and personal items, among others. The judge of the investigation requested expert analyses that could provide the greatest possible detail about each piece of evidence in order to support the process of victim identification and the determination of the cause and circumstances of death.

The process consists of keeping the chain-of-custody, and documenting all of the expert analysis process through written, photographic, and sometimes video record. A qualitative (descriptive) and quantitative (measurement) analysis is carried out after recovering trace evidence (earth residues, study of stains, firing residues) and manual cleaning, taking care that the evidence is not damaged.

At the same time, the condition of the clothing and the recovered fragments is established; the color is determined through the use of a textile pantone; the type of fiber (natural or synthetic) and the type of fabric is also determined. The process continues with the reconstruction of textile fragments until rebuilding the clothing as much as possible has been completed, then continues with the determination of trauma, not related to taphonomic damage, such as the passage of a projectile.

Along with this, a judicial procedure for the recognition of associated evidence is usually performed with the participation of families linked to the case and the appearance of the judge.

Finally, the conclusions of the expert process are compared with ante-mortem information provided by the family and the judicial investigation.

This type of analysis can mean an important contribution to the inquest, given the peculiarity of some objects and articles of clothing and findings related to trauma in them, are useful information in determining the cause of death and the circumstances surrounding it.

In this sense, it is important to note that the consideration of the contribution of the associated evidence analysis depends on all of the elements of the investigation, its correlation with each other, the interpretation of the findings of each discipline and the integration of all information gathered.

On the other hand, the recovery and reconstruction of the clothing and personal belongings of the victims is of inestimable emotional significance for the family. Many times, the family can even restore a link with their beloved ones, when after a long time they see the woollens or garment that they may have made for, lent to, or helped choose for the victim while they were still alive.

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**Associated Evidence Analysis, Human Rights Violations, Recognition Process**

## **D64 The Role of the State and the Clarification of Human Rights Violations in Chile**

*Patricio Bustos, MD, Servicio Medico Legal, Av. La Paz 1012 Independencia, Santiago de Chile, CHILE; and Dina A. Jimenez, DDS\*, Medical Legal Service, Recoleta, Av. la paz 1012, Santiago de Chile, CHILE*

After attending this presentation, attendees will understand the value of the forensic sciences in the investigation and prosecution of human rights violations.

This presentation will impact the forensic science community by showing how forensic science, at the service of justice within the framework of a State policy to investigate gross violations of human rights, opens a real possibility for justice, truth, and reparation. Above all, it can be seen that the constraints imposed by the circumstances of the atrocities are overcome by an adequate scientific work of humanitarian outreach. It is the obligation of states to investigate crimes against humanity, which are by nature imperishable.

The judicial investigation, detection, recovery and analysis of thousands of tiny and fragmented skeletal remains hidden in the desert, military compounds, illegal mass graves, cemetery for unidentified bodies, corresponding to victims of gross human rights violations that occurred during the dictatorship have been analyzed from the forensic point of view, to determine its identification, cause, manner and circumstances of death.

After 35 years, only now is it possible to respond to injunctions and families by using all currently available methods of the various forensic science disciplines, to analyze small fragments of bone remains that resulted a strategy implemented by General Augusto Pinochet, to make the bodies of victims disappear.

The political will of the Chilean state and the provision of the necessary resources gave origin to set up the Human Rights Program in the Servicio Médico Legal, devoted to perform expert analyses of the recovered remains, with the participation of the international scientific community, the creation of a family members genetic bank, the implementation of all forensic disciplines, continuous education, and ongoing communication with family members. The team has worked closely with investigators and judges.

Physical evidence recovered is extremely scarce and fragmented, with taphonomic changes left by the passage of more than 17 years before recovering. This has imposed the need to use everything that is within reach of science and to interpret and integrate all the information in understandable language single report with the judge and other parts of the judicial process.

At present, Chile has incorporated the international protocols to the analysis processes such as Istanbul, Minnesota, Missing ICRC, and genetic analysis in accredited laboratories using the three markers. The techniques of forensic archeology, anthropology, medicine, dentistry, ballistics, associated evidence analysis, chemistry and other areas of forensic interest are also used in the resolution of cases. From the humanitarian point of view, close ties with the families involved in this painful process have been developed.

In addition and pursuant to the complexity of the cases, an international committee of experts including leading forensic medicine, anthropology, and genetics, scientists made recommendations for each case to ensure transparency and credibility to the actions undertaken by the program.

**Human Rights, Forensic Science, Commingled Remains**

## **D65 Setting up and Running a New Forensic Student Organization Within a University Setting**

*Erin L. Berdanier, BA\*, 3909 Creekway Trail, Dayton, OH 45440*

After attending this presentation, attendees will learn about the challenges and strengths of organizing a local university group for current and potential forensic students. Attendees will understand the importance of creating these groups in locations that have student interest but do not have a forensic science program, and how the program helps the students personally.

This presentation will impact the forensic science community by enhancing the quality of students lives in order that they are better prepared for the work force when they have completed their bachelors degree.

To encourage students who are interested in the field but for whatever the reason could not attend formal programs, this presentation will include details on how to: set up a student organization, get students motivated to do independent learning, deal with legal problems such as students with felony charges, and get students to support and learn from one another.

This presentation focuses on organizing a university based student-group's members. This will include the officer positions and their interactions, and how every member earns titles (levels) within the group as a symbol of their academic achievement. Attendees will also hear about getting students motivated to present at meetings and to do more than just the minimum requirements for their university. This will help when attending conferences like the American Academy of Forensic Science (AAFS) and the International Association of Forensic Science (IAFS). Ultimately this will produce members who are willing to do individual projects and present the findings to further our knowledge in forensic sciences.

At the conference, attendees will see a sample of portfolios made by students within the university organization. These binders will allow the attendees of the conference to see how the program is helping the students on a personal level, and take suggestions back to their own student groups or staff. Some of the feedback from the students to the university group will also be available at the conference; therefore, listeners may learn some "do's and don'ts" within forensic student organizations.

Attendees will discover how to host small conferences that are specifically targeted to students. By learning this skill, attendees can train students, interns, or new employees faster and cheaper than traditional methods. Skills and motivational techniques to get students to help run the conferences will also be taught to attendees making planning and follow-through significantly easier.

Having a member who ultimately cannot work in the field because of a felony conviction is problematic. Attendees will learn how to remove member from a student group in a considerate and ethical manner. Other legal implications and concerns will also be addressed in the presentation.

The ultimate goal of this project is to support students at the university level who do not have a forensic science program yet by fostering learning, sharing, and the development of new techniques in the forensic sciences. By teaching attendees at the American Academy of Forensic Sciences meeting how to set up and run a student group, the worth of the students within the group will may rival the quality of the students in the established forensic science programs. Since our future as investigators will be eventually resting in the hands of these students, attendees will learn how to set up or improve their programs so that the overall quality of investigators will advance.

**University, Education, Organizations**

## **D66 Distinguishing Peri-mortem and Postmortem Fracture Patterns in a Mass Grave Scenario**

*Kenda K. Honeycutt, BA\*, North Carolina State University, Raleigh, NC 27695; and Ann H. Ross, PhD, North Carolina State University, Sociology & Anthropology, Campus Box 8107, Raleigh, NC 27695-8107*

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The goal of this presentation is to perform experimental research to simulate a mass grave with the intention of identifying the effects of taphonomy on fracture characteristics with relation to time-since-death.

This presentation will impact the forensic science community by showing how the research in this experimental study is novel in its goal to establish fracture characteristics and taphonomic changes within the context of a mass grave. This is significant in that the findings can be used to help establish time-since-death and cause-of-death for investigations involving human rights abuses.

Forensic anthropologists are often asked to assist in the investigation of international human rights violations. A review of the literature indicates that little research has been conducted on the occurrence of features as it pertains to distinguishing the peri-mortem interval from the postmortem interval. The purpose of this study is to perform experimental research to simulate a mass grave with the intention of identifying the effects of taphonomy on fracture characteristics with relation to time-since-death.

The research in this experimental study is novel in its goal to establish fracture characteristics and taphonomic changes within the context of a mass grave. This is significant in that the findings can be used to help establish time-since-death and cause-of-death for investigations involving human rights abuses. Despite the fact that such a study will only record a fraction of lethal injuries, it provides a better understanding of the manner in which taphonomic variables (in a mass grave setting) have the potential to mask evidence of a crime.

To replicate the conditions of a mass grave, a sample of ten intact pig carcasses were used. The sample of ten were euthanized in the same manner (by captive bolt pistol) and were transported to the NCSU field facility to observe taphonomic processes and the effect they have on fracture patterns. In addition to the original injury from the captive bolt pistol, each of the ten pigs received both blunt force and sharp force trauma. All ten pigs were then haphazardly interred together in an open grave to allow for observation and accelerated decomposition. In addition, to evaluate bone degradation, all ten pigs underwent a full body bone density scan prior to placement within the mass grave; they will be scanned again following skeletonization.

Documentation of trauma was conducted through gross morphological assessment, photography, diagrams, and charts; allowing for a detailed comparison to their appearance after exposure. However, this research remains within the initial data collection stage. Prior to the meeting, several macroscopic features will be observed: fracture outline, fracture edge, fracture angle, surface weathering, and color of the fracture surface—fractures will be determined as peri-mortem or postmortem based on these indicators. Observations were also made regarding stage of decomposition, insect activity, scavenging, and daily temperatures/precipitation.

In terms of interpretation, both statistical and morphological analyses will be implemented in order to find correlations between fracture characteristics. Categorical data analysis, such as logistic regression, will also be utilized to determine which specific taphonomic variables have more influence in disguising the peri-mortem interval for each type of trauma (gunshot wound, blunt, sharp). Additionally, variation between peri-mortem and postmortem changes will be examined using chi-square and odds ratio.

The null hypothesis tested is that taphonomic processes do not affect the appearance of peri- and postmortem fractures and it is expected that due to moisture retention from bodily fluids, fractures will appear peri-mortem into the postmortem interval. In addition, the pattern of skeletonization will be evaluated using three-dimensional spatial analysis (GIS). It is expected that the pigs on the periphery of the grave will skeletonize more quickly and the bone damage (fractures) will appear to be more characteristically peri-mortem than the pigs located in the center and bottom of the grave. Thus, this research should display how the conditions of a mass grave will

simulate bone damage that will appear peri-mortem for an extended period of time and help establish guidelines for forensic scientists working globally in post armed conflict arenas.

**Mass Grave, Fractures, Taphonomy**

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## **D67 Categorical Analysis of Pennsylvania SPCA Humane Law Enforcement Animal Cruelty Cases: 2008-2011**

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After attending this presentation, attendees will have a better understanding of the trends seen in cases investigated by Pennsylvania humane law enforcement officers over the course of several years. This will highlight animal crime as a subset of the overall crime statistics within Philadelphia and across Pennsylvania. It will show what types of animal crimes have demonstrated increases and decreases, and will therefore allow for future study into the connections of these crimes with additional ones, or reasons or explanations for the trends as they relate to accompanying crimes. Attendees will also be provided insights into the job responsibilities of a humane law enforcement officer within the state of Pennsylvania, some of their investigatory techniques including surveillance, and court appearances, and will also hear an overview of the state's animal-related laws.

This presentation will impact the forensic science community by serving as an example of anti-cruelty investigatory trends and by establishing a starting point for further study into the interdisciplinary aspects of animal cruelty and other crimes.

This study is an attempt to demonstrate trends in suspected animal cruelty cases investigated by the Pennsylvania SPCA's (PSPCA) humane law enforcement division both state-wide and in the city of Philadelphia from the years 2008 to 2011. The types of cases were evaluated categorically and include "unsanitary confinement," "lack of food/water/shelter," "lack of veterinary care," animal fighting," "abandonment," "commercial breeders," "stables," and "pet stores." The categories were then compiled graphically to provide visual illustrations regarding the numbers of cases investigated per year. In this way it was easy to see the prevalence of each category of cases within each year. However, it should be noted that the Pennsylvania areas (counties) served by PSPCA humane officers has changed drastically throughout the course of the study period due to budget constraints, and that the numbers may reflect these changes. PSPCA humane officers are authorized to carry weapons, obtain search or arrest warrants, issue citations, and make arrests for violations of Pennsylvania's anti-cruelty laws. They often work in conjunction with traditional law enforcement officers. There are ten PSPCA humane law enforcement officers working within Philadelphia (some are authorized to work in other counties as well), and two officers for the remainder of the state.

Investigations into cases of animal cruelty are more common nationwide now than in years past, due to increased public awareness and the resulting pressure from communities armed with more knowledge and a changing attitude toward animals. A growing enthusiasm for animal rights, as well as more research and literature related to animal welfare, and even popular reality television shows have helped shed light on what was once an underreported and often unacknowledged occurrence but is now a recognized crime. As awareness of animal abuse and aversion for it has grown, laws have evolved and expanded, resulting in harsher punishments for offenders. This in turn has prompted a greater need for forensic investigatory practices which may aid in successful prosecution.

Animal abuse has long been seen as a connection to other crimes and it is often a precursor to more severe offender behavior. Veterinary and wildlife forensics are new branches of forensic science that incorporate the

applicability of science to animal laws. While the use of forensics in animal-related investigations is still in its infancy, it will continue to increase as the number of reported cases of animal cruelty and the resulting investigations grow. Further research is merited with regards to categories of cases not reported in this study but encountered with increasing frequency by PSPCA's humane officers, including hoarding and ear cropping.

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**Animal, Cruelty, Investigation**

## **D68 Database of Family Members of Human Rights Violations Victims in Chile**

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After attending this presentation attendees will have a raised awareness regarding the need to build a solid normative basis and validated scientific protocols a bank for a Sample of Families, to enable identification if this information is required with relatives who are no longer alive.

This presentation will impact the forensic science community by increasing the understanding of how this bank represents a heritage and a legacy for future Chile generations that tell about what happened and the hope of the families to know the fate of their loved ones and their whereabouts. It also allows having the necessary samples in the face of new findings, to advance in the identification process irrespective of the time of the find.

Knowing the history, context, circumstances, and time when the disappearance and execution of the victims occurred allows modeling a forensic approach strategy and clarifies the needs of the process to succeed in the identification of victims.

In the initial days following the Coup d'État, some of the deaths and arrests followed by disappearance were selective, affecting leaders of political movements, trade unions and neighborhoods, indigenous people, students, and supporters of Salvador Allende. Most of the deaths in the specified time period were people without political connections, paupers, young men who lived on the streets, persons accused of committing misdemeanors or for violation of curfew.

During the 17 years of dictatorship, most of the remains were not found. With the advent of democracy in Chile, the search and recovery of remains began with findings of mostly commingled, fragmented, without medical and dental characteristics, individualizing features, except in few cases.

The first antemortem data bank of the victims was set up by a non-governmental body in 1976, the Vicariate of Solidarity, where families are interviewed by social workers and volunteer lawyers. In 2006, facing the difficulties of making a comparative process of identification considering medical, dental characteristics, clothing, etc. and due to the state of the remains recovered, the international scientific community recommended the establishment of a bank of blood samples from family members with the limitations of time such as the absence of parents of the victims who have already died.

Despite the time elapsed and the distrust on the State, family members, with a vote of confidence and hoping for news of their beloved ones, donated blood samples. The process is carried out according to the international standards (national and international regulations; ethical, technical, and humanitarian aspects). In the sampling process, thousands of families have been received, going to their places of residence in Chile and also abroad through consulates, have launched a communication campaign with informative videos, and to date, all this has resulted in 3,200 samples with genetic profiles.

Each family will be giving at least three samples depending on the availability of relatives, with four FTA cards; all information is encrypted

and protected according to the rules of sensitive data, and all have been obtained by genetic profile at least at two markers.

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**Antemortem Data Bank, Genetic Profiles, Family Members**

## **D69 Humanitarianism and Human Rights in the Context of Post-Conflict Forensic Investigations**

*Adam R. Rosenblatt, PhD\*, Champlain College, 163 South Willard Street, Burlington, VT 05401*

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After attending this presentation, attendees will understand the distinctions that have been drawn between international forensic investigations serving the cause of human rights and those motivated by humanitarian concern for families of the missing. They will acquire background in the historical circumstances, particularly in Latin America and the former Yugoslavia, that lead to a perceived competition between these two purposes, as well as the roots of the human rights/humanitarian split in a broader, non-forensic context.

This presentation will impact the forensic science community by providing a critical look at how terms borrowed from international law and politics can ill-serve the specific context of scientific investigations of violence, and by fostering dialogue amongst practitioners about the basic terminology they employ in the field.

The current use of a humanitarian/human rights distinction for international forensic investigations tends to confuse practical questions of institutional and political constraint for questions of political philosophy. In Argentina, where the first wholly independent forensic team was created in the mid-1980s to deal with persons missing as a result of repression, impunity laws made it next to impossible to put the perpetrators of dictatorship-era human rights abuses on trial until decades later. The Argentine Forensic Anthropology Team thus focused its efforts on identifying individual victims and accompanying families in their grief—a set of priorities they shared with other organizations conducting forensic investigations throughout Latin America, where impunity laws have been widespread. On the other hand in the former Yugoslavia, initial forensic investigations into genocide in the 1990s were sponsored by the International Criminal Tribunal for the former Yugoslavia, which gave priority to prosecutors' need for evidence of war crimes. The situation was nearly the reverse of the one in Latin America, with many families of the missing and some forensic experts viewing the investigations as too focused on legal outcomes and not enough on the needs of families and other mourners.

A number of experts from the forensic field have proposed that this perceived tension between evidence-collection and identification be described as a difference between human rights and humanitarian priorities. In this view, collecting evidence for trials constitutes "human rights work," while the identification and repatriation of individual bodies is a matter of "humanitarian" concern for the families of the missing. A human rights/humanitarianism distinction that is itself hotly contested in other areas of international activism and intellectual discourse is thus altered and imported into the context of international forensic investigations.

Drawing on years of interdisciplinary research into international forensic investigations, this paper argues that the distinction exacerbates, rather than ameliorates, the perceived power disparity between international institutions and families of the missing. By using a purely legalistic definition of human rights that associates human rights investigations with war crimes trials, it ignores the many human rights claims that families of the missing have traditionally made in other venues—including some rights, such as the right to know the fate of the missing and the right to mourn, that are directly related to identification and repatriation efforts. At the same time, by associating the needs of families of the missing solely with the humanitarian tradition of political neutrality (as embodied by the International Committee of the Red Cross), it also minimizes the stake that

families often feel in trials—which has even motivated some family members, such as members of Argentina’s Mothers of the Plaza de Mayo, to forgo efforts at exhumation or identification until they are assured that all perpetrators will be held accountable. The association of human rights with court cases and humanitarian concern with identification thus proves to be too simplistic for the complex reality of post-conflict forensics.

The paper concludes by suggesting that evidence-collection and identification are both bound up with human rights as well as a number of other political projects and moral values, even when they are applied unevenly or at different times. Planning for their implementation in any given post-conflict context can only occur by drawing on experience in previous locations, understanding the specific limitations of the new context, and working directly with families of the missing to understand their priorities.

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#### **Human Rights, Post-Conflict, Missing Persons Identification**

### **D70 The Contribution of the Clothing Analysis in Determining Cause and Manner of Death in Two Cases of Political Executions in Chile**

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After attending this presentation, attendees will understand the value of the clothing analysis as support to establish the cause and manner of death in human rights violations cases.

This presentation will impact the forensic science community by showing the analysis applied produced relevant results 38 years after the event.

In September 1973 three people were executed in a northern city of Chile by state agents under conditions of political repression that prevailed in the country. The two males, who are the focus of this presentation, were university students. Once executed, the bodies were taken to a cemetery where there was allegedly performed an external examination, recognized by relatives and friends, and buried in the same cemetery in a perpetual niche.

On August 25, 2010, upon a court order, these three victims were exhumed and subsequently transferred to the Unidad Especial de Identificación Forense of the Servicio Médico Legal in Santiago, to determine the cause and manner of death. In the historical context of that time, these executions were justified as a result of the detainees escaping (Escape Act); although this was not consistent with the circumstances.

The expert analysis performed included the analysis of clothing, which purpose was to help in the determination of the cause and manner of death. The three bodies were dressed, and had to be undressed in the laboratory of the Unit. This presentation discusses only the analysis of the two male victims, as the third one, female, showed no peri-mortem alteration of forensic interest in her clothing.

In the analysis of the clothes, the macro morphological characteristics were taken into account to determine the type of garments and its historical context, as well as the individualization and characterization of the alterations that are directly related to the time of death.

The clothes had tears consistent with bullet holes, which matched-up with bone trauma of the same origin. The support of a chemist and a ballistic expert was requested to determine the trajectory and distance of the shot by applying the inductively coupled plasma-mass spectroscopy method to samples taken from the aforementioned tears, in search of metal particles present as gunshot residue, mainly Barium (Ba), Antimony (Sb), and Lead (Pb).

Despite 38 years having passed since the time-of-death, with the help of the clothing and chemical analyses, the judge had more scientific evidence to aid in the clarification of the facts, since the chemical results obtained show that in some tears the level of metal elements detected (Ba,

Sb, and Pb), were consistent with short range shots with contact, typical of an execution.

This presentation shows the forensic impact that the clothing analysis can have as a supporting tool, in the determination of cause and manner of death in a human rights violation context, despite the time elapsed since the event.

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#### **Clothing Analysis, Cause of Death, Human Right Violations**

### **D71 Is it Possible to Identify 15 Victims in a Mass Grave With More Than 5,000 Human Skeletal Remains? Lonquén Case, Chile**

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After attending this presentation, attendees will understand the importance of a comprehensive approach in human rights violation cases. The analyses carried out by the various disciplines as well as continuous work with the families of the victims is highlighted, as a part of contributing in a better way to the judicial objectives, it provides emotional reparation to persons affected by political violence.

This presentation will impact the forensic science community showing how interdisciplinary work could help to resolve complex forensic cases associated with grave human right violations.

In the role of the expert in relation to the elucidation of the truth in cases of human rights violations, lies the need of an entire community to build trust and social ties.

This work presents the methodology used to identify 15 victims of the military dictatorship (1973-1990) illegally buried in a mass grave containing more than 5,000 commingled skeletal remains.

In 1978, as a consequence of an anonymous report to the Archbishopric of Santiago, semi-skeletonized human remains of 15 individuals were found in an old lime kiln in the area of Lonquén. This was the first finding of bodies reported through the mass media, as an explicit evidence of something that was already known in the depths of the society but which had steadily been denied by the authorities of the time.

At that time, the court ordered the recovery of remains and their transfer to the Instituto Medico Legal of Santiago, work that was carried out by unskilled staff. This situation resulted in the dismemberment of the bodies and the subsequent loss of individualization of the same.

The technical and political conditions neither allowed the victims to be identified (only one of them was identified) nor elucidating the cause of death; however, the investigation determined that the bodies belonged to 15 individuals arrested in the town of Isla de Maipo, near Santiago, in October 1973, who were mostly farmers that had participated in the Agrarian Reform process promoted by the two previous governments. Finally, following the public uproar, the Military Justice acknowledged the illegal inhumation of the remains in a mass grave of the cemetery of Isla de Maipo.

In March 2006, thanks to the perseverance of the families and according to the request of the Court, all of the content of the mass grave was exhumed, exceeding 5,000 elements corresponding to at least 30 adult individuals. Documentary, anthropological, dental, clothing, and genetic analyses were performed for almost four years, establishing the effective presence of the 15 individuals recovered in 1978 from the Lonquén kilns. Also established was their cause of death as well as the identification of 13 of the victims. On March 28, 2010, a funeral was celebrated for the victims with a massive and emotive act in the cemetery of Isla de Maipo.

At present, genetic analysis that would determine the identification of the two victims who are still missing is still pending.

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#### **Commingled Human Remains, Human Right Violations, Multidisciplinary Forensic Analysis**

## **D72 America's National Tragedy: The Missing and Unidentified**

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After attending this presentation, attendees will have a greater understanding of the importance of gathering comprehensive dental record data efficiently and compassionately while conducting missing person investigations. Attendees will learn of the significant variance in state-to-state dental record retention guidelines and/or mandates, thus allowing for enhancement of future investigations.

This presentation will impact the forensic science community by increasing the knowledge of the most critical elements of the antemortem dental record required, as well as useful supplemental records.

As of January 2011, the FBI reported 84,352 active Missing Person records (MPR) and 7,539 active Unidentified Person (UIP) records in the National Crime Information Center (NCIC). The problem: only 8,060 (10.47%) of MPR have dental data associated with them. It is reasonable to conclude that hundreds or thousands of registered missing persons are no longer missing but rather recovered and unidentified due to incomplete antemortem data collection.

The specific evidence a dental record provides to the antemortem and postmortem comparison review is critical to positive identification. An ideal dental detail entry into the NCIC facilitates the highest accuracy in possible correlations between unidentified and missing persons. All correlations, a "cross match" strong and weak, are generated as \$.M (dollar "M") analysis reports delivered daily from the Criminal Justice Information Services (CJIS) to the investigating agency. Case detectives and consulting experts review the reports for plausibility in meriting further investigation. Thus, the individual translating the dental record from written form into NCIC code for upload is as important to the case management as is the record itself.

This delegation should be given to a specifically trained NCIC dental coding expert; this is normally not the dentist who has supplied the dental record to investigators. A comprehensive antemortem dental record provided to an Odontologist will give the necessary data for postmortem comparison and expedites this reliable, scientifically sound, and cost-effective method of positive human identification. Often this data can be easily obtained by asking the correct questions of immediate family and the primary care dental provider for additional record collection.

Furthermore, during the course of new and cold case missing and unidentified person investigations, it is also important to note that dental record retention laws and/or guidelines vary significantly from state to state, and this can significantly impede the recovery of antemortem dental records even when a valid source for the record is located. This further delays identification, as well as forces more time and revenue consuming methods to be employed if dental identification cannot be utilized.

It will be shown that in several states there is no law or mandate to prohibit a dentist from destroying a patient record at any time he or she is inclined to do so. Data and graphs will be presented that are representative for the United States and Canada. Recommended antemortem dental record data that should be collected for missing person investigations and their transcribed results as entered into NCIC and the National Dental Image Repository (NDIR) will be discussed, as well as The Health Insurance Portability and Accountability Act (HIPAA) and its impact on dental record release for forensic investigations. The organizations NamUS, The Doe Network and the North American Missing Persons Network will be reviewed as adjuncts to the NCIC. These are available to law enforcement and the public for investigative lead development and community contribution to solving missing and unidentified person cases.

Other forensic investigations effected by dental record retention include: state conducted investigations into patient complaints of malpractice and/or diminished standard of care; patient or employee initiated reports of insurance fraud, and illegal record amendment or destruction. Experts of jurisprudence and forensic accounting will be able

to apply the knowledge gained regarding dental record retention mandates in their practice states immediately for plaintiff, defendant, and expert witness deposition in civil casework. The knowledge gained may prioritize the process of evidentiary discovery within a state with a low or non-existent requirement for dental record retention, or when a statute of limitation for a claim being investigated approaches the threshold for legal dental record destruction within a state.

Police and law enforcement, medical legal investigators, cold case teams, family assistance facilitators, medical examiners, coroners, and forensic consultants have a significant focus upon unidentified persons, and the investigation of criminal acts against them. A criminal and/or homicide investigation begins with the identity of the decedent, and the process is significantly impaired without it. The NCIC is a powerful investigative tool in the process of identification when the information uploaded into it is accurate, and knowledgeable experts review the reports created to keep investigative resources focused on likely matches.

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**Missing, Dental, NCIC**

## **D73 Assisted Suicide Teams: A Final Exit Case Study**

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After attending this presentation, attendees will understand how assisted suicide teams, also known as "Exit Guides," are being used in order to provide hands-on assistance in executing a suicide as well as removal of all evidence from the death scene in order to elude detection that the death was an intentional act.

This presentation will impact the forensic science community and legal community by providing information regarding the methods used by the Final Exit Network and their "Exit Guides."

Assisted suicide is a controversial topic with many supporters on both sides of the debate. However, what is not debatable is that in most of the United States and other countries all over the world, assisted suicide is considered to be illegal; with some areas considering it to be a homicide to assist someone in ending their life. Physician-assisted suicide is legal in certain instances in three states which include Oregon, Washington, and Montana. Many cases have documented family/friends assisting a loved one to commit suicide. This presentation will focus on volunteer Exit Guides from the Final Exit Network and provide information regarding the signs/evidence to look for when investigating a death which may initially appear to be from natural causes.

The Final Exit Network is the only organization in the United States that will help individuals who have non-terminal illnesses to hasten their deaths. The best known "how-to" guide is Derek Humphry's 1991 *Final Exit: The Practicalities of Self-Deliverance and Assisted Suicide for the Dying*, which was a New York Times best seller.<sup>1</sup> Individuals considering taking their own life are able to obtain assistance from this network after they become a member of the Final Exit Network and undergo an application process. Once approved to receive services they are then assigned two Exit Guides to assist them with the preparation of, completion of, and clean-up after their suicide. Methods for hastening death are varied with the most notable being the use of helium and plastic bag asphyxiation.<sup>2</sup>

A case study of the death of a 64-year-old woman whose death was initially thought to be from natural causes will be presented. Months later, the woman's name appeared on a list obtained by the Georgia Bureau of Investigation (GBI) of individuals who contacted the Final Exit Network for assistance with their suicide. The GBI contacted the South Carolina State Law Enforcement Division (SLED) who notified local jurisdictions of

potential cases that may need to be reviewed. The case was re-opened and investigated. This presentation will show participants the forms used by the Final Exit Network, the information they gather before agreeing to provide exit assistance, and the instructions provided to those seeking their assistance which ensure that the suicide is not detected. It is recommended that medicolegal death investigators become familiar with the methods used by the Final Exit Network and volunteer Exit Guides as deaths, which may initially appear to be from natural causes, may be a suicide which was covered up by "Exit Guides" who remove evidence from the death scene. Exit Guides have been criminally charged in multiple states after further investigation into the deaths of individuals whose names appeared on the list obtained by the GBI.

#### References:

1. Humphry, D. 1991. *Final Exit: The Practicalities of Self-Deliverance and Assisted Suicide for the Dying*. Eugene, OR: The Hemlock Society.
2. Ogden, RD. & Wooten, RH. Asphyxial Suicide with Helium and a Plastic Bag. *Am J Forensic Med Pathol*, 2002; 23(3), 1-4.

#### Assisted Suicide Teams, Final Exit, Death Investigation

## D74 Death Investigation and the Scientific Method

*James M. Adcock, PhD\*, 1304 Mandalay Parkway, McDonough, GA 30253*

After attending this presentation, attendees will: (1) learn about the frequency of homicides in the United States and the clearance rates associated with them; (2) will learn about some of the reasons why so many cases are still unresolved; and, (3) will learn how to apply a scientific approach to death investigation by utilizing a Death Investigation Protocol specifically designed to incorporate the scientific method.

This presentation will impact the forensic science community by addressing some of the concerns relating to investigative failures and how these concerns may be remedied through the application of the scientific method to each investigation. It will also outline a scientifically based model to be utilized during the conduct of any investigation that will help to reduce the number of unsolved murders.

At the AAFS meeting in Chicago, in February 2011, Dr. Adcock presented a paper entitled "Managerial Responsibilities in the Homicide Investigation Process: Making a Case for Periodic Reviews of All Ongoing Death Investigations" that is also relative to this presentation but with more of an emphasis on the actual investigative process versus the managerial responsibilities. It is believed that coupling supervisory oversight of death investigations that utilize a scientifically based model will enhance the resolution rate and make other cases stronger for court.

The "scientific method" as practiced by science scholars, researchers, and practitioners is quite straight forward and has proven itself time and time again as the approach to use above all other possibilities. While some have variations, the following steps are commonly accepted: (1) state a problem; (2) observe; (3) form a hypothesis; (4) conduct experiments; (5) collect data; and, (6) draw a conclusion.

However, according to Dr. Thomas Young, "The scientific method, a time-honored approach for discovering and testing scientific truth, does not and cannot work for the forensic sciences in its standard form because it does not work for past events. Past events cannot be observed, cannot be predicted or deduced from physical evidence, and cannot be tested experimentally."<sup>1</sup> It was this premise and writings by Young that were the impetus for the design of the Scientific Method for Investigators in hopes that investigators would realize this process of analysis is not just for scientists and that utilization of it would in fact make stronger cases and increase solvability. Therefore, the Scientific Method for Investigators: (1) obtain from witnesses the accounts of what happened; (2) based on these accounts anticipate the questions you will be asked by others so you can properly collect and record the physical evidence; (3) collect and record the physical evidence: (4) formulate hypotheses about the events that occurred and anticipate the questions you will be asked; and, (5) determine whether

the witness statements are consistent with the physical evidence; gather more information or evidence as needed.

Through the process of verifying witness statements, admissions/confessions, consider the evidence at hand and disprove as many hypotheses as you can. Formulate an assessment (final hypothesis) to a reasonable degree of certainty, recognizing the existing limitations.

While keeping those seven steps in mind, the scientifically based model called the Death Investigation Protocol was also designed. It includes all of the steps in a simple format that is easily followed. In its simplest form the death act begins with behavior from two people, a suspect and a victim. Then law enforcement is brought into the picture where the investigation begins at the scene with the collection of the physical evidence and the informational pieces of the puzzle. The standard process moves into the autopsy, crime lab analysis of all evidence, and hopefully the design of a victimology. Once those are all collected and created, the preliminary analysis begins where hypotheses (to some theories of the crime) are promulgated.

The next phase of the model is to evaluate these hypotheses through reconstruction of the physical and informational aspects of the investigation to see if a final analysis can be attained. In the interim, a review of all the pre-offense, peri-offense or "crime behavior," and post-offense behavior should be reviewed and evaluated. Once all this is accomplished the investigator needs to identify the hypotheses that are confirmed; if not, then the process returns to the preliminary analysis. If confirmed are all future questions answered? If not then back to the preliminary analysis, etc. If all questions that can be answered are answered and the hypothesis is validated then one can state a more accurate conclusion as to what happened.

#### Reference:

1. Young, Thomas, Heartland Forensic Pathology, LLC, Forensic Science and the Scientific Method, <http://www.heartlandforensic.com/writing/forensic-science-and-the-scientific-method>, retrieved July 18, 2011.

#### Death Investigation, Scientific Method, Protocol

## D75 Suicide, Homicide, or Death by Misadventure: Requirement for Collaborative Relationship Between Forensic Investigators and Forensic Pathology

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After attending this presentation, attendees will gain a better understanding of the collaborative relationship that should exist between forensic investigators and forensic pathologists in relation to suspicious death investigations

This presentation will impact the forensic science community by demonstrating the importance of removing bias or "tunnel vision" when conducting homicide investigations.

Currently in the United States, laws requiring all motorcyclists to wear a helmet are in place in 20 states and the District of Columbia, while 27 states require only some motorcyclists to wear a helmet. Only three states have no motorcycle helmet use law. Most helmet laws require motorcycle riders to use a helmet that meets the DOT Federal Motor Vehicle Safety Standard (FMVSS) 218. FMVSS 218 requires that in a 138.4 cm drop test (5.2 m/s, meters/sec) onto a hemispherical anvil, and on a 182.9 cm drop test (6.0 m/s impact speed) onto a flat anvil, the peak head acceleration shall not exceed 400g. If the peak head acceleration exceeds 200 g, the duration at 200 g's must be less than 2 ms (milliseconds), and if the peak head acceleration exceeds 150 g, the duration must be less than 4 ms. Helmets that are similar in form to a motorcycle helmet designed for on-road use, but that are not certified by their manufacturer to meet the requirements of FMVSS 218 are often referred to as "novelty" helmets.

According to the National Highway Traffic Safety Administration (NHTSA), 2006 NOPUS survey, a probability-based observational survey of motorcycle helmet use in the United States, found that 14 percent of motorcycle riders use helmets that do not comply with FMVSS 218.

To combat this problem, NHTSA issued a final rule on May 13, 2011, that changes the labeling requirements on helmets, making it more difficult to sell helmets with markings that resemble current DOT labeling. However, novelty helmets remain a safety hazard. In the current study, the performance of a DOT certified half helmet and a similar looking novelty helmet were evaluated using a drop tower system. Six drop tests were conducted using a novelty helmet and a DOT certified half helmet. The helmets were placed on a crash test dummy head that was instrumented with a tri-axial accelerometer at its approximate static center of gravity. The head was suspended from a drop tower and dropped onto an asphalt test bed. It was suspended such that the head was free to rotate on impact. The drop height was 152.4 cm, to simulate the height of an average-sized rider on a cruiser-type motorcycle. All data was recorded at 10 kHz. Axis orientation and data filters were used in accordance with SAE J211 Recommended Practice. For the novelty drop tests, the peak resultant head accelerations were 451g, 358g, and 473g for the left, right, and top impacts respectively. The corresponding HIC36 values for the novelty drop tests were 3677, 2260, and 4201. For the DOT drop tests, the peak resultant head accelerations were 143g, 142g, and 243g, for the left, right, and top impacts respectively. The corresponding HIC36 values for the DOT drop tests were 739, 595, and 1681.

For this test series, the DOT certified helmet met the DOT criteria described above in all three impacts; however, the novelty helmet did not meet the criteria for any impact. The peak resultant g's from the novelty helmets were 1.9 to 3.2 times higher than those of the DOT certified helmets, depending on head impact orientation. The HIC36 for the novelty helmets ranged from 2.5 to 5 times that for the DOT certified tests, again depending on head impact orientation. These results show that the DOT helmet was at least twice as effective at reducing the potential for head injury when compared to the novelty helmet. A study by Scher et al., (SAE#2009-01-0248) was similar to the present study with the exception that the head orientation was fixed during impact, so that all head motion was constrained to one direction, similar to FMVSS 218 testing. On average, for the Scher study, the peak resultant g's from novelty helmets were 2.6 times those of DOT certified half helmets, and on average the HIC15 for novelty helmets was 2.9 times those of DOT certified half helmets. Thus, based on the average data presented by Scher, both studies

Short Title: D.O.T. and Novelty Helmet Comparison [For Reference Purposes Only] showed similar reductions in head accelerations and HICs when using a DOT certified helmet rather than a novelty helmet. The present study appeared to have higher magnitudes for peak acceleration and HIC for all drop tests when compared to the Scher study. However, because only average data was presented in the Scher study, and because the present study was limited to six tests, direct correlation cannot be accomplished. Possible explanations for the discrepancy between the studies include a constrained head versus a freely rotating head and head impact orientation. Further testing and more information regarding the Scher testing are needed to determine the basis for this discrepancy.

Drop testing clearly demonstrates that DOT certified motorcycle helmets are much more effective at protecting the head than novelty helmets.

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**Collaborative, Forensic, Pathology**

## **D76 Assessing the Need for Family Assistance Services within the Medical Examiner System**

*Bethany Lynn Bless, MS\*, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054*

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After attending this presentation, attendees will be familiar with the current role of the Harris County Institute of Forensic Science as it pertains

to family assistance. The challenges of assisting grieving families will be outlined and current limitations of assistance within the Harris County Institute of Forensic Science will be identified. This presentation will assess the need for improved family assistance services.

This presentation will impact the forensic community by contributing to the understanding of challenges in assisting grieving families and identifying the need for a specialized program to assist with family assistance services.

By Texas Law, (CCP Art. 49.25), the medical examiner is mandated to determine the cause and manner of death in all cases of accident, homicide, suicide, and undetermined death. In cases of natural death, when the person is not under a doctor's care, or the person passes away in less than 24 hours after admission to a hospital, the medical examiner must be notified, as an autopsy may be required. The forensic investigators are responsible for conducting death investigations by developing organized, concise, and accurate death reports in accordance with Texas Code of Criminal Procedures 49.25. The investigators are also responsible for handling proper identification of the decedent and notification of family. The medical examiner must use all information available to make a determination about the death. This may include information from his/her own investigation, police reports, staff investigations, and discussion with the family and friends of the decedent.

Medical examiner investigators may be the first official to contact the family of the deceased individual and as such, need to be sensitive and exhibit an understanding of the initial grieving process. The sudden and unexpected death of a family member/friend can bring a range of emotions including guilt, shock, anger, sadness, resentment, and regret, as well as a number of unanswered questions. There is no time frame for grief and no right or wrong way to grieve. It is important to understand that everyone grieves differently. Grief is an individual experience that is unique to each person and to the relationship he or she had with the deceased. The act of informing family members of a death requires a responsible, well-trained, and sensitive individual who can manage to cope with this mutually traumatizing experience. Family members of deceased victims have a wide range of needs and reactions to the sudden and untimely death of their loved ones. Consequently, the individuals who deliver death notifications and the manner in which they carry out this duty factors significantly in the trauma experienced by the family.

The Harris County Institute of Forensic Science currently has limited resources to locate, notify, and support families. There are more than 2,000 violent deaths per year in Harris County. Approximately 85% percent of families are located and notified within 24 hours of death. However, the remaining 15% require in-depth searches to ensure timely identification and notification of death. Families often require ongoing communication to obtain further information and to provide final determination of cause and manner of death. There is no formal means for the Harris County Institute of Forensic Science to assist families in identifying local victim related services and resources.

A specialized family assistance program is necessary in the medical examiner's system. The program would ensure timely notification of death, compassionate assistance in understanding the medicolegal death investigation process, and early access to local relevant victim family related services. The program would assist in the collaboration and coordination of local governmental and not-for-profit victim assistance service providers to meet the emotional, physical, mental, and financial needs of those affected by untimely violent death.

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**Medical Examiner, Grief, Family Assistance**

## **D77 I Shot Him: A Case for Murder or Self-Defense**

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After attending this presentation, attendees will have an understanding of the challenges faced by investigators and prosecutors in a homicide case where the perpetrator is the sole survivor witness and claims self-defense. Attendees will be presented with the facts of the case and an evaluation of these facts with regard to murder or self-defense.

The presentation will impact the forensic science community by articulating the investigative steps necessary in sound crime scene processing and the theory underlying the claim of necessity of self-defense in a criminal case.

On April 3, 2009, the decedent was shot to death by his wife in their rural Meigs County home. The wife told her stepson and responding investigators that her husband was showing her how to shoot a gun and she didn't know that the safety was off, and the gun went off and then kept going off multiple times until empty. The weapon was a 9mm semi-automatic pistol. The wife told the responding investigators that she and her husband had a good marital relationship, had had no argument, and that the gun "just kept going off."

The decedent was found in the home living room reclining in a cloth-covered recliner chair. He suffered gunshot wounds of the upper and mid chest, abdomen, left arm, right leg, and right thumb. The room and home was neat and orderly with no signs of a struggle. The weapon and ejected casings were found nearby the body.

At trial the wife was charged with aggravated murder. Rather than to assert an accidental shooting due to weapon misfire as she had originally stated to investigators, she alleged self-defense and that she had been a victim of "Battered Wife Syndrome." A jury found her not guilty of aggravated murder, but deadlocked regarding the lesser included offense of murder and a mistrial was declared.

The wife was subsequently tried before a second jury for murder. At the second trial she again asserted self-defense stating the decedent became increasing abusive after retiring several years previously. At trial, both the prosecution and defense provided expert testimony both with regard to the movements and positions of the defendant and decedent at time of the shooting and also about Battered Wife Syndrome. The defendant claimed the decedent, a very large man, attacked her with a paddle and was standing over her, after knocking her to the floor, when she was forced to shoot him in self-defense. Prosecution maintained the decedent did not assault his wife, that she had no assaultive injuries at time of the initial investigation, and he was either in his chair or coming out of it when the shooting occurred. The defendant was found guilty of murder and sentenced to life with parole.

In the State of Ohio for a defendant to assert the self-defense claim of necessity the defendant must show by a preponderance of the evidence that she reasonably believed herself to be in imminent danger of death or serious bodily harm and that a reasonable person would feel the same way in the same or a similar situation. In this case the evidence did not support a history of violence or battering, nor did the evidence support the weapon misfiring.

**Murder, Self-Defense, Gunshot Wounds**

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## **D78 Multiple Gunshot Suicides**

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After attending this presentation, attendees will have a better understanding of the prevalence, features, and implications of multiple gunshot suicides.

This presentation will impact the forensic science community by showing how reviewing literature and case studies will reveal several key features that are similar in multiple gunshot suicide case outcomes. Understanding these factors has the potential to significantly impact medicolegal death investigations of multiple gunshot suicides.

Multiple gunshot wounds are found in 6-10 % of all gunshot suicides. Most cases involve two gunshots, but as many as nine gunshots have been reported. The alternate possibility of homicide may be suspected when there are multiple gunshot wounds. As a result, case management problems often exist. Problems may be related to the skepticism and disbelief of family, media, and others. As in all death investigations, a thorough scene investigation and team approach is critical in determining the manner of death. The position of the body and gun, wound entrance and exit locations, wound characteristics, blood spatter pattern, past and recent medical and psychiatric history, statements from household members, and the presence or absence of a suicide note are important features in trying to determine evidence of suicidal intent.

Research shows that multiple gunshot wound suicide features are similar to those found in single gunshot wound suicides. Wounds are contact or near contact and involve classic entrance wound sites of the head and neck. Soot or back spatter can be present on the hand(s). Multiple gunshot wounds to the head are relatively rare, but do occur. The most common wound area is the left chest or precordial region. Wound characteristics must be documented to determine instant versus delayed incapacitation. Several types of weapons have been documented in suicide cases, including long rifles and shotguns. Twenty-two caliber handguns are used most frequently according to case findings. Four case reports will be presented and characteristics will be compared to the literature findings:

**Case #1** - an 86-year-old male who suffered from Depression and Parkinson's disease. The investigation determined a self-inflicted GSW to the chest with a .22 caliber handgun, followed by a second gunshot wound to the head.

**Case #2** - a 42-year-old male with a history of Bipolar Disorder, occasional erratic behavior, and past suicide attempts. History revealed verbal threats of shooting himself first in the chest and then in the head to "do it right." The male went into the bedroom, and was found with gunshot wounds to the head and chest from a 9 mm pistol.

**Case #3** - a 41-year-old male who was found in a pickup truck at an isolated, rural location. He apparently died from a self-inflicted shotgun wound to the head. Secondary scene investigation revealed a first, unsuccessful shot in the head with a .22 handgun while at home. He then drove to a rural location, and shot himself in the head with a 12 gauge shotgun. The bullet lodged in the head, but did not perforate the skull bone, and was recovered at autopsy.

**Case #4** - a 53-year-old male left a suicide note and apparently shot himself four times in the chest with a low power handgun.

Review of the literature and case studies reveal several key features that are similar in multiple gunshot suicide case outcomes. Understanding these factors has the potential to significantly impact medicolegal death investigations of multiple gunshot suicides.

**Suicide, Multiple Gunshot Wounds, Self-Inflicted**

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## **D79 A Survey of Forensic Professionals and Law Enforcement on Synthetic Drugs**

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After attending this presentation, attendees will gain an understanding of the prevalence of synthetic drug samples commonly observed in the law enforcement field and forensic laboratory. In addition, current testing protocols for the samples will be discussed based on information obtained from a given survey.

This presentation will impact the forensic science community by highlighting the distribution demographics of synthetic drug samples in the United States, trends of synthetic drugs, and the laboratory procedures used for testing these samples.

In recent years, synthetic drugs have increasingly grown in popularity in the United States and subsequently, more samples are being seized and tested in the forensic science community. A survey was sent to a group of forensic professionals who participated in online continuing education courses from August 2010 through November 2011, regarding the prevalence of synthetic drug samples in their work environment. The questions in the survey were used to gather information regarding the frequency of synthetic drug samples by demographic region and laboratory testing capabilities.

The survey is comprised of the following questions:

1. In what state do you work?
2. Do you work for a local or state agency?
3. What is your job title?
4. Have you encountered synthetic drug (e.g., synthetic cannabinoids, and bath salts), samples in your position?
5. What synthetic drug compounds are you specifically seeing?
6. How often are you working with synthetic drug samples?
7. Do you perform testing in-house for synthetic drugs?
8. If yes, can you elaborate on your testing protocol?
9. If no, what is the protocol for synthetic samples in your agency?
10. What further information can you provide regarding synthetic drugs?

The survey was distributed to forensic professionals and law enforcement with ranging experience levels and job positions in order to gain a wide range of information. Due to the growing number of synthetic drug compounds and samples, it is important for the forensic community to understand and become familiar with the trends of synthetic drugs. Once trends are recognized, the forensic community can understand what they may be experiencing in the variety of compounds being seen and testing methods being used. Trends are important for law enforcement and analysts to be aware of in order to recognize what the drugs and their packaging look like, where they are sold, what drug compounds are currently controlled, how the samples should be collected, and what testing protocols need to be established. Because synthetic drug manufacturers can change the compounds that are being used or where they are distributed, it is important to have regional contacts in order to share information.

When information is shared amongst the field, progress can be made towards the standardization of protocols and methods. Legislation can build off of the standardization by working towards scheduling the entire class of synthetic drugs and not only single compounds and their analogs. By understanding current trends, the community may be able to determine future synthetic drug trends and be prepared for new drugs in the future. Preparedness is an important aspect in the forensic community, especially with drugs like synthetics, where a slight change in a compound can make an illegal drug, legal again.

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#### **Synthetic, Drugs, Trends**

## **D80 Medicolegal Elderly Death Trends Within Harris County, Texas: Are Changes in Practice Warranted?**

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After attending this presentation, attendees will learn to identify categories of elderly death trends and barriers that inhibit thorough death investigation of the elderly. Understanding these categories of death as well as recognizing potential barriers will impact death investigators by allowing them to target improvement efforts within the medicolegal death investigation system.

This presentation will impact the forensic science community by providing information on interesting trends in elderly deaths and case study examples to illustrate key points. Initiatives to improve investigation of the elderly will be presented which could be implemented within any medicolegal death investigation system.

The demographics of our population are changing. As our society ages, in part because of advances in healthcare services, people are living longer than at any other time in history. At the same time, the aging population requires additional healthcare resources or family support. The role reversal of children caring for their parents can be overwhelming and unexpectedly stressful. The majority of deaths in the elderly population are due to natural causes; however, often the people reporting these deaths are not aware of nor did they consider prior or ongoing Adult Protective Service (APS) investigations. When investigators are unaware of an APS history during the initial death report, the likelihood of further investigation into the cause and manner of death subsides. The impacts would include those deaths from non-natural manners and especially elder mistreatment.

This retrospective study was initiated in efforts to describe death trends and barriers of the elderly falling within medicolegal jurisdiction in Harris County, Texas. The study is a four-year longitudinal descriptive study looking at deaths of people aged 65 and above (N=38,827) where medicolegal jurisdiction was retained and an examination did occur (n=4,400). The purpose was to describe any differences within the decedent sample with no APS reports (n=4208) and the decedent sample that had an active or closed APS investigation (n=192). There was no distinction made between APS cases in which the decedent was investigated for self-neglect or whether there was a caretaker who was investigated by APS. Of the 38,827 deaths reported to the Medical Examiner's Office, there were 4,400 cases in which jurisdiction was retained and an examination occurred. Of the 4,400 cases surprisingly only 192 had APS history; the initial report only included information about active or past APS investigations in 28 of those cases. Additionally, after cross-tabulating medical examiner cases with APS database it was discovered that of the 34,427 elderly deaths that were reported but which jurisdiction was released, 1,310 cases had a history of an APS investigation. In some of those cases, jurisdiction may have been retained and a physical examination performed if knowledge about APS history was attained during the initial investigation. The consequences of not having this APS history available at the time of the initial report may have led to deaths being released from medicolegal jurisdiction. Efforts were initiated to identify and remove barriers impeding investigators from obtaining APS history. Two of the initiatives included improving communication with APS and providing additional education regarding elder mistreatment to death investigators.

Some interesting trends in elderly deaths and case study examples provided to illustrate key points will be presented. Initiatives to improve investigation of the elderly will be presented that can be implemented within any medicolegal death investigation system.

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**Medicolegal Death Investigation, Elder Mistreatment, Adult Protective Services**

## **D81 Body Donation and Death Reporting: Three Case Studies**

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After attending this presentation, attendees will gain an understanding of how body donation programs interact with coroners and medical examiners in the state of California through three specific case reports.

The presentation will impact the forensic science community by raising awareness of body donation programs and role and their interactions during death reporting.

Each year over 1,000 individuals donate their bodies after death to the University of California (UC). These donations are made to one of five campus locations at Davis, Irvine, Los Angeles, San Diego, or San Francisco. Most of these deaths are from natural causes; typical for the aged donor population. They result from long-term illness such as cancers, heart disease, or pneumonia, but occasionally, the circumstances surrounding the death are not typical and require further inquiry or formal investigation.

Standard protocol for receiving a donor at UC includes death notification to the program personnel and confirmation of registration in the donor program. A medical and social history screening is performed during the notification phone call. If the donor meets the criteria for donation, the remains are then transferred to the program. A serology sample is taken and submitted for communicable disease testing. Simultaneously with the preparation of death certificates and burial permits, donor program personnel conduct a thorough external examination and photograph the remains; noting trauma, surgical history, identifying markings, and other significant findings. The following are three cases that required further investigation.

Case number one is a 70-year-old Caucasian female who died in a nursing home. The attending physician provided cause of death information, the local coroner provided a case number and the death certificate was accepted by the local health department. During preparation for scientific use, donor program personnel found a foreign object and reported the case back to the coroner.

Case number two is an 85-year-old female. This case was received after a residential death and was transported by a contracted mortuary service. Program personnel determined that there was no doctor to sign the death certificate, concluding that the decedent had not seen a doctor in a number of years. The case was referred to the coroner's office for follow up at which point, that office determined that the death was not caused naturally. Case number three, a 71-year-old Caucasian male, was received after his death occurred in his residence. The local coroner had responded to the death, but declined to investigate the case. Upon external examination by the donor program personnel, evidence of traumatic injury was identified and the decedent was returned to the coroner's office for investigation.

Of the estimated 4,500 deaths accepted for donation in the UC System since 2008, 1,142 of them have been assigned coroner case numbers. The vast majority of these are assigned case numbers due to required reporting circumstances such as an emergency room death, hospice death or similar circumstances. In some cases, donor program personnel identify atypical circumstances that result in reporting to obtain a referral number or initiate a full investigation. The UC Davis donor program reports approximately 30 cases per year for further investigation.

It is essential that donor program personnel be trained to recognize normal postmortem processes so that abnormal cases can be appropriately referred to local officials for full review or follow up. It is possible that whole body donors for scientific use, who account for less than one percent of the total annual deaths in the state of California, may not always be subject to the same postmortem reporting process as those who choose a more traditional disposition. Theoretically, this may be due to caretakers who want to facilitate the donors' disposition wishes or for other, less obvious, reasons such as incomplete information provided in the initial death report. Further analysis of donation data may reveal reporting patterns, or a lack thereof, for decedents who do not follow a typical disposition path.

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**Body Donation, Death Reporting, Death Investigation**



ATLANTA 2012

# JURISPRUDENCE



ATLANTA 2012

## E1 Sanity and Insanity in a Criminal Trial: The European Experience Seeks the American Experience

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After attending this presentation, attendees will learn from the data presented in this thorough and comprehensive examination the legal and scientific definition of “sanity” and “mental instability” to be evaluated within the criminal process, both during the investigation phase and in a courtroom.

This presentation will impact the forensic science community by providing an easy examination of the different categories of sanity and mental instability, in order to use them during their studies and professional practice.

A correlation between the existing Italian system between liability and social dangerousness intended to understand the authentic predisposition of the suspect/defendant to commit a crime is observed. The notion of insanity in our legal system is correlated with the legal institution of imputability: an essential condition for an offender to be punishable (Articles 88 (total defect of mind) and 89 (partial defect of mind) of the Italian Criminal Code). The concept of “crime” as a criminal act will be explained, and therefore what typical action is perpetrated with criminal intent and perceived as such by the community that suffers. Following, the guilt of a crime as a key element connected to the psychic sphere of the subject in the form of malice, misconduct, or involuntary act will, be defined on a level very different from liability, where an non-indictable (a minor) has acted with intent.

Otherwise, liability is correlated to the ability to be in full possession of one’s faculties given that Article 85 in the Italian Criminal Code stating that no one can be punished for an act seen by law as a crime “if, at the moment of the act, was not liable” and that “is liable those who are in full possession of their faculties.”

The ability to be in full possession of one’s faculties is linked in the Italian penal system, like those in Europe, to the concept of criminal responsibility, recognizing that the offender is capable of understanding and self-determinating their actions, performing them “with consciousness and will.” Thus it is necessary to distinguish the ability to understand (perceive the external reality and understand the worthlessness of their actions/omissions) from will (to control the impulse to act). Italian legislators have introduced two presumptions of capacity: an absolute (relative to an adult capable of understanding) and the other relative (the minor who is over fourteen years of age, whose capacity will be evaluated case by case). The scientific aspect of liability and guilt is then determined, being able to distinguish between consciousness and the will to act on the ability to understand what is accomplished.

The Law, in line with science, has created a concept of insanity associated to the medical criteria of a mental disease. In Italy, magnetic resonance imaging is used in the forensic field to highlight structural changes in the cerebrum of the brain that can lead to crime. From the ethological point of view, it has been observed that primates are able to recognize subjects with mental and physical impairments and therefore are not addressed with the same punitive attitudes in response to behavioral deficiencies. Moreover, “the partial defect of mind” as understood by the Cassazione Penale (Italian Supreme Court) will be examined, a vice compatible with the existence of intent (consciousness, will and prediction of the act). In this context the “reactions to short-circuit” can also be

induced by particular emotional or passionate and pathological states (Art. 90 of the Italian Criminal Code) which can predispose the individual to perform acts of brutal violence. The criminal is evaluated as such via the application of three benchmarks: the first related to the biology of the brain (area where the study of genes as potential source of violent behavior is essential); the second associated with the personality of the offender (sector in which psychology and forensic psychiatry are already active); the third is identified with the environment in which the criminal lives or has lived (under jurisdiction of behavioral sciences and judiciary bench).

The data obtained are compared with the American penal system. Finally, it examines the conscious participation of the accused at the trial in an Italian context, also in light of “neuroscience,” and then is compared to the American system.

**Criminals, Biology, Criminal Law**

## E2 Explaining DNA and DNA Results to Judges and Juries

*Pamela A.W. King, JD\*, 400 South Broadway, Suite 15, Rochester, MN 55904*

After attending this presentation, attendees will have new ideas about how to present complex, often technical concepts in DNA litigation to judges and juries, using technology in the courtroom.

This presentation will impact the forensic science community by encouraging lawyers to explore new approaches to presenting important yet technical concepts to judges and juries. This will provide the fact finder with the skills necessary to be able to understand and apply scientific evidence in a particular case, thus raising the quality of DNA forensic testimony in the courtroom.

Assuring that judges and juries understand the scientific evidence presented to them, including an assessment of the weight that should be given to any particular piece of forensic evidence, is where lawyers play a crucial role in the forensic science community. In any given case the fact finder is asked to understand DNA evidence involving complex mixture analysis, different statistical analysis designed to assist the trier of fact in assigning weight to a particular DNA match, inclusion or exclusion, and consider different processes such as STR typing, YSTR, and Mitochondrial DNA analysis. They may be confronted with a multitude of issues surrounding sample collection, serology test results, machine malfunctions, etc. Many jurors and judges have little to no scientific background. Typically, their “knowledge” of forensic science is founded on what they see in pop culture and the media. As such jurors and to some degree judges must rely heavily on the ability of scientist and attorneys to explain these concepts in a manner that is simple and approachable.

The research that has been done in a multitude of forensic science disciplines looking at whether jurors understand and properly assign weight to particular types of evidence suggests that jurors in particular do not understand much of what the forensic scientist and the lawyers are trying to relay to them. Examination of exoneration cases also suggests that in some cases where the value of forensic evidence is being overstated or misrepresented by scientist or lawyers, juries haven’t recognized these flaws or it may be the evidence was simply misunderstood by the finder of fact. Therefore, lawyers must attempt to find more effective tools to explain scientific concepts and present scientific evidence in the courtroom.

How can this material be presented in a more effective manner? What tools can be provided to the fact finder to allow them to properly assess the weight of this evidence? What role can the attorney play in helping judges and juries understand DNA evidence? This presentation will explore the

\* Presenting Author

particular challenges faced when DNA evidence is presented to the fact finder. It will then look at examples of how DNA evidence has been presented using tools such as visual aids to explain to the jurors how DNA typing is done and the challenges presented by things such as complex mixtures. These tools can be used by the prosecution as well as defense to assist the trier of fact in better understanding how DNA typing works, its limitations and what the forensic scientist is assessing in reaching their conclusions. This presentation will focus on specific cases and how the information was presented. The effectiveness of these tools has not been measured in any formal study. The suggestions are designed only to provide lawyers and forensic scientist with ideas and to encourage creativity on behalf of those presenting this evidence to assure the trier of fact has the tools necessary to effectively make a decision in a particular case.

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**DNA, Expert Testimony, Juror Comprehension**

### **E3 Evidence Retention/Post-Conviction DNA Testing: Issues and Answers**

*Rockne P. Harmon, JD\*, 2846 Lincoln Avenue, Alameda, CA 94501; and Melissa Mourges, JD, District Attorney's Office, New York County, One Hogan Place, New York, NY 10013*

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After attending this presentation, attendees will understand the challenges facing policymakers, police, defense attorneys, and prosecutors who must determine what evidence retention policies to put in place, in light of the role DNA testing plays in criminal cases.

This presentation will impact the forensic science community by illustrating the problems presented by evidence retention issues. Prosecutors, who employ new forensic techniques to develop cold cases, and defense attorneys, who hope to expand post-conviction testing, need a reliable system to maintain evidence. Police need to know what they need to save, for how long, and must find a way to do it all in an era of shrinking resources.

In light of the attention recent exonerations have brought to post-conviction DNA testing, significant efforts are being made to draft new evidence retention statutes in states that do not already have them. This presentation will discuss these efforts in light of the overall need for the statutes, the nature of previous exonerations, the infrequency of those exonerations, and the complicated legal issues that arise when legislators attempt to fashion remedies for defendants whose evidence is unavailable for testing.

Complicated issues abound. What is the scope of post-conviction testing? Should it be available only to those who are convicted after trial, or should defendants who plead guilty be entitled to post-conviction testing as well? And in what type of cases? Only in serious felonies like rapes and homicides? In all felonies? In misdemeanors? Only in cases where the defendant is still in jail? Should there be a statute of limitations on a defendant's right to request testing? Should he/she be allowed to request testing only where the results would be a "game changer," or should testing be permitted in every case?

Police agencies, which in most jurisdictions are responsible for maintaining evidence, have an enormous task. Policies differ - some agencies keep property through the final appeal, some keep homicide evidence and destroy the rest, and some discard evidence after the statute of limitations has run. Sexual assault evidence kits may be stored indefinitely, stored until tested, destroyed without testing depending on the investigator's opinion of the case, or destroyed if no arrest is made within a specific time.

Besides deciding what to store (guns and knives are an easy call, but what about cars and other vehicles?) must police departments build climate - controlled facilities to maintain biological evidence in case testing is ever requested? Who will pay for those facilities, or for the computerized, bar-coded or RFID systems to track the evidence? What remedies, if any,

should be available to defendants whose evidence was destroyed in compliance with former or present-day policies?

Finally, who should make these decisions? Should policymakers rely only on advocates for defendants who hope to be exonerated or to have their sentences reduced, or should prosecutors, lab personnel and police be at the table?

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**Evidence, Retention, Exonerations**

### **E4 Issues in Eye Witness Identification From Composite Sketches to Photo Array Lineups**

*Thomas L. Martin, BS\*, Crime Scene Forensics, LLC, PO Box 515, Red Hook, NY 12571*

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After attending this presentation, attendees will understand the issues being raised nationwide regarding the reliability of eyewitness descriptions, identifications and photo array line-ups.

This presentation will impact the forensic science community by defining the purpose behind using composite sketches and witness descriptions as investigative leads, and explaining the variables and limitations that exist when asking a witness or crime victim to describe the physical characteristics of an offender.

It is a common investigative practice to ask a victim or witness of a crime to describe the offender alleged to have committed that crime. Often these descriptions will result in either the generation of a composite sketch or with the victim or witness viewing a series of photographs in the form of what is commonly called a photo array or photo lineup. Eyewitness identifications are commonly used to support probable cause for arrest and as evidence against a defendant in trial; however, *erroneous* eyewitness identifications are commonly cited as reasons for false convictions. The variability by which eyewitness identifications are made increases the importance that investigators understand how to properly qualify the offender descriptions and identifications.

In any criminal case there is a commencement of an investigation, and an eventual trial in which evidence will be presented and an outcome will be determined. There are different stages involved in an investigation, and consequently, evidence and information will be obtained during that investigation that will amount to different levels of proof. In other words, certain investigative tools or techniques employed by investigators may not necessarily rise to the level of evidence required to demonstrate proof beyond a reasonable doubt; however these investigative tools may prove useful in generating information that allows for the investigation to proceed to the next level.

Composite sketches are a good example of an investigative tool. Composite sketches or artist's renderings are generated by a qualified artist and are based upon the descriptions of an offender as detailed by the victim or witness of a crime. The final drawing will be disseminated to the general public, in the hopes that information will be obtained regarding the unknown person responsible for the offense.

While composite sketches may prove to be a valuable investigative tool, they should remain just that; an investigative tool. The accuracy of the composite however, may prove quite useful in the investigation, therefore it is important that the rendering be as accurate as possible. To that end, the investigator taking the information must qualify the descriptions given by the victim or witness.

People perceive things differently, and consequently, descriptions of the same offender may differ as given by different witnesses. Techniques should be employed to ensure that the witness is accurately describing the offender, and can accurately identify that offender at a later time. Even in the case of a "positive identification" follow up investigative steps are necessary to ensure that the correct person is identified.

This presentation will discuss the role that composite sketches and photo arrays play in a criminal investigation as well as their respective limitations. Corroborative steps that can be taken to ensure that accurate

details are obtained in order to verify the accuracy of eye witness identifications will also be detailed.

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#### **Eyewitness Identification, Photo Lineup, Investigations**

### **E5 Juror Expectations for Scientific Evidence in Criminal Cases: Empirical Studies of the “CSI Effect” Myth**

*Donald E. Shelton, JD, PhD\*, PO Box 8645, 101 East Huron, Ann Arbor, MI 48107; and Gregg Barak, PhD, and Young S. Kim, PhD, Eastern Michigan University, Department of Sociology, Anthropology, and Criminology, Ypsilanti, MI 48197*

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After attending this presentation, attendees will learn that the results of two empirical juror studies do show increased juror expectations and demands for scientific evidence but do not support the simplistic accusation that “CSI” and similar television programs are the cause of those heightened expectations. Attendees will be presented with the suggestion of a larger cultural “tech effect” on jurors and to the suggestion that law enforcement and forensic scientists must adapt to the increased juror expectation generated by that phenomenon.

This presentation will impact the forensic science community by revealing the nature and extent of juror expectations for scientific evidence and confronting ways in which the forensic science community must adapt to those heightened expectations and demands.

The so-called “CSI effect” has many meanings but its most popular connotation, called the “prosecutor version,” is that jurors are wrongfully acquitting criminal defendants when the prosecution does not present the sophisticated (and perhaps non-existent) types of forensic science evidence featured on popular crime scene investigation television programs. Prosecutors blamed jurors when they lost cases. The news media picked up on these complaints, accepted them as factual, and quickly labeled it the “CSI effect.” The mass-media-created CSI effect was repeated again and again, almost always in the context of blaming the television programs for what prosecutors claimed was a *crisis* of misguided juror demands for scientific evidence. But is it true?

The presentation focuses on two large empirical studies of Michigan jurors in diverse jurisdictions, finding that this “prosecutor version” of the so-called CSI effect cannot be substantiated empirically. In the first study, 1,027 persons called for jury duty in Washtenaw County, Michigan were surveyed as to their television watching habits, their expectations for scientific evidence in certain types of criminal cases, and their likelihood of conviction in several evidentiary scenarios. In the second study 1,219 jurors were surveyed in Wayne County (Detroit), Michigan. The survey was similar but also included questions designed to test the earlier suggestion of a “tech effect” as the cause of juror expectations and demands.

Statistical analyses of the survey results reveal that modern jurors do have high expectations that the prosecutor will produce scientific evidence and that, in some cases, jurors will demand such evidence before voting to convict. However, there is no significant statistical relationship between those factors and the television viewing habits of the jurors. It is suggested that the increased expectations and demands arise from a much greater cultural shift toward the awareness and use of technology, described as the “tech effect.” Analysis of the separate, and then the merged, data from the two studies supports the suggestion of this “tech effect” based on cultural changes, rather than any direct impact from viewing certain specific or genre television programs.

It is suggested that while the prosecutor version of the CSI effect is a myth, there are indeed increased juror expectations that arise from the combination of the tech effect, the general media portrayal of forensic evidence, and the misperception of attorneys and judges that the CSI effect really does exist. Possible justice system responses to that combined effect will be explored.

It is suggested that the legal system, and in particular the role of forensic sciences in the criminal justice system, must adapt itself to modern juror expectations rather than blaming jurors for “unreasonable” expectations and demands for forensic science evidence. In our legal system, jurors decide what is proof beyond a reasonable doubt and jurors have decided that what is “reasonable” to expect from the prosecution in a criminal case is very different from what was considered reasonable just a few years ago. To meet those expectations, the government will have to expend a significant amount of resources and energy, both before and during trial, and the cost and methods for meeting those expectations will be discussed.

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#### **CSI Effect, Jurors, Evidence**

### **E6 Contextual Considerations for Evidence Collection and Testing Decisions: A Conceptual Framework for Investigators, Analysts, and Attorneys**

*Ted R. Hunt, JD\*, Jackson County Prosecutor’s Office, Jackson County Courthouse, 415 East 12th Street, Fl 7M, Kansas City, MO 64106*

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After attending this presentation, attendees will be familiar with a framework of heuristic factors for analyzing and interpreting the contextual significance of items located at a crime scene.

This presentation will impact the forensic science community by providing a framework with which to help facilitate logical and relevance-based evidence collection and testing decisions by investigators, analysts, and attorneys.

The term “context” may be defined as “that which surrounds, and gives meaning to, something else.” In forensic science, an item’s evidentiary context consists of the presence or absence of a probative relationship between the item and a criminal act. Depending on the facts of a particular case, this relationship may be direct and explicit, circumstantial and inferential, or a combination of each. For purposes of this discussion, a candidate object, article, or substance under consideration for forensic collection or testing will be referred to as an “item.”

Many times, the critical foundational question of a crime scene item’s contextual significance is bypassed or ignored. This may stem from investigative efforts that increasingly emphasize testing an ever-larger number of collected samples. The result is a technology-based, high throughput approach primarily focused on the question of source determination. This practice can be characterized as *forensic question begging*—drawing an inference of significance from a *testing conclusion* that includes or excludes a particular individual *absent* preliminarily establishing the *contextual probative value* of the item from which the test result was obtained.

To avoid such fallacious reasoning, an essential foundational question must be asked: Given the sum of the collective situational circumstances surrounding the item and the extrinsic information about the item provided by the victim(s), witness(es), and suspect(s), can a reasonable inference be drawn that will support the conclusion that the item is or is not related to the criminal activity being investigated? In some cases, the answer will be obvious; in others, that will be far from the case. It is for this reason that a conceptual framework for analyzing and interpreting evidentiary context is helpful.

The analysis of evidentiary context is composed of three distinct levels of significance: Level I, the intrinsic attributes of the item and its surrounding contextual circumstances at a crime scene; Level II, extrinsic information about the item provided by the victim(s), witness(es), and suspect(s) that tends to establish, enhance, diminish, or destroy its Level I significance; and, Level III, the presence or absence of an associative relationship between the item and a relevant person, place, or other crime scene item determined by forensic testing. This includes the statistical significance of a match, if any, that gives the item its evidentiary weight.

The ten Level I factors involved in contextual evidentiary analysis are: (1) the item's **Environment**, be it open or closed; (2) whether the item is **Native or Foreign** to the environment; (3) the **Nature** of the item, such as blood, semen, saliva, hair, or tissue; (4) the **Location** of the item, relative to other relevant persons, places, objects, or substances; (5) the **Relation** of the item, either direct or circumstantially inferred, that *connects* it to other relevant persons, places, objects, or substances; (6) **Action**, an inference of movement, force, or velocity associated with the item; (7) the **Quantity** of the item, meaning its relative abundance or scarcity at the scene; (8) the **Rarity** of the item, meaning its commonality or infrequency in general or in the crime scene environment; (9) the **Portability** of the item, meaning its transferability or fixity; and, (10) the **Condition** of the item, meaning the ability to reasonably infer the general period of time it has been present at a crime scene based on an examination of the item's external and/or internal characteristics and properties.

Level II of contextual evidentiary analysis is extrinsic to the item. It concerns assertions, omissions, or denials by the victim(s), witness(es), and suspect(s) regarding items recovered at a crime scene. A witness' assertion about an ostensibly insignificant and prosaic item located at a scene can *transform it into evidence*. Likewise, a witness' statement about an item apparently possessing strong Level I significance can relegate it to the status of a meaningless crime scene artifact. Level II assertions, omissions, and denials have the ability to enhance, diminish, or entirely eliminate the prior analytical interpretation and significance of the Level I factors.

Level III of contextual evidentiary analysis concerns the testing conclusion and statistical weight, if any, attached to a match. Any item recovered from a crime scene, depending on the facts of the case and analysis under Levels I and II, has a certain starting quantum of contextual significance prior to forensic testing. Forensic testing may then "individualize," "match," "include," be declared "inconclusive," or "exclude" a particular individual, object, or substance. Additionally, the item may be qualitatively or quantitatively insufficient to answer the question posed. Although a testing conclusion alone will not alter the preexisting contextual significance of the item analyzed, the probative value of that item, as an aspect of contextual relevance, can be exponentially enhanced or diminished.

The usefulness and applicability of the foregoing three levels of contextual analysis by investigators, analysts, and attorneys will be discussed. It is desired that these factors will help promote logical, relevance-based, and targeted evidence collection and testing decisions.

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#### **Evidence, Context, Collection and Testing**

### **E7 Michigan MRE 703 a Study in Fairness**

*Michael J. Nichols, JD\*, 3452 East Lake Lansing Road, East Lansing, MI 48823*

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After attending this presentation, attendees will have a better understanding of the boundaries for an expert witness to present the factual basis of an opinion.

This presentation will impact the forensic science community by discussing the difference between what facts or data must be admitted in evidence when testifying as an expert in a Michigan case. It will also allow forensic examiners to understand the "backdoor hearsay" rationale behind the rule and the difference between underlying facts or data that must be admitted as evidence and learned treatises and studies that do not need to be admitted.

Courts all over the country are presented with the problem of how to assess the reliability of proposed expert testimony and opinions and most importantly, whether the opinion applies reliably to the facts of the case. In Michigan, the facts or data upon which the expert relies in forming the opinion "shall" be in evidence by virtue of the requirement of Michigan Rule of Evidence (MRE) 703. The Federal Rule of Evidence is inconsistent with this requirement. It states: "The facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived

by or made known to him at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence."

Often, the ability of an expert to provide the baseline facts upon which he or she relied allows the expert to introduce testimony that would be otherwise inadmissible but is introduced to the jury only because the expert was allowed to provide it as a baseline fact. Often, these facts are assumptions or hypothetical and further, do not necessarily apply to the facts of the case for which the expert is providing an opinion.

The sufficiency of the application of the opinion to the facts of the case as a threshold matter is addressed in the voir dire process of the expert; however, the reliability of the expert's facts or data to support the ultimate conclusion of the expert can only be analyzed through requiring sufficient foundation for the admission of the witness who can authenticate those facts or data and be subject to cross examination on the admissibility of those baseline facts or data in the case for which the expert is called to testify.

A case study involving an expert's proposed testimony in an area called "retrograde extrapolation" will be presented. Retrograde extrapolation is a technique in which a person with knowledge in the field (often a toxicologist or pharmacologist) uses a subject's assumed bodily alcohol content at one point in time to opine as to what the bodily alcohol content was at an earlier point in time that has specific relevance to a contested fact in a case. The attendees will learn how the proponent of the opinion failed to meet the threshold of admissibility and why the judge's ruling was fair by preventing the admission of expert testimony that was not supported by facts that were reliable.

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#### **Expert, Hearsay, Reliability**

### **E8 Go or No Go: Screening Scientific & Technical Studies and Reports Presented in Court**

*Joseph J. Maltese, JD\*, New York Supreme Court, 130 Stuyvesant Place, 3rd Floor, Staten Island, NY 10301*

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After attending this presentation, attendees will have an understanding of the factors judges and attorneys consider in screening scientific studies and expert witness report.

This presentation will impact the forensic science community by providing a better understanding of how judges scrutinize studies and reports presented by expert witnesses in court.

When Jason Daubert and Eric Schuller were born, with serious birth defects, their parents sued Merrell Dow Pharmaceuticals, Inc., the manufacturer of a prescription anti-nausea drug known as Bendectin, which was prescribed to their mothers during pregnancy to limit "morning sickness." The plaintiffs claimed that Bendectin caused birth defects in their children. Before the case was able to be tried by a jury, the defendant manufacturer made a motion for summary judgment before the judge, asserting that Bendectin did not cause birth defects in humans. In support of that assertion, the manufacturer presented the affidavit of a physician, who was also an epidemiologist that had reviewed all of the 30 plus published studies involving over 130,000 patients. The doctor concluded from his review of the studies that the use of Bendectin in the first trimester of pregnancy, when nausea is at its worst, had not been capable of causing malformations in fetuses.

While the plaintiffs did not contest the characterization of the published Bendectin studies, they opposed the motion for summary judgment and presented eight well credentialed experts in the fields of pediatrics, veterinary medicine, pathology, toxicology, developmental biology, clinical pharmacology, epidemiology, biostatistics in reproductive epidemiology, biometry, and pharmacology.

But, the trial judge rejected the plaintiffs' proffered opinions of those eight experts in favor of the sole defendant's expert, who had summarized

all of the studies concerning whether Bendectin taken by pregnant mothers could cause birth defects in their children. The judge granted the defendant-manufacturer summary judgment, in essence in dismissing the case and did not allow the case to proceed to trial where a jury could evaluate all of the expert opinions. Clearly, under Federal Rule of Evidence §702, each expert was well qualified in their respective fields by education, training, skill, experience and knowledge. Yet, the trial judge found that their proposed testimony was insufficient to rebut the more than 30 studies that failed to find a causal link between the ingestion of Bendectin and birth defects in children.

Why were the plaintiffs' eight experts muzzled by the judge?

What the plaintiffs' experts presented was a recalculation of the data from some of the previous human epidemiological studies, which demonstrated no causal relationship. After the recalculation it should show a positive correlation between the drug and the cause of the birth defects. But those recalculations were never published or subjected to peer review. They also presented animal studies and pharmacological analysis to demonstrate that there was a statistically significant association between a component of Bendectin and birth defects. Others would testify that the chemical composition and physiological activity of a drug are important in ascertaining whether it is a teratogen, capable of causing birth defects. Because the plaintiffs did not present any contrary epidemiological evidence to counter the published studies in the field, the court rejected the plaintiffs' contention.

The *Daubert* case was ultimately appealed to the U.S. Supreme Court.<sup>1</sup> The Supreme Court outlined that judges as the gatekeepers of evidence should consider some or all of the following flexible factors to determine: (1) whether proposed expert testimony or evidence was tested or capable of being tested or refuted; (2) whether there was a margin of error; (3) whether the evidence was ever published and subjected to peer review; and, (4) whether the evidence or theory was generally accepted in the discipline to which it belongs. While scientists distinguish validity – does the principle support what it purports to show? – from reliability – does application of the principle produce consistent results – in law where a case involves “scientific evidence,” the evidentiary reliability will be based upon scientific validity. Moreover, it must be relevant to the case presented. That is, the evidence or theory fits the facts of the case at hand.

The Supreme Court established or reasserted a judge's role as a gatekeeper of evidence, especially evidence concerning science, technology, and other specialized knowledge; it did not give the trial judges a definitive framework or checklist of factors to consider before admitting such evidence into a trial to be scrutinized by a jury.

The trial judge is confronted with a “go” or “no go” determination as to whether the proffered expert testimony or evidence is scientifically valid. That is, that it followed established scientific or technical methods and procedures to arrive at a conclusion that is relevant to issues of the case before the court. There is no middle ground – the evidence is either admissible, that is sufficiently reliable to go before the jury or inadmissible and will not go before the jury. Such decisions are not just another evidentiary ruling. Most decisions concerning the admissibility of scientific evidence are dispositive of the entire case. Therefore, judges need guidance in determining whether to go or not go with scientific evidence before a jury.

This presentation will provide several considerations for judges and attorneys to consider when scrutinizing scientific studies or reports. Since this is a time consuming endeavor for any judge, studies and reports asked to be admitted or adopted as the basis to support an expert opinion ought to be presented and scrutinized well in advance of a hearing or trial with time allowed for opposing studies and reports to be submitted by the opposing side.

#### References:

- <sup>1</sup> *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579 [1993].

Screening Studies, Expert Witness Reports, Admissibility

## E9 The Exclusion of Forensic Identification Science Evidence Since *Daubert vs. Merrell Dow Pharmaceuticals, Incorporated*

Mark Page, BDS<sub>c</sub>, DipCl\*, University of Newcastle, Department of Oral Health, Ourimbah, New South Wales, AUSTRALIA; and Jane Taylor, PhD, and Matt Blenkin, MDSc, University of Newcastle, Ourimbah, AUSTRALIA

After attending this presentation, attendees will be able to describe incidences and patterns of judicial reasoning in cases where forensic science evidence has been excluded since the decision in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*

This presentation will impact the forensic science community by presenting how analysis of judicial reasoning for exclusion of forensic evidence reveals that pure deference to the *Daubert* factors is not the primary means by which to avoid potential exclusion of forensic science evidence in court. Judicial reasoning in decisions to exclude forensic identification science can be categorized in such a way as to be of relevance to forensic scientists as they conduct their analysis and present their evidence. These criteria for exclusion, derived from case law examples, are explained to participants so that they may take heed from other forensic scientists' experiences and avoid exclusion of their evidence in court.

The 1993 United States Supreme Court decision in *Daubert v. Merrell Dow Pharmaceuticals, Inc.* transformed the way scientific expert evidence was reviewed in courts across the United States. Five hundred forty eight judicial opinions were analyzed from cases involving a challenge to forensic identification evidence since the *Daubert* decision in order to gauge its impact on the admission of such testimony. Eighty-one (15%) of these cases resulted in exclusion or limitation of evidence. These eighty-one cases were then coded according to the reasons given for exclusion or limitation. Relevancy issues accounted for exclusion of evidence in fifteen of these cases (19%); and, the witness was deemed not qualified as an expert in a further sixteen (20.3%). A failure to meet the state or federal requirements for reliability was cited in fifty of the eighty-one cases (65.7%), mainly due to lack of an underpinning scientific foundation (twenty-seven cases), and inappropriate or unsupported witness conclusions (seventeen cases). Further analysis revealed that forensic odontology was the discipline most likely to be excluded due to reliability issues (100% of excluded odontology cases), followed by handwriting analysis (72%), fingerprint analysis (58.3%), and firearm and tool mark analysis (52.8%). The greater incidence of exclusion or limitation due to a lack of demonstrable reliability compared to other reasons suggests that there is a continuing need for the forensic sciences to pursue basic research validating their underlying theories and techniques of identification in order to ensure their continued acceptance by the courts.

Following a statistical analysis of these cases, those in which forensic science evidence was excluded were analyzed qualitatively in an attempt to discern patterns in judicial reasoning. The results reveal that exclusion of forensic science evidence is not simply based on superficial application of the *Daubert* indicia to the evidence in question. The use of unfounded statistics, a failure to address the reliability of the evidence as it relates to the case at bar, an inability to clearly explain the methodology behind analysis, and the failure to adhere to recognized standards have all been fatal to the admission of forensic science evidence in the United States since 1993. In addition, the existence of observer bias, unrealistic proficiency testing, a lack of objective standards, custom experiments and implausible error rates have also contributed to decisions to exclude fingerprint, firearm and tool mark, odontology and handwriting evidence. A reliance on general acceptance alone has also been cited by several cases as reasons for rejection. None of these reasons for exclusion can successfully be addressed by the legal community. It falls to the researchers and practitioners in forensic science to discern ways in which to overcome these shortcomings.

Forensic Science, *Daubert*, Law

## **E10 Bringing the Complex Crime Scene Into the Courtroom: Using Animation and Still Illustrations to Simplify Testimony in Criminal and Civil Litigation**

*Rod Englert, BS\*, Englert Forensic Consultants, PO Box 605, West Linn, OR 97068*

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After attending this presentation, attendees will understand the use of moving diagrams and still illustrations in the courtroom.

This presentation will impact the forensic science community by showing how a complex crime scene can be explained meaningfully to jurors through the use of demonstrative aids in the form of moving diagrams and still illustrations.

Drawings (illustrations) in cave dwellings date back to historic periods and have demonstrated many sequential events to help the observer understand the occurrence(s). These illustrations have provided us insight into the ancient world, the Renaissance period, and into the Twenty-First Century where illustrations have been animated beyond cartoonish figures into realistic people and places. The use of moving diagrams (animation) and illustrations (art) is not that common, but is becoming more so, and is highly dependent upon the accuracy of the event to be explained.

Demonstrative aides (moving diagrams/illustrative art) in jury trials are, by their very nature, a method to help juries understand what occurred during an event, be it criminal or civil.

Sometimes the event involves multiple parties or actions and necessitates more of an explanation of the facts beyond what photographs, physical evidence, charts or graphs can do. Development of animation and illustrations help the expert witness better explain the case to the triers of fact.

This presentation will show how to go about reconstruction of several officer-involved shootings and a homicide. Because of the complexity of the events, moving diagrams (animation) of each scene are digitally created representing what actually occurred. The re-creation is based upon facts from reports, photos, and detailed documentation from each scene. The accuracy of the animation/illustration is important to withstand the scrutiny of opposition motions to suppress.

The cases used as examples can be used in a variety of expert disciplines to simplify what may sound complex if only verbally presented from the witness stand. Some of the examples are in real time, accomplished by time clocks on the viewer's screen and with voice transmissions built into the diagram, such as radio calls to dispatchers. Examples involving airplane disasters, motor vehicle collisions, industry accidents, and miscellaneous cases will be shown where both illustrations and moving diagrams are used.

Some of the examples have animated figures built into the actual scene photographs of buildings, vehicles, blood patterns, and physical evidence at the scene. Some walk the viewer through a living body.

Often, moving diagrams (animation) are not as valuable a tool as an artist's rendition and illustration of the event. Illustrations also involve the development of stills based upon measurements and facts for the visual re-creation. For example, the wound path of a bullet through the body on its destructive path through vital organs could best be described in one or several artistic poses in still frames.

When to use animation and/or illustrations, along with the cost of producing the rendition will be discussed. Some renditions become very expensive.

Additional areas to be covered will be suppression motions that are often encountered from opposing counsel. Also discussed will be what happens when both sides produce moving diagrams, both quite different from each other. When two moving diagrams are presented, the one likely to survive the court's scrutiny will be the one most accurately portrayed. There can be no guesswork in the final product.

**Animated Scenes, Still Illustrations, Demonstrative Aids**

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## **E11 Casework Peer Review Evidence: Understanding What It Really Means and When Such Evidence Will Be Precluded**

*Andrew Sulner, JD\*, Forensic Document Examinations, LLC, 220 East 57th Street, Suite 200, New York, NY 10022*

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After attending this presentation, the attendees will learn about the different types of casework peer reviews that occur within the various forensic disciplines and the standards that define the meaning of each type of case review. Attendees will learn about the legal basis for excluding peer review testimony by the actual reviewer.

This presentation will impact the forensic science community by providing forensic scientists with examples of the problems and challenges they can face when testifying in court about peer reviews of their own casework. All stakeholders in the administration of criminal or civil justice – experts, lawyers, and judges – will benefit from learning about expert testimony, casework, and peer reviews; how it is disingenuous and misleading; and when courts will preclude such testimony.

Expert witnesses from various forensic disciplines often testify that their casework is subjected to peer review, without ever describing the particulars of the so-called "peer review." Such testimony, generally elicited during direct examination, invariably conveys the impression that the testifying expert's casework is regularly scrutinized and verified by another expert, and that some other, non-testifying expert independently examined the same evidence and reached the same conclusion as the testifying expert in the case at hand. Most lawyers seek to challenge such testimony by establishing that the casework peer review was not conducted anonymously and that the reviewer was a "friendly" colleague or co-worker, often working in the very same laboratory unit or office.

However, in most instances, expert testimony about casework peer review is misleading because the so-called "peer review" did not consist of a thorough and complete re-examination of the evidence, and often comprised little more than a "spell check." Lawyers and trial judges frequently assume that the mere mention of the words "peer review" equates to a comprehensive, independent verification of a given opinion or conclusion. This presentation will clearly establish the fallacy of that assumption.

This presentation will examine expert testimony from actual cases to demonstrate instances of self-serving, disingenuous testimony about peer review(s) of casework that arise in criminal and civil cases. Although this presentation focuses on expert testimony concerning peer review of casework of forensic document examiners, the information disclosed in this presentation applies to many other forensic disciplines.

The ASCLD-LAB standard for performing case reviews, as well as a proposed standard for conducting case reviews that was drafted by the Scientific Working Group on Documents (SWGDOC) and submitted for approval to ASTM, will be examined and analyzed. Attendees will become knowledgeable about the different types of "peer review" occurring within the forensic community and the true meaning of each type of case review. Attendees will also learn about the legal basis for excluding peer review testimony by the actual reviewer.

**Peer Review, Case Review, Disingenuous Testimony**

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## **E12 Selecting and Verifying Experts**

*Gil Sapir, JD\*, PO Box 6950, Chicago, IL 60680; and Mark G. Giangrande, JD, DePaul College of Law, Vincent G. Rinn Law Library, 25 East Jackson Boulevard, 5th Floor, Chicago, IL 60604*

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After attending this presentation, attendees will understand and recognize the use of available resources and strategies for locating and evaluating experts.

This presentation will impact the forensic science community by enabling interested persons to readily obtain background information and history of the expert witness.

Litigation periodically requires expert testimony to explain technical or scientific issues that are a substantive part of the case. Attorneys handling these types of cases will need to engage an expert or defend against one. The Supreme Court said in its *Daubert*<sup>1</sup> opinion that expert witness testimony is the most persuasive evidence. Experts, in some circumstances, are permitted to state opinions regarding the ultimate issue of case.

Expert testimony is regulated by Federal Rule Evidence 702 and state counterparts. Federal Rule of Civil Procedure 26 requires additional disclosures by attorneys regarding the experts they retain, including records of past contributions to literature, litigation performance, and other documentary evidence. The applications of these rules reveal information about experts to assist federal judges fulfill their gate keeping role as defined by *Daubert*. The same disclosures give attorneys a framework for discovery and litigation strategies.

There are numerous sources for locating expert witnesses. These include traditional print directories that list experts, commercial research services, such as Lexis and Westlaw, and various expert witness directory sites available through the Internet. Each source has different advantages and disadvantages. One factor that is common to all of them; however, is reputation.

Print directories from a reputable source, such as Martindale-Hubble, have a history and some editorial control over the listings. The disadvantage of print; however, is its information cannot be changed or updated until a new edition appears.

Lexis and Westlaw also perform some editorial control over expert listings in their database by licensing content from other commercial providers. The fact that both of these research services would license content from others, rather than create their own, implies that they would attach their high reputations for accuracy to these listings. Lexis, for example, licenses content from the Daubert Tracker, a respected Internet site for expert research.

Lexis and Westlaw have an additional research advantage through their leverage of extensive collections of case law and litigation documents, acting as a comprehensive source for research on individual experts. A researcher can search categorical material on expertise, locate resumes, access prior reports and other documents filed with courts. Additional analytical tools are available to evaluate an expert's performance. The disadvantage is that they require expensive subscriptions to access this content.

The internet has any number of expert directory sites. Many of them are businesses, which sell other legal services, such as continuing legal education materials. These sites often use expert directories as a draw to advertise other services to attorneys. As such, these sites tend to encourage individuals to list their services as a way of increasing the size of their roster, without offering any kind of oversight on qualifications. Too often attorneys only rely upon these listings or the person's resume to assess the expert's qualifications. Expert witness directories, or agencies, generally do not verify credentials and the background of the expert. Therefore, it is necessary for the reviewing attorney to conduct additional research using other information sources to confirm the listed expert's background and suitability. The selection and pretrial examination process must validate the expert witness' credentials, credibility, competency, and charisma.

This presentation will offer strategies for selecting and evaluating experts. It will cover available sources of experts, including the Internet, commercial research services, and other sources. It will detail ways to explore and corroborate the background of potential experts, taking into consideration additional points such as an expert's personality, communication skills and ethical obligations. It will also take into account how to locate materials that document an expert's record. An expert's viewpoints on a technical subject are often available in his or her scholarship. This type of literature is usually the more difficult type of material to assemble compared to litigation documents. The presentation

will offer strategies locating and acquiring copies of scholarship. The selection and verification of experts is integral to litigation and corresponding success or failure – it should not be minimized.

**Reference:**

<sup>1</sup> *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 595 (1993)

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**Experts, Credentials, Selection**

## **E13 The Breath Alcohol Test: A Continued Call for the Pretest Observation Period**

*Jay Zager, HS\*, Jay Zager Forensic Consultant, 10638 NW 69th Street, Parkland, FL 33076*

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After attending this presentation, attendees will understand the importance of pretest observation and deprivation period in forensic breath alcohol testing in driving under the influence (DUI) cases.

This presentation will impact the forensic science community by reinforcing the necessity of conducting a proper pretest observation period in breath alcohol DUI cases.

A pretest observation period is defined as a continual, uninterrupted period of 15 or 20 minutes during which a person suspected of DUI is observed by law enforcement personnel.<sup>1</sup> It is designed and intended to eliminate the possibility of introducing a contaminant into the breath sample before analysis.

Breath alcohol concentration (BrAC) testing is an indirect form of measuring the soluble gas exchange of alcohol between the blood and lungs relying on established partition ratios.<sup>2</sup> Alcohol is absorbed into smooth tissue between 10 to 12 minutes.<sup>3</sup> Therefore, a 15 to 20 minute uninterrupted deprivation and observation period is required to decrease possible contamination of the evidentiary BrAC test result.<sup>4</sup> Common sources of contamination include: chewing tobacco; smoking; vomit; mouth and breath fresheners; dentures; gum; wet belches; gastric reflux; regurgitation and residual alcohol in the throat; nasal cavity; and, mouth. If the pretest observation period is compromised, then the BrAC result is unreliable. "Although all aspects of the testing process are important in a Q.A. sense, the scientific safeguards are the most critical."<sup>5</sup> If the observation period is removed, then the test result's evidential or confirmatory attributes are compromised and reduced to those of a presumptive indication.

Contamination of the oral cavity prior to collection of breath alcohol specimens may cause false high or boosting BrAC results. Therefore, simple analytical safeguards are used to minimize sample collection contamination during the statutorily mandated pretest observation period. Law enforcement uses a portable breath alcohol test device (PBT) as a screening tool for roadside testing in DUI cases. It is commonly used as a screening device to inform law enforcement that alcohol like substances may, or may not, be in the test sample. A PBT test result is not a substitute for the pretest observation period or an evidentiary BrAC result. When the observation period is compromised, for any reason, reasonable doubt is created regarding the BrAC test result.

Law enforcement agencies frequently begin the observation period when the officer first comes into contact with the suspect and lasts until driving to a breath alcohol testing facility. Even though this may be a deprivation period, it is not a proper observation period. It is impossible for the officer to focus his attention during the entire time period on the DUI suspect. Any number of events, or acts, during this period may compromise the observation period and BrAC test result.

An improper pretest observation period creates a conflict between science, law and public policy. When the observation period has been compromised the government often endeavors to still justify the BrAC test result. Too often law enforcement, or the prosecution, attempts to minimize or abridge the pretest observation period by extolling the virtues of approved, technologically sophisticated, evidential breath testing equipment to minimize violations of the required observation period.<sup>6,7</sup>

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\* Presenting Author

There is a paradox between case law and forensic science. Legislators have changed *per se* legal intoxication levels downward from .15 to .10 to .08 g/210L, in an attempt to remove DUI violators. However, the basic laws of BrAC science testing, (e.g.) Henry's Law, Charles Law and Lambert-Beer's Law) are inviolate. Ergo, the paradox – public policy versus good science and equal justice.

Although modern evidential breath alcohol testing machines employ detectors to screen for potential interfering substances and mouth alcohol, they are not perfect. Violations of pretest observation period may cause false positive or boosting of BrAC results. The observation period was instituted to ensure these factors do not occur. Law enforcement must comply with all DUI procedures. Without the appropriate pre-test observation period and mouth alcohol detectors being effectively utilized, the reliability of BrAC results are suspect.

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**Alcohol, Observation Period, DUI/DWI**

## E14 Drug Recognition Experts are not Qualified to Testify as Experts and Should Only Testify as Fact Witnesses

*David M. Benjamin, PhD\*, 77 Florence Street, Suite 107N, Chestnut Hill, MA 02467-1918*

After attending this presentation, attendees will learn why Drug Recognition Experts (DREs) should testify only as fact witnesses and not as experts.

This presentation will impact the forensic science community by reviewing how the lack of scientific training and bias have allowed DREs to testify incorrectly about the effects of drugs in OUI-Drug cases.

**Background:** State prosecutors should be prosecuting meritorious cases and not oppressing or burdening citizens with cases that cannot be won in court. There is no way to meet your burden of proof in many cases, and large amounts of money and time are wasted in pursuit of “unwinnable cases.”

**Case Law:** The prevailing case law regarding OUI-Drug cases and the use of so-called Drug Recognition Experts (DREs) has stated that DRE examinations are not scientific and that they are well within the ability of a juror to understand (see *Williams v. State of Florida*, 710 So. 2d.24 (1998)). If they are not scientific, then the testimony DREs offer cannot come in under FRE 702 as “expert testimony” but only under 701 as testimony from a fact witness. Moreover, a Drug Recognition Expert (DRE) who never examined the defendant at the time of the alleged infraction cannot be smuggled into court as an expert witness and, accordingly, should never get to testify in the prosecution’s case.

DREs are employed as police officers and work in a para-military organizational structure. Their job descriptions state, “to support prosecution” not “to determine the truth.” They are inherently biased. Their testimony is intrinsically unreliable. Unlike the defense witness, the DRE cannot tell the state’s attorney that the data do not support the case because that would be insubordinate, and perhaps grounds for dismissal, despite the prospect for a subsequent civil suit for wrongful dismissal

**An Example of an Unwinnable OUI-Prescription Drug Case:** One such case involved defendant ED who was stopped for crossing a road line. When her toxicology report came back, it was found to have been positive for a small amount of butalbital, a barbiturate found in common anti-migraine medications.

Unfortunately, butalbital has a half-life of 1.5 – 3.5 days. Based on generally accepted pharmacokinetic principles, it takes 6-10 half-lives to rid the body of a drug. This means that she could have taken the drug more than a month before she was stopped and still had a positive urine test on the day she was stopped, even though its pharmacologic effects on migraine relief and impairment last only a few hours.

The commonwealth in that case, enlisted the assistance of a DRE who had not conducted an assessment of the defendant at the time of the police stop. The state planned to have the DRE testify at trial that the defendant had been impaired, an opinion that could not be supported by the urine test results, and one to which the DRE was not percipient, as she had never met or assessed the defendant.

It is important that defense attorneys educate themselves about the pharmacologic properties of butalbital, and to assist with the preparation of a motion to suppress the urine test result and either strike the DRE or significantly limit her proposed testimony.

On the day of the motion hearing, the commonwealth reconsidered its position and agreed to let the defendant off with probation.

To this day, it is not known why the defendant did not drive in an acceptable manner, but it is believed that it had nothing to do with her prior use of the migraine medication.

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**Drug Recognition Expert, Bias, Insufficient Scientific Training**

## E15 Understanding Latino Youth Gang Violence: From the Prison Yard to the Streets

*Cliff Akiyama, MA, MPH\*, Philadelphia College of Osteopathy Medicine, Department of Forensic Medicine, 4170 City Avenue, Philadelphia, PA 19131-1694*

After attending this presentation, attendees will be able to explain the organization of Latino prison and street gangs. Understand and interpret common graffiti and tattoos. Discuss some of the recent gang enhancement laws including gang injunctions that several states have in place to help tackle the gang problem.

This presentation will impact the forensic science community by informing and educating attendees about youth gangs. Particularly Latino youth gangs in order to keep safe while investigating the deaths of these youths.

One cannot open the newspaper or watch the local news without hearing of another casualty of gang violence. Throughout the country in urban, suburban, and rural communities gang violence has reached epidemic proportions according to the Department of Justice, Office of Juvenile Justice and Delinquency Prevention. Furthermore, gangs have been identified in every single state, meaning that gangs are no longer a California problem. Nationwide there are 24,500 gangs with a gang membership of over 750,000, while the ethnic composition of these gangs include 47% Latino, 31% African American, 13% Caucasian, 7% Asian, and 2% Mixed. In particular, youth gang violence in the Latino community has had a dramatic increase in the last two years. In Los Angeles County, California alone, there are currently 650 documented Latino gangs with a gang membership of over 83,000. Demographics show a gang member average age of 15 with a range of 8-22 years. Other counties in California

and nationwide have seen increases in Latino gangs, especially in rural communities. The Mara Salvatrucha (MS 13) an El Salvadorian gang originating in the Pico-Union area of Los Angeles, California since 1983, has seen the largest increase in membership nationwide with over 60% in some states. Over 400 gang members out in the streets, jails, and juvenile halls in California and Pennsylvania were interviewed. This study identified eight distinct manifestations of gang violence and nine ethnic differences and similarities among Latino gangs i.e., definite cultural differences between Latino gangs and various other ethnic gangs; drugs; weaponry; killing over turf/territory; extortion; defacing property/graffiti; and, women in gangs).

This study found that Latino gangs are motivated by a state of mind driven by “La Raza” which translated means “for the race.” It is important to note that La Raza is more of a cultural ideology than a gang related motto. Latino gangs are extremely territorial and unlike African American gangs where the individual is important, for Latino gangs it is the gang as a whole that is important and not the individual. To illustrate this point, when Latino gang members go to prison, they are run and controlled by one of two prison gangs depending on the geographic location of the prison: La Eme or Nuestra Familia. La Eme otherwise known as the Mexican Mafia is a prison gang originating in California and considered the leadership arm of all Latino gangs in Southern California. The letter “M” in Spanish is pronounced “eme” and is the 13<sup>th</sup> letter of the alphabet. Consequently, throughout Southern California, Latino gangs will often call themselves by the City or area that they represent, followed by the number “13” to indicate “La Eme” or “Southern” by giving respect to the Mexican Mafia. In Northern California, the Nuestra Familia which is translated to mean “our family” is the prison gang that controls every Latino gang north of Fresno, California and is often indicated by the number “14” representing the letter “N” for Nuestra and Northern. It is important to remember that Latino gangs are not just a California problem, although California is where these gangs originated. Rural, suburban, and urban communities across the nation are now seeing an increase in Latino gangs which mimic the California based Latino gangs, such as the MS 13.

In direct response to the increase in gangs, the state of California, North Carolina, Virginia, and Idaho have passed gang enhancement laws, which increase the penalty if an individual is found to have committed for the benefit, association, or direction of a criminal street gang a felony. Other states including California have also filed a civil injunction or “gang injunction” against specific gangs, whereby removing the leadership of the gang and disbanding its members.

The purpose of this paper is to present timely data on Latino youth gangs; offer strategies on how to recognize and interpret various tattoos and graffiti associated with these gangs, which could assist the medical examiner/coroner and death investigator in the positive identification of the decedent out in the field and/or in the autopsy room. This paper will also discuss some of the recent gang enhancement laws including civil injunctions that California, North Carolina, Virginia, and Idaho have in place to help tackle this deadly problem.

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**Youth Gangs, Gang Enhancement Laws, Tattoos**

## **E16 Biological Evidence Storage and Disposition: A Discussion of Legal Implications and Trends**

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After attending this presentation, attendees will learn about the legal considerations and trends regarding biological evidence storage. Further, this presentation will provide an overview of Technical Working Group on the Preservation of Biological Evidence activities to date and preliminary recommendations.

This presentation will impact the forensic science community by bringing awareness to legal and policy issues in evidence storage and the solutions being proposed by the working group. Recent headlines have highlighted significant problems with the storage of potentially exculpatory biological evidence in property and evidence storage units across the country. Court orders for the location of evidence have demonstrated inadequacies in the packaging, storage, and tracking process of some evidence. Investigations into these inadequacies reveal underlying factors such as: capacity of the storage facility; laboratory backlog; materials available for packaging; geographic distance between the collecting and storage facility; and, the selected tracking system. While preserving and readily retrieving biological evidence from adjudicated and unsolved cases is a goal and has clear benefits for all members of the criminal justice system, the management of retaining and eventually disposing of biological evidence requires that each state and jurisdiction consider the relevant legal and policy issues.

In August of 2011, the National Institute of Justice (NIJ) and the National Institute of Standards and Technology’s Law Enforcement Standards Office (OLES) convened the first meeting of the *Technical Working Group on the Preservation of Biological Evidence Preservation*. The primary objective of the working group is to establish best practices, based in science, to reduce the premature destruction and degradation of biological evidence, thus ensuring its availability for future analysis. A key dimension to the work of the group is the legal and legislative landscape of biological evidence retention. The purpose of this presentation is bring awareness to these issues and introduce the preliminary recommendations being proposed by the working group.

Most states have laws that provide guidance for the evidence disposition process but these laws vary widely. This process may include getting a court order, district attorney approval, notification of the law enforcement agency, or notification of the defendant/defense attorney or attorneys of record. Recent Supreme Court decisions including *Melendez-Diaz v. Massachusetts* and more recently *Bullcoming v. New Mexico* set out the importance chain of custody documentation and the importance of evaluating the integrity of evidence and the circumstances of testing. Creating the appropriate sanctions for evidence destroyed in violation of relevant policy and ensuring remedies for the wrongly convicted are also issues under consideration by the working group based on its analyses.

The group’s key deliverables will include a report on legislative considerations, a handbook outlining best practices and standardized protocols for property and evidence clerks, a report discussing current technological trends and possible applications, and a web-based clearinghouse for biological evidence handlers in the property rooms, courts, and law enforcement agencies.

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**DNA, Evidence, Storage**

## **E17 State Crime Laboratories — Open or Closed to Criminal Defense Attorneys?**

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After attending this presentation, attendees will begin to appreciate the conflict between science-related decisions and policy-related administrative decisions made by a crime laboratory as to its desire to be transparent in its process and its data reporting.

This presentation will impact the forensic science community at large by providing a starting point for discussing and focusing the debate between those who are in favor of transparency and those who are not. The impact to the forensic science community is to provide data to sharpen and highlight this debate. To date, this is the first formal and systemic experiment that tests this apparent divergence of thoughts.

Interaction at previous AAFS meetings between criminal defense attorneys and forensic scientists employed by various state crime laboratories has led to collegial interactions that have suggested criminal defense attorneys would be welcome to visit such laboratories. Such visits would include interactions with the laboratory administration, bench analysts performing the forensic assays, photographs of instruments, examining procedures, and observing actual forensic specimens being processed.

The present study was designed to test the following hypothesis: State crime laboratories will permit criminal defense attorneys to visit individual laboratories, observe forensic specimen analyses, photograph pertinent laboratory equipment, have interactive sessions with employed forensic scientists, and access to standard operating procedures.

For evaluation of the hypothesis, a standardized letter was sent to heads of crime laboratories in all 50 states plus the District of Columbia. The letter identified requesters as criminal defense attorneys who practice DUI defense. The letter asked the following:

- Will you grant us a tour of your laboratory?
- Will you allow us to take pictures of your laboratory?
- Will you give to us a current uncontrolled copy of your policy, procedures and instructions used for blood ethanol analysis?
- Will you give us evidence of the validation of your assay and method? If not, why not?
- Will you grant us an interview (no more than 30 minutes) with your most proficient analyst who routinely performs blood alcohol analysis?

As in any questionnaire research, less than 100% response was observed. Findings suggest that there exists a gap between what bench forensic scientist perceive as openness to criminal defense attorneys and what the laboratory administrators are willing to permit.

Data collected included:

- Date the letter was sent;
- Date the letter was received (tracked by certified letter, return receipt requested with restricted delivery);
- The date of the response (if any);
- The nature of the response.

The follow through with any commitments the laboratory made as to the requests of one through five above.

The substantive responses to the above five requests and the actual fulfillment of any commitments that were made related to the five requests above were compiled. This data was collected and analyzed for the edification of the forensic science and legal defense communities and to enhance their spirit of cooperation. A fact-based, data-driven presentation that outlines the findings of the above will be presented. The underlying raw data will be available for attendees to review.

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**Transparency of Crime Laboratories, Administrative Control of Crime Laboratories, NAS Recommendation #4**

## **E18 The Design and Analysis of Calibration Experiments and the Reporting of Prediction Errors**

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After attending this presentation, attendees gain an appreciation of the design and analysis of calibration experiments and how to construct an appropriate prediction interval of the result.

This presentation will impact the forensic science community by serving as an introduction to the importance of expressing not just a number, but an interval to explain the uncertainty in the experimental work and setting appropriately derived quality standards rather than choosing an arbitrary percentage agreement.

The design of calibration experiments is critical in deriving an estimate of the prediction error in reporting a drug analysis result. The reporting of only a single number gives the impression that the result has a negligible error when in fact the error is dependent on the design of the calibration experiment and the number of predictions to be made from the calibration curve. A high R-squared from the regression of the calibration curve is often misinterpreted as showing linearity and a correct model though neither is correct. Neither does a high R-squared imply that the regression model will be an accurate predictor. It is often forgotten that randomization is the basis for many statistical tests and this is also true with the design of a calibration experiment. Failure to randomize will confound instrument drift and other problems that may arise in the analysis of samples limiting the conclusions that can be drawn from the experiment. An analysis of the residuals is important since this can highlight problems such as instrument drift and outlying and influential points. Usually the instrument software will allow the calculation of the calibration curve and then use that information to print out the associated values from the samples. One should resist this since just because the software lets you do it, doesn't mean that it should be done. A thorough analysis of the regression equation should always be done and this means using appropriate statistical software and evaluating coefficients from a statistical standpoint and examining various residual plots. The calibration experiment is a model and thus an appropriate statistical analysis should be done to verify the model. Furthermore, since this experiment will be repeated at regular intervals, a control chart of the results should be produced and updated after every run and this will provide further insights into the quality of the work and the predictions.

Predicting the appropriate result is more complicated than doing a simple linear regression and allowing the software to report a number. The prediction error on the result depends upon the number of standards in the calibration curve and the number of samples being tested. Running samples in duplicate may or may not provide additional information since it depends upon how the samples were prepared in the first place. Splitting the sample into two gives the error of the machine, not the error on the sample. A complete analysis must have a well delineated standard operating procedure that clearly explains every step of sample preparation and analysis. The standard operating procedure should also define the mathematical and statistical model being tested with the appropriately validated statistical software to be used. Some analyzed examples will be presented using alcohol and ecstasy (MDMA) to show both what to do and what not to do.

**Calibration, Prediction Error, Experimental Design**

## **E19 ARMD and Dangerous: Adverse Reaction to Metal Debris, Metal on Metal Joint Replacements, and Present Toxicological and Legal Challenges**

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After attending this presentation, attendees will learn about a newly identified complication of joint replacement surgery currently identified by a number of different and confusing names. The information gap on the toxicology of low levels of heavy metals will have short term and long term consequences for clinical medicine, public health, medical device innovation, and regulatory policy.

This presentation will impact the forensic science community making attendees aware of data that is currently lacking on the biological effects of long term low level heavy metal concentrations in the body. Legal, political, public health, and regulatory debates are filling this vacuum with polemics and speculation. It is recommended that attorneys in this medico-legal debate be mindful of the potential harm yet recognize the limits of

current knowledge. Clinicians and researchers should seize upon this opportunity to advance the state of knowledge and fill this vacuum.

**Hypothesis or Proposition:** Shedding of metal microparticulates from so-called metal-on-metal joint prostheses is a clinical phenomenon with potentially toxic consequences. Toxicologists must identify the biomedical significance of elevated levels of metals used in joint replacement surgery. Inconsistent naming conventions for the phenomenon are hampering clinical assessment of its import to patients, physicians, and regulators.

**Synopsis of Content:** Medical science has been experimenting with suitable materials for human joint replacement since 1891 when a German doctor tried to substitute ivory for the head of the femur in a hip joint. Since then, various mundane and ultra sophisticated materials have been tried and tested with varying degrees of success. Use of cobalt-chrome surfaces to replace both the hip and femoral sides of the ball and socket hip joint, so-called “metal-on-metal (MoM)”, began in the 1950’s and continues today with the application of harder alloys and precision surfacing equipment.

Yet friction forces that degrade the metal surfaces are inevitable. The tribology of friction, wear, and lubrication in these anatomical bearing surfaces dictate the toxicological potential of the bearing materials. Micro particulates and metallic ions migrate from the immediate joint space throughout the body and across the placental barrier. Blood and serum levels of cobalt and chromium that equal or exceed EPA and OSHA permissible exposure limits (PELs) are increasing in frequency. Certain devices using the MoM design are reported with higher than expected failure rates due to component loosening.

Toxicological screening now measures heavy metal levels in the low parts per billion ranges. The pathological significance of these findings is uncertain, but they have become an indication for costly and risky revision surgery in the absence of other signs or symptoms of prosthetic failure.

Local tissue toxic reactions are identifiable by gross visual inspection and characterized by frank tissue necrosis and cellular organization into pseudotumors. Various names are used to link their pathological features to their identified cause: metallosis, adverse reaction to metal debris (ARMD), arthroplasty-related metal disease (ARMD), periprosthetic cobaltism, aseptic lymphocytic vasculitis- associated lesions (ALVAL), and others. No uniform set of diagnostic criteria has yet been devised.

The actual number of U.S. patients that have received or will have joint replacements with metal-on-metal joint couples is unknown. Medicolegal concerns about alleged design defects and inadequate warnings to patients and physicians have discouraged but not eliminated use of the metal-on-metal design. Public health policies recommending medical monitoring for affected patients will be valued in the millions of dollars.

Diverging, if not conflicting, regulatory pronouncements by the FDA and its European counterparts leave device manufacturers and their customers at a loss to decide what is or is not safe and effective. Political debate over a stricter or more lenient regulatory policy governing device approval reflects the contest between the free market and the public health. Litigation, rather than science, is now driving research into the toxicology of metallic ions widely disseminated throughout the human body.

**General Statement of Conclusion:** The science of toxicology needs to re-capture the initiative on the biological consequences of a body burden of metallic ions and microparticulates. Pending litigation that is seeking damages for short term and long term medical monitoring is based in part on extrapolation from existing industrial exposure investigations. Defining what are safe and unsafe heavy metal levels have tremendous economic and medical consequences for individual patients, health care providers, medical device innovators third party benefit payors, and litigants.

#### **Metal-on-Metal, Joint Replacement, Cobalt Poisoning**

## **E20 No Hit Left Behind: Getting the Most Out of CODIS**

*Melissa Mourges, JD\*, and Martha Bashford, JD\*, District Attorney’s Office, New York County, One Hogan Place, New York, NY 10013; and Noelle J. Umbach, PhD\*, Office of the Chief Medical Examiner, Department of Forensic Biology, 421 East 26th Street, New York, NY 10016*

After attending this presentation, attendees will understand how use of a NYC-developed email system for the distribution of information about combined DNA index system (CODIS) hits can prevent match information from falling through the cracks.

This presentation will impact the forensic science community by illustrating deficiencies in most notification systems, where only police agencies get notified of a CODIS match, and where many DNA matches are not acted upon.

Many CODIS matches occur months or even years after the crime scene samples are uploaded into the forensic index. Many public labs routinely send notifications of these matches back to the police officer or detective who originally submitted the evidence. Unfortunately, detectives retire or get transferred, and often the notification sits in a fax machine, tossed into the “circular file” or thrown unread into the original case folder, never to be seen again. Many matches are therefore not acted upon. This creates several serious problems. One is that perpetrators who should be charged with crimes remain free to commit new ones. Second, some CODIS matches serve to exonerate the convicted. Third, in some states, legislators who learn that hits remain uninvestigated are reluctant to budget money for DNA labs or to expand DNA databanks.

To battle this problem, the mayor’s Criminal Justice Coordinator in New York City decided to develop a notification system that would accomplish several tasks: notify police and prosecutors simultaneously of DNA matches; eliminate the use of fax machines that could malfunction; and create a system where all stakeholders could view and update information. The result is “DNA Hits”, an email system that notifies police and prosecutors simultaneously of DNA matches that occur in the local, state, and national CODIS database. The system, which went online in 2006, also has a “create reports” function to make statistics readily accessible. This is vital in tracking outcomes for policymakers and for grant purposes.

Observe a “real time” notification, which comes in via email to participants’ smart phones. The actual information about the match is on a secure database where police agencies, prosecutors, and the OCME all have access. On the opening screen, the database provides the following information: lab case number, convicted offender swab ID number, victim’s name, date of crime, police report and precinct number, evidence type, perpetrator name, criminal ID number, and whether he was a named suspect at the time of testing. Any particular “hit” can be opened to reveal the name of the criminalist, contact information in the case of out of state hits, and whatever additional updates prosecutors or police may add. Simultaneous notifications greatly reduce the chance that a DNA match will fall through the cracks and ensures public safety.

#### **CODIS, Matches, Simultaneous Notification**

## **E21 Uncertainty Analysis in Forensic Practice: How to Apply It Wherever Scientific Integrity Demands Its Use**

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The goal of this presentation is to educate forensic practitioners who have not yet introduced uncertainty analysis into their work and to hone the skills of those who have.

This presentation will impact the forensic science community by providing a general understanding of uncertainty/error analysis, enhancing their ability to quantitatively assess the reliability of forensic evidence, and to knowledgeably demand such assessments in evidence presented by others.

“In science, the word *error* does not carry the usual connotations of the terms *mistake* or *blunder*. Error in a scientific measurement means the inevitable uncertainty that attends all measurements. As such, errors are not mistakes; you cannot eliminate them by being very careful. The best you can hope to do is to ensure that errors are as small as reasonably possible and to have a reliable estimate of how large they are.”<sup>1</sup>

Those in the general scientific and engineering communities are astonished when they hear some forensic practitioners claim: “I don’t know what error analysis is; I cannot find its definition” or “There are so *many* definitions of error analysis, it cannot have any significance” or “There is no need to use numbers in describing reliability.” The fact is that the presentation of the results of a key measurement without a quantitative characterization of its uncertainty is non-scientific, that is to say, meaningless.<sup>2</sup> Now that the courts are increasingly recognizing this truth,<sup>3</sup> all forensic and legal practitioners need to develop a facility understanding of and a facility with uncertainty/error analysis—especially in the wake of the 2009 National Academy of Sciences Report on forensic science. The effort here is directed at facilitating that development, starting and ending with the defusing of a number of mistaken concerns about applying uncertainty/error analysis in the forensic context.

The existence of varying definitions for uncertainty/error is to be expected. As with most important concepts, scientific and otherwise, the definition of uncertainty/error varies with the context in which it is applied. Indeed, different definitions can be used within the same context, as will be described below. The important point is not this variety but rather that one be aware of the specific definition being used at a particular time. A statement of the uncertainty/error in one’s results is incomplete without a statement of the method you used for estimating the uncertainty/error.

One often hears concern that once error/uncertainty analysis is universally required in the forensic setting, the practitioner will have to carry it out for every measurement made, no matter how mundane or preliminary. This concern is misplaced, as the results requiring a statement of uncertainty/error are those going to the determinations to be made by a trier-of-fact. Those results can be divided into two broad categories: quantitative and qualitative.

- Quantitative results include such things as the blood-alcohol concentration (BAC), the speed of a car involved in a crash, the time elapsed between time of death and the discovery of a corpse, etc.;
- Qualitative results include such things as the match/no-match determinations encountered in signature, fingerprint and DNA analysis.

Uncertainty/error analysis in the first category is the more amenable to short definitions; however, even here one must take care to ensure that the meaning of a statement is understood. For example, what does the statement “the defendant’s BAC was measured to be 0.12±0.02 g/100ml” mean? When dealing with the statement of uncertainty/error, that is, the number following the ±, the wise person will withhold judgment until its definition is revealed. Is this a maximum range outside of which one would not expect the “true” value to fall? Or does it refer to a more rigorously determined range within which the “true” value is expected to fall with a given probability? The answers to these questions will lead to the most important question of all: How was the uncertainty/error determined? Forensic scientists and attorneys must know and understand these issues in order for the results of forensic measurements to be competently presented to the parties to litigation and the ultimate triers of fact.

In the DUI/OUI field, it is common to be presented with a result such as 0.12 g/100ml, with no characterization at all of the number’s uncertainty/error. This is an indefensible practice from a scientific perspective, which should be the forensic perspective. The party adverse to the one presenting it is on firm ground in making a motion *in limine* to

exclude any statement about this number until its reliability has been revealed through a statement of uncertainty/error.

The determination of the uncertainty/error of a BAC result is relatively simple compared to doing so for the speed of a car involved in a crash, often a key number in criminal prosecutions for vehicular homicide. In general, the ultimate number testified to involves a several independent measurements, each of which carries a its own range of uncertainty/error. Once one has all the individual values for uncertainty/error, there are a number of methods for combining those values so as to determine the uncertainty/error of the final result. The most comprehensive and appealing, but one which most attorneys shy away from identifying by name in court, is the Monte Carlo approach. We will discuss this method as well as two simpler ones, one of which is acceptable and one of which should be attacked when it is introduced. Fortunately or unfortunately, it is the latter that seems to come up most often in court.

When dealing with qualitative tests, such as signature, fingerprint or DNA analysis, the result itself is often of the nature match/no-match, with the ultimate conclusion of whether this implicates or excludes a particular suspect or defendant left to the trier of fact. When the *Daubert* court and its progeny wrote of “reliability” of a forensic result, it was referring to the likelihood that the result was what it purported to be. Thus, reliability of a qualitative statement is usually quantified by stating the probability that it is correct. Conversely, it can be stated in terms of the probability (which should be low) that the result shows a match where it should not (“false positive”) or a non-match where it should show a match (“false negative”). How are such probabilities to be determined? To what do they relate? Should one distinguish between the uncertainty/error of the result, that is, the match/no-match, and the ultimate conclusion of association?

Pattern evidence, such as expert testimony that a fatal wound was made by a four-inch blade with a broken-off tip just like the blade found in the defendant’s possession, is also to be considered. This evidence is proffered for its indirect role in identifying a perpetrator. The probability of a false positive in this instance necessarily requires at least two questions to be answered. What is the probability that the weapon’s characterization is correct? If it is correct, what is the probability that the wound was made by the defendant’s knife?

Finally, if the fact is considered that uncertainty/error analysis is never absolute and relies, to a large extent, on judgment and the use of prior measurements and information not necessarily obtained during the course of the forensic test in question. The fact is that one never has complete knowledge concerning anything, including the probability that a particular result deviates from physical reality. Contrary to arguments seeking to block legitimate attempts to characterize uncertainty/error, this state of affairs is precisely what requires such efforts. Rational inferences can be made only when our incomplete knowledge/information is adequately understood and characterized. To see this, reflect on the fact that to one assessing the survey of a particular parcel of land, it is useful to know whether Surveyor A or Surveyor B did the work, in light of the knowledge that Surveyor A has a record of doing very good work and Surveyor B a record of not-so-good work. Even though this knowledge of past events does not tell us with certainty that new work by A will be good or that new work by B will be poor, there are few people who would use that as an argument for not taking account of who did the surveying in question. Indeed, no rational person would do so.

#### References:

- <sup>1</sup> An introduction to Error Analysis; The Study of Uncertainties in Physical Measurements, 2d ed., J.R. Taylor, University Science Books, 1997, p.3.
- <sup>2</sup> See, for example, Chapter 4, “The Principles of Science and Interpreting Scientific Data,” in Strengthening Forensic Science in the United States: A Path Forward, National Academies Press, Washington, D.C., 2009.
- <sup>3</sup> See, for example, State v. Fausto, No. C076949, Order Suppressing Defendants Breath Alcohol Measurements Under in the Absence of a Measurement for Uncertainty (King Co. Dist. Ct. WA 9/20/10),

City of Kent v. McDaniel, No. K81862 Order Suppressing Defendants Breath Alcohol Measurements in the Absence of a Measurement for Uncertainty (Kent Muni. Ct. WA 5/4/11), People v. Jabrocki, No. 08-5461-FD (79th Dist. Ct. Mason Co. MI 5/6/11)

### **Error, Uncertainty, Measurement Reliability**

## **E22 Global Gatekeeping?: A Comparative Analysis of the Judicial Process for Determining the Reliability of Proffered Expert Testimony**

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After attending this presentation, attendees will have an international perspective on the admissibility of scientific evidence in courtrooms across the globe and will be able to compare and contrast the gatekeeping standards of their own jurisdictions with those in several other countries. Attendees will learn what factors judges in other jurisdictions consider when admitting expert scientific evidence.

This presentation will impact the forensic science community by showing how similar and dissimilar various jurisdictions are across the globe in admitting expert scientific evidence. Members of the forensic community across the globe will learn various types and kinds of factors which can be considered in evaluating whether scientific evidence should be admitted in their courts. The various forensic communities across the globe can learn from each other regarding how to better evaluate expert scientific evidence in order to have the most reliable and relevant scientific evidence enter our courtrooms regardless of the geographic location of the court and science. Science and Law should intersect at the same location or venue regardless of where or who is evaluating the expert scientific evidence in order to render justice—at the truth where reliable and relevant scientific evidence exist.

The American judicial system has developed specific processes to regulate the admissibility of forensic science evidence and has focused on the role of the trial judge as the gatekeeper for such evidence. It is useful to compare those processes and that trial judge role, with the approach of the judicial systems of other nations. This presentation presents some of that comparison.

Initially, this presentation describes the United States gatekeeping process as a base for comparison. The 1923 standard announced in *Frye v. United States*, 54 App. D.C. 46, 293 F. 1013 (1923), established a requirement that proffered scientific evidence must have received “general acceptance in the particular field in which it belongs.” Some version of *Frye* is still the applicable law in several states. In 1993, the Supreme Court established the trial judge as the gatekeeper for scientific evidence and announced new tests for admissibility in *Daubert v. Merrell Dow Pharm., Inc.*, 509 U.S. 579 (1993). In addition to “general acceptance,” *Daubert* requires judges to assess: (1) whether the underlying methodology is scientifically valid; (2) whether it can be and has been tested; (3) whether it has been subjected to peer review and publication; and, (4) whether there is a known or potential error rate. The *Daubert* standards apply to all federal courts and most State courts.

Recently, the role of forensic science has received considerable criticism. The National Research Council Report found serious deficiencies and called for major reforms. Post-conviction DNA testing has exonerated a number of persons who were convicted based on forensic science evidence. This debate over the reliability of forensic science evidence is also a currently active debate in a number of other countries.

In Canada, scientific evidence is also a topic of considerable concern. The Canadian system mostly parallels the U.S. process. While expert

testimony is treated as a part of opinion evidence generally, Canadian courts have recognized the “gatekeeping” role of the trial judge. *R. v. Mohan* [1994] 2 S.C.R. 9. They have required evidentiary hearings that are similar to, and perhaps even more demanding, than a typical *Daubert* hearing. The Canadian evidentiary requirements of relevance and balanced probativeness are also similar. The reliability of proffered testimony appears to be decided on factors similar, but not identical, to *Daubert* tests. It appears however that the Canadian courts have, much like the United States, applied those standards less stringently to the prosecution than to the defense.

In England and Wales, the focus was traditionally on the experts rather than the science, although there is a stated requirement that the field of expertise must at least be “sufficiently well established to pass the ordinary tests of relevance and reliability;” *Dallagher* [2002] EWCA Crim 1903, [2003] 1 Cr App R 12 at [29]. Recently, highly publicized and the proposed closure of major forensic science facilities have spawned a movement to a system more resembling *Daubert*. The Law Commission, a government advisory organization, reported this year (2011) recommended statutory action that would require criteria similar to FRE 702, notably omitting however an analysis of error rate. The government appointed “Forensic Science Regulator” and the Forensic Science Society are also working toward the establishment of criteria for analytic procedures, testing and accreditation within the various disciplines.

In Australia expert testimony is regarded as a part of opinion evidence generally. The admission of opinion testimony is controlled by statutory rule, s79(1) of the *Evidence Act* 1995 (NSW). That rule has language strikingly similar to the United States rule and provides: “If a person has specialised knowledge based on the person’s training, study or experience, the opinion rule does not apply to evidence of an opinion of that person that is wholly or substantially based on that knowledge.” The expert must demonstrate to the satisfaction of the Court how the proffered opinion is based upon the training, study or experience and that such training, study, or experience permits the witness to provide an expert opinion (*Dasreef Pty Ltd. v. Hawchar* [2011] HCA 21).

### **Evidence, Admissibility, International Standards**

## **E23 Australian Bloodstain Pattern Analysis (BPA) Training and Education: A Response to Judicial Scrutiny**

*Mark E. Reynolds, PhD\*, Western Australia Police, 2 Clayton Street, Midland, Perth, Western Australia 6152, AUSTRALIA*

After attending this presentation, attendees will understand the Australian education and training levels in place for forensic investigators of major crime who perform bloodstain pattern analysis (BPA) as part of their crime scene examination duties. The rationale and drivers for the training and education package’s development will be explained as will the implementation process and the likely future educational direction for Australian BPA practitioners.

This presentation will impact the forensic science community by describing a robust and sustainable education and training model for BPA practitioners, the components of which lend themselves to generic adoption and implementation by most forensic disciplines.

Recent judicial rulings in Australia have clearly articulated the need for robust scientific education and training for those involved in the forensic investigation of major crime. In a system where detectives and pathologists are invited guests to a crime scene controlled by forensic investigators (usually sworn police officers), the need for elevated practitioner training and education standards has never been greater. Further, typically many of the pattern recognition disciplines have been the purvey of ‘police officers’ who culturally have had little pressure to justify any opinions or conclusions they have derived.

In 2006, under the umbrella of its National Institute of Forensic Science, Australia embarked on a three year journey that resulted in the development of four (4) levels of BPA training and education for its forensic investigators of major crime. Foundational to those levels are: demonstrated competence, continuing proficiency, discipline and practitioner succession planning, sustainability and most importantly, a strong scientific basis. As a reconstructive “crime event” tool, the forensic discipline of BPA necessitates a contextual and holistic investigative approach and as such introduces levels of complexity over and above a number of other forensic disciplines. Subsequently, practitioners require high levels of educational and experiential exposure in order to derive the maximum, but more importantly, reliable results from the discipline.

In late 2009, the following four (4) levels of training and education were universally adopted by law enforcement jurisdictions across Australia:

- BPA Awareness: An introductory training level for all forensic investigators of major crime
- BPA Level II: Equivalent to the Basic 40 Course designed to provide a scene technician level of competence but prevent the provision of expert opinion “reconstructive” evidence
- BPA Level III: An advanced level of training designed for the provision of expert opinion “reconstructive” evidence
- BPA Level IV: LIII Mentor and Senior Instructor

The advanced levels of training (Level III and IV) are based on a curriculum including exhaustive scene analysis and case work exposure, laboratory focused exhibit examination particularly surrounding fabrics and other textiles, hypothesis testing and reconstructive considerations and in a vision for the future, the inclusion of a fluid dynamics curriculum. The adoption of these levels is already assisting to crystallise the judicial acceptance of the forensic discipline of BPA and its practitioners within Australia. Furthermore, the levels have been suggested as a demonstrative model that could be adopted by a number of forensic disciplines such as tyre and shoe outsole identification and forensic investigation of firearm related matters.

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#### **BPA, Education, Training**

## **E24 Virtopsy®: Its Associated Legal Parameters and Impact**

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After attending this presentation, attendees will receive an overview about the legal implications of Virtopsy® and postmortem computed tomography (pmCT) or magnetic resonance imaging (pmMR) in Australian, Swiss, and U.S. American legislation.

This presentation will impact the forensic science community by emphasizing the necessity to legally qualify that state-of-the-art procedure in forensic medicine and serving as its (worldwide) legal approach.

The Virtopsy® project was launched at the Institute of Forensic Medicine in Bern, Switzerland, more than ten years ago with the goal of replacing or supplementing traditional forensic autopsy examination techniques with a multi-modality approach comprising three dimensional photogrammetry-based optical body surface scanning (3D surface scan), pmCT, pmMR, pmCT-guided angiography, and targeted tissue sampling by pmCT-guided biopsy. Virtopsy® is used to document cases of sudden or unexpected death of (unknown cause) or unnatural deaths (homicides, suicides, accidents, medical malpractice) and identification of bodies. Since that time pmCT scanning (and in some centers pmMR) has been incrementally introduced in many forensic death investigation facilities all

over the world (e.g., Australia, Denmark, Germany, Japan, United States) and now assumes an important role in case management protocols. Both Virtopsy® and pmCT (or pmMR) scanning allow more sophisticated approaches to death investigation and in many cases may obviate the need for autopsy. Advantages of these imaging technologies include: the ability in some circumstances to determine a cause of death without dissection, visualization of body areas not easily examined at autopsy (e.g., pelvis and base of skull), safer examination of contaminated or infected bodies (e.g., tuberculosis), transmissibility of data for (second) opinions, a permanent digital record of the state of a body at the time of presentation, and 3D pictorial demonstrations of complex pathological processes for evidentiary/court purposes. CT, MRI, and 3D surface scan have also found application in the forensic medical setting of several institutes where they have been used to record and analyze injury patterns, examine areas of trauma (e.g., to the neck in cases of attempted strangulation) and digitally record crime scenes.

The introduction of novel imaging/diagnostic techniques into the time-honored and arguably conservative legal processes associated with forensic death investigation may have interesting and unanticipated consequences although literature on this particular area is sparse. Specific legislative provisions are also uncommon. While traditional court processes such as coronial hearings and criminal prosecutions are increasingly utilizing imaging data derived from Virtopsy® or similar technologies, in the English or German speaking world, no court decrees or published rulings relating specifically to forensic imaging have emerged to date.

This presentation will consider legal issues relating to the current practice of Virtopsy® and pmCT (or pmMR) in a comparative study of three jurisdictions (Australia, Switzerland, and United States), with specific reference to statutory interpretation in different fields of law including criminal procedure and coronial legislation. In conclusion, it will examine whether this new technology is satisfactorily accommodated by current law or whether amendments might be necessary. Thus, the legal issue whether Virtopsy® and pmCT/MR can be qualified as an inspection (external examination) – to triage if an autopsy should be done – or as an autopsy (adjunct) is an important part of that legal analysis.

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#### **Virtopsy®, Legal Bases, Australian, Swiss and U.S.-American Legislation**

## **E25 Honor Killings in Turkey: Facts and Figures**

*Itir Erkan, MSc\*, Yeni Yuzyil University, Faculty of Health Sciences, Cevizlibag, Topkapi, Istanbul, TURKEY; Raket Rozant, MA, Maltepe University, Institute of Social Sciences, Psychology Program, Istanbul, TURKEY; E. Hulya Yukseloglu, PhD, Istanbul University, Institute of Forensic Sciences, Cerrahpasa, Istanbul, TURKEY; S. Sebnem Ozcan, PhD, and Gavril Petridis, PhD, Yeni Yuzyil University, Faculty of Health Sciences, Cevizlibag, Topkapi, Istanbul, TURKEY; and Ersi Abaci-Kalfoglou, PhD, Yeni Yuzyil University, Faculty of Health Sciences, Yilanliayazma Cd., No 26 Cevizlibag, Istanbul, TURKEY*

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After attending this presentation, attendees will be informed about the idea of honor killings and the way it is covered by the Turkish Law.

This presentation will impact the forensic science community by alerting attendees to this cultural phenomenon.

Many countries all over the world are experiencing honor killings for different reasons. Depending on the culture, the level of education, the environment, the living conditions, the written or simply verbal rules and the position of women in society, this issue is named differently. Honor killings are called “the passion killings,” in Europe, whereas Asia and Africa call them “honor killings.” Data in Turkey shows that between 2000 and 2006 there has been 1,091 honor killings. The reported reasons for honor killings are vendetta, family conflict, sexual harassment, prohibited intercourse, rape, and simply honor. According to the survey, the number of cases increases as education level decreases. Victims are not solely women and the number of male killed seems to be higher than those of

females. The age of the perpetrator ranges between 19-35 years. The highly populated cities of the country are more likely to host the killings, because the action generally does not take part in its origin but in a big city where the parties have migrated. Particularly inadequate housing facilities, lack of education, unemployment, and poverty are triggering factors. Honor killings vary from country to country. Nearly twenty women are murdered per year because of honor violations despite the opposition of the Royal Family in Jordan. Whereas in Lebanon, the murder of a person based on an honor issue may be saluted. In Europe the situation is somewhat different. The problem in Europe is seen much more in immigrant populations. Germany, Sweden, Italy, Denmark, France, Belgium, Great Britain, and the Netherlands have to face the honor killing issue in their immigrant populations. The reported number of the honor killings by the United Nations Population Fund is approximately 5,000 women victims per year. But it should be noted that a part of honor killings is disguised as suicide or accident which if included would elevate the actual number. Although the old Turkish penal code underestimated honor killings and envisaged a lower penalty in the new Turkish Penal Code, "honor killings" are considered as regular homicides and the perpetrators are sentenced to life in prison. As a result, the economic structure the level of development, the education/training programs and their application, the legal arrangements, the status of women's rights and women's awareness, the cooperation between institutions and organizations, and the media coverage are important factors affecting the issue.

#### **Honor Killings, Woman's Rights, Turkey**

## **E26 Facebook® and the Faceless: Authorship in an Electronic Society**

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After attending this presentation, attendees will learn about the role of authorship issues involving blogs, emails, Facebook®, and other documents in both high profile and low press cases. Attendees will attain information regarding two different methods of language-based authorship identification forensic stylistics and computational linguistics. Attendees will also gain information on validation test results for these methods and the linguistic community's reaction to these methods since the 2011 *Ceglia v. Zuberberg* Facebook-ownership case.

This presentation will impact the forensic science community by being able to accurately assess authorship identification methods by both the *Frye* and *Daubert* criteria, giving them an additional tool for handling electronic evidence.

For most of the general public, many attorneys and most crime laboratories, the term "forensic linguistics" is so unfamiliar that it evokes images of argumentative linguini or the speech of dead people. But anyone who has received an email from a Nigerian scam artist, a vixen selling herbal compounds, has read blog posts that scorch the computer screen, or has received digital applications to fill out over CAPTCHA boxes might also wonder about the reality of email addresses, screennames, and digital documents. Authorship in an electronic society provides pseudonymity, anonymity, and blithe shape-shifting.

Some recent high profile cases which have involved an authorship issue include the JonBenet Ramsey homicide, the 2004 precedent-setting case of *Cahill vs. Doe* (Shaeffer), the 2008 *Best Western International vs. John Doe* (Dial, Furber et. al.), settled for over \$2M, and now the latest Facebook® suit, *Ceglia vs. Zuckerberg*. Cases which have little or no press, but are just as pressing for societal concerns and forensic science include, from 2011, the Masters homicide and the Isaacs custody-related trials which focused, in part, on a pseudonymous Facebook post.

There are currently two main methods for author identification: forensic stylistics and computational linguistics. Forensic stylistics has

practitioners in the United States, Britain, Australia, and Spain. The computational linguistics approach has practitioners and researchers in the United States, Israel, Germany, France, Greece, Spain, and Ireland. These two methods differ radically in their procedures, data requirements, implementation tools, and most importantly, in the validation testing results. The differences between authorship identification in forensic stylistics and computational linguistics have been documented by Crystal<sup>4</sup>, Chaski<sup>2-6</sup>, Koppel and Schler<sup>7</sup>, Nunberg<sup>8</sup> and now in relation to the latest Facebook® case, Zimmer<sup>9,10</sup> and Liberman<sup>11,12</sup>. In this presentation, we review these differences by references to the specific cases mentioned above will be reviewed, highlighting the core difference of validation testing. While computational linguists employ validation testing as a primary means of software and method development, forensic stylistics practitioners have never offered any test results or error rates.

The *Frye* standard of general acceptability has been subsumed by the *Daubert* criteria, not abandoned. The relevant scientific community for both authorship identification methods, forensic stylistics, and computational linguistics, is linguistics. Linguists who function independently of the forensic world and some linguists who do forensic work are not in agreement with forensic stylistics as a method or as a representation of linguistics. The forensic science community, including crime laboratories and the judicial system, should begin to pay heed to what linguists are saying about these methods. Attorneys are the key personnel for presenting the documentation reported to the judges and juries who face issues of authorship in an electronic society.

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#### **Authorship Identification, Computational Linguistic, Frye and Daubert Criteria**

## E27 International Child Abduction

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After attending this presentation, attendees will develop an awareness of the Hague Convention on the Civil Aspects of International Child Abduction. Attendees will also learn the importance of integrating the disciplines of law, forensic science, and child psychology when dealing with abduction cases, as well as the problems that can arise when dealing with cultural differences among signatory countries.

This presentation will impact the forensic science community by fostering awareness of the importance of international conventions that create a common language and deal with the humanitarian aspects of child abduction cases, in a way that fosters a child-centered system free from cultural bias.

In a world that reached seven billion people by mid-2011, nearly 2.9 billion, or 42% of the world's population are children up to 18 years-of-age. Not surprisingly, children are often exploited, and in some areas of the world, child labor, child soldiers, and child abductions are rampant.

In addition, ease of travel and communications has affected not only economic and business relations, but has led to an increase in international marriage, and its counterpoint, international divorce. Unfortunately, children sometimes become pawns in these struggles, and one parent, intent on causing pain to the other, may resolve a child custody dispute by kidnapping the child in question. When this happens, everyone suffers.

For parents, there is nothing more terrifying than losing a child in this fashion. Words cannot describe the torture the parent feels—is the child lost, hurt, hungry, afraid, or alone? Will the child ever come back? And the kidnapped child is suddenly isolated from all that is familiar, wrenched from home and thrust into an unknown world. Oftentimes, the child is told that the “left behind parent” no longer wants him, or even has died.

The kidnapped child can face serious psychological and emotional problems. These include anxiety, nightmares, sleep disorders, aggressive behaviors, and phobias as a direct result of being neglected and exploited.

The Hague Convention of the Civil Aspects of International Child Abduction of 1980 aims to create a legal solution to international custody disputes. Often, that results in an order returning the child to his “habitual residence” as being in the best interests of the child. Factors to be weighed include an objective viewing of the child's spiritual, psychological, and educational needs. Indeed, actions of a kidnapping parent who curtails a child's access to a loving “left behind parent” and cuts off his relations with other family and friends could be described as both physical and emotional negligence. Such children may respond to this family stress by exhibiting anti-social behavior and suffer a marked lack of self-confidence. The “habitual residence” analysis, aided by a combined psychological, legal and forensic approach, may help repair the damage inflicted by fractured international families.

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**Law, Child Abuse, Exploit and Neglect**

## E28 How Can the Question of Relevancy Ensure an Ethical Use of Forensic Science?

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Often cited, implicitly used, but not clearly defined, relevancy is a concept that may be understood differently depending on the legal structure

and proceedings. The goal of this presentation is to define such a concept in the European forensic and legal communities, and what questions such a definition raises in terms of communication between scientific experts and lawyers, and in terms of right to a fair trial for a defendant.

This presentation will impact the forensic science community by raising its awareness to relevancy as a manifold concept that might be understood very differently by scientific experts and lawyers, and this discrepancy in definition can influence the course of a criminal proceeding.

The inquisitorial nature of the European criminal justice systems will be introduced in order to emphasize the importance of the role of the instructing magistrate in the evaluation of the forensic data used as evidence. After confronting the Anglo-American and European notions of relevance in the criminal investigation context, the importance of the concept will be outlined. It will be argued that relevancy from a legal standpoint is a rather vague concept in European criminal justice systems, leaving ample latitude to the investigating magistrate to determine which forensic information is relevant and which is not. Such a “laissez-faire” approach often results in an a priori evaluation of relevancy, which is hardly compatible with the scientific reasoning required by the forensic work and may, in the end, be prejudicial to the defendant.

It will be argued that the legal reasoning applied to a concrete case could be enhanced by the formalization of the concept of relevancy. To achieve this goal, one must go back to the investigative phase, the very beginning of the process, instead of considering only adjudication. Indeed, it is expected that a better understanding of the concept at an early stage of the criminal justice system will allow more relevant questions to be raised and will strengthen the interactions between the different stakeholders (forensic scientists and magistrates). In short, such an approach would produce more relevant clues from the forensic point of view and stronger evidence from the legal point of view.

To do so, it is necessary to go back to the notion of relevancy in science and specifically forensic science. The concept of relevancy represents one of the cornerstones of forensic science. This principle is understood as a key point between three entities: the trace, the clue, and the evidence, that are related within a context. The relationship between these entities will be discussed, and it will be argued that each is specific to a particular step of the forensic reasoning process. They all share a common aspect: they exist within a context specific to a case investigated, and this is aimed finally to insure that the right to a fair trial is safeguarded.

Confronting the legal and forensic definitions of relevancy would, eventually, allow a better definition of the expectations and needs of the instructing magistrate in terms of forensic information. This attempt to formalize such a manifold concept is aimed at showing that focusing on the initial phase of the process will help strengthening the information provided and, thus, the principle of equality of arms. Indeed, the concept of relevancy, if taken from a scientific point of view, is independent of the legal systems, whether European or Anglo-American will be shown. This principle is considered universal and should be considered as a scientific “guardrail” which guarantees an ethical use of forensic information by the justice systems.

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**Forensic, Legal, Relevancy Concept**

## E29 Paul Gregory House: Death Penalty Exoneration in Tennessee Through the “Gateway to Innocence” Concept

*Linda K. Baden, JD\*, Law Office of Linda Kenney Baden, 15 West 53rd Street, Apartment 18 B/C, New York, NY 10019*

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The goal of this presentation is to provide attorneys with information on how to proceed once a new trial is granted subsequent to a murder or other major felony conviction in order to obtain a dismissal before retrial.

This presentation will impact the forensic science community by providing attendees an avenue to shepherd a case where a defendant may

be innocent and understand the legal procedures and pitfalls in such a case. The presentation will also enhance knowledge about the expert testimony and investigative domains needed in order to properly prepare such a case for both the appellate court and retrial. In 2006, the United States Supreme Court in *House v. Bell*, 547 U.S. 518 (2006) reversed the conviction of Paul Gregory House and remanded to the State Court for a new trial in a case where House had previously been convicted of a death penalty murder and was awaiting execution. Unlike most actual innocence cases, the opinion by Justice Anthony Kennedy in a 5-4 decision, ruled that DNA testing and other investigation may give a defendant the right to argue to a jury that he is innocent even if the DNA did not prove actual innocence. The House decision was unique in two ways: (1) it enveloped the concept of “gateway to innocence”; and, (2) it allowed House to proceed on his claims even though the State Court had previously procedurally rejected him.

In 2009, the Tennessee State Court dismissed the murder indictment against Paul Gregory House. Mr. House had been on death row since 1986 for the brutal murder and alleged attempted rape involving a neighbor, Carolyn Muncey. Mr. House appealed from this conviction and had been rejected by courts for years. DNA testing had been blocked through procedural barriers; however, as a result of the efforts of the Federal Public Defender’s Office through a *habeas corpus* petition, House was finally able to obtain DNA testing and also argued through investigation that the evidence presented at the first trial was improper. Even though DNA testing seemed to indicate that House was not the murderer due to the fact that a rape had never been consummated, the Supreme Court was presented with the issues as to whether DNA testing on the clothing of the victim, which could be argued to support the fact that House was innocent, could be cause for reversal of a death penalty conviction and retrial. As a result of additional investigative leads pointing to a third party, Justice Kennedy in a landmark decision sent the case back for retrial in an Opinion of the United States Supreme Court in 2006, *House v. Bell*, 547 U.S. 518 (2006).

While the federal public defender had been charged with handling the investigation up to the United States Supreme Court decision, the local public defender’s office was charged with continuing House’s legal representation, the additional testing of forensic evidence, and preparation of the case for retrial. With the aid of additional organizations who assist wrongfully convicted defendants, along with forensic experts and attorneys who donated their time on a *pro bono* basis, the case was prepared for retrial. This resulted in the unique marriage of a branch of the Tennessee Public Defender’s Office and the legal *pro bono* assistance. Additional investigative interviews were conducted, additional DNA testing was performed, additional review of the evidence was undertaken, additional unfettered review of the evidence was permitted by all branches of law enforcement, and the Tennessee Court allowed the involvement of all out of state experts and attorneys.

Through the avenues set out in the Supreme Court opinion and the efforts that continued from 2006 through the date given for retrial in 2009, on the eve of retrial, the charges against Paul Gregory House were dismissed. This presentation will discuss the legal parameters that attorneys and experts should be aware of when handling such cases and the actual step-by-step footprints that need to be performed when representing a client in a wrongful conviction case.

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**Wrongful Conviction, Gateway to Innocence, Paul Gregory House**

## **E30 The Innocent Rapist: The Story of a Prosecution-Led Exoneration**

*Melissa Mourges, JD\*, and Martha Bashford, JD\*, District Attorney’s Office, New York County, One Hogan Place, New York, NY 10013*

After attending this presentation, attendees will understand how re-submitting a “negative” rape kit during backlog testing produced an “uploadable” DNA profile, which in turn led to an exoneration.

This presentation will impact the forensic science community by illustrating how a “negative” rape kit in a closed case yielded important

results when it was swept up in a “fork lift” backlog testing project, and provides a road map for a fast and efficient prosecution-led exoneration when the results proved the wrong man was in prison.

On March 19, 1991, in upper Manhattan, a 15-year-old girl was attacked by a man who followed her into the elevator of her building, then dragged her onto the roof and brutally raped her at gunpoint. After reporting the crime and giving a detailed description of her assailant, the victim stayed with friends and did not return home for three months. On the date of her return, she saw Michael Mercer outside the building, recognized him as her attacker, and called for help. Neighbors held him for police.

The sexual assault evidence kit was tested, and the vaginal, oral, and anal swabs were negative for sperm. Even the slides retained by the hospital were tested, with the same results. While there was no forensic evidence to corroborate the victim’s identification of Mercer, some details did match—the defendant admitted to being in the building on the date of the rape, visiting a friend who lived on the same floor the attacker pressed, and said he recognized the victim as a girl he rode with in the elevator. He also fit the detailed physical description given by the victim. Mercer was convicted after trial and sentenced to 20.5 to 41 years. From prison, Mercer repeatedly requested post-conviction DNA testing, but those requests were denied since the kit results were negative.

In 2003, his kit was part of the NYPD backlog project, which employed a “forklift” approach and tested every kit in storage, even those from closed cases. This time, one of the four swabs in the kit tested positive for sperm, a profile was developed and uploaded to CODIS, where it hit to Arthur Brown, a convicted rapist.

Prosecutors were notified of this “hit” on Wednesday, May 14, 2003. First, it was confirmed that the convicted offender samples from Mercer and Brown were collected in different prisons and developed on different days, so there was no possibility of mix-up. The victim was contacted, who had become a police officer, and determined Brown was not a consensual partner. A DNA swab was taken from her, tested, and confirmed that results were the same as initial testing. This proved the integrity of the kit and contents were intact and had not become comingled with other evidence over the years.

The victim was shown a photo array containing both Mercer and Brown and she identified Brown as her assailant. She recognized Brown immediately as the real rapist and also understood that she had misidentified Mercer. Prosecutors drafted a motion to dismiss and sent detectives to the prison in upstate New York where Mercer had spent 12 years. On Monday, May 19, 2003, he was brought into court and the charges against him dismissed. Five days from the date of the DNA match, Mercer was a free man.

Learn why initial testing was negative, the victim’s reaction to her mistake, what the Manhattan DA’s office did for Mercer, Mercer’s reaction to events, what Arthur Brown had to say for himself, and why Mercer’s court-appointed attorney was extremely happy.

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**Backlog, Exoneration, CODIS**

## **E31 An Expert for the Court: Testimony in the Magdalena Dzubia Case**

*Mark E. Reynolds, PhD\*, Western Australia Police, 2 Clayton Street, Midland, Perth, Western Australia 6152, AUSTRALIA*

After attending this presentation, attendees will understand principles governing the role of the expert witness and the difficulties that can be encountered when attempting to fulfill the responsibilities to the judicial process that surround the role of an expert witness.

This presentation will impact the forensic science community by improving practitioner knowledge regarding the provision of expert opinion evidence.

Providing expert opinion evidence within a criminal trial can be a daunting and lonely experience. Often the testimony of an expert becomes

pivotal to the acceptance, or otherwise, of scenario(s) consistent with the guilt or innocence of an accused person and in many instances becomes the decision fulcrum used by the jury in coming to a determination. In this presentation, the forensic component of investigations surrounding a murder case to highlight the value of expert opinion evidence to the criminal trial process and demonstrate the overarching responsibility code those experts must apply to the judicial process, irrespective of party advocacy.

At about 9:00 a.m. on Wednesday, November 30, 2005, Magdelana Dzubia returned home from work to an improvised covert alarm positioned across her entry door with security locks on doors between her and her male housemate which had been rendered inoperable with the insertion of cardboard into components of the doors locking mechanisms. Within five minutes of entering the house she was set upon by her housemate who attempted to bind her with rope thus rendering her incapable of resistance and who then advised her that it was his intention to rape and then kill her. She managed to escape from the bindings, and in fighting for her life she subsequently killed her housemate by stabbing him in the chest with the very knife he intended to use upon her. At postmortem, the deceased housemate was found to have several incised and penetrating injuries to his chest and leg. Dzubia suffered a single superficial incised injury to her left index finger.

The assessment and analysis of the bloodstains and bloodstain patterns along with complementary DNA results from more than 60 of those bloodstains spoke of that fight, particularly when the results were schematically mapped to the floor and wall plans of the house. The physical evidence assessed during the course of investigations surrounding the death of the housemate, and in particular the bloodstains and bloodstain patterns, became vital to the issue of “acting in self defense” that was raised and argued at Dzubia’s subsequent trial for murder.

The presentation of this case example, articulates an unlikely “event” scenario proffered by the prosecution and its subsequent attempts to mitigate the evidence of the state’s own bloodstain pattern analysis (BPA) expert. A highlight of the trial was the cogent exposé of the bloodstain evidence by the defense team following extensive pre-trial dialogue with that same BPA witness. Following less than five hours of jury deliberation, Magdelana Dzubia was acquitted of murder on the grounds of self defense.

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**Expert, Evidence, Bloodstain**

## **E32 Who Killed Ruby Jean Johnson? Unleashing the Power of Y-STRs**

*Melissa Mourges, JD\*, and Martha Bashford, JD\*, Distric Attorney’s Office, New York County, One Hogan Place, New York, NY 10013*

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After attending this presentation, attendees will understand how, despite statistical limitations, the use of Y-STR DNA testing can identify the perpetrator in a rape/homicide case.

This presentation will impact the forensic science community by illustrating the development of Y-STR DNA testing by the New York City Office of Chief Medical Examiner’s Forensic Biology lab and presenting its first use in a courtroom, leading to the conviction of Elbert Mitchell for the rape and murder of an 81-year-old woman.

On June 26, 1998, the home health aide for 81-year-old Ruby Jean Johnson was worried. She had been unable to enter Ms. Johnson’s Harlem apartment for two days. Police climbed in through a fire-escape window and found Ms. Johnson’s body. She was bent double over the bathtub, with a dog’s leash wrapped around her neck and a fur coat draped over her body. Decomposition was well underway. Based on her state of undress and dried secretions on her thighs, police suspected she had been sexually assaulted. Although the apartment was cluttered with a lifetime’s worth of stuff, police also believed the killer stole property.

A fingerprint in the bathroom led to suspect Elbert Mitchell, a convicted felon who claimed he often helped Ms. Johnson around the

house. DNA testing was still pending when Mitchell was first questioned two weeks after the crime, and he vehemently denied harming Ms. Johnson, who he described as “like a mother” to him. He did offer up another suspect; however, a neighborhood man who did maintenance work around the building, and who was easily identified by police.

Meanwhile, serological testing was positive for semen on vaginal, anal, and oral swabs, as well as on the fur coat, but only the victim’s DNA was detected through multiplex STR testing. Fearing that any male DNA was “masked” by the victim’s female DNA, OCME scientists employed a “home brewed” Y-STR kit validated to attempt to identify male DNA on the crime scene evidence. The results were then compared with exemplars from both Elbert Mitchell and the maintenance man.

Prior to trial, defense attorneys made a motion for a *Frye* hearing to exclude the Y-STR results, claiming the science was novel and the statistics unproven. Prosecutors countered that the technology was exactly the same as traditional STR testing, and that statistics, reached through the “counting method,” were the same as those used in mitochondrial DNA testing. The prosecution prevailed.

The presentation includes filmed interviews with participants in the case. The investigating detective, who teaches interview techniques to NYPD investigators, explains the secret of getting a suspect to talk. The relationship between strangulation and sexual assault will be explained. The early attempts at Y-STR testing, how results were achieved in this case will be described, as well as the future utility of Y-STRs. Mitchell’s videotaped statements are presented, demonstrating his changing explanations as the unfolded evidence, ending with his claim that sex with the 81-year-old disabled victim was consensual. Finally, prosecutors explain how to prove time-of-death, theft of property, and forcible rape when the only witness to the crime is dead.

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**YSTRs, Homicide, Rape**

## **E33 Disingenuous Testimony in the Forensic Toxicology Community**

*David M. Benjamin, PhD\*, 77 Florence Street, Suite 107N, Chestnut Hill, MA 02467-1918*

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After attending this presentation, attendees will be able to: (1) define disingenuous testimony; (2) summarize the forensic expert’s duties under the testimonial oath; and, (3) recognize why laws on *per se* levels of drugs are oppressive.

This presentation will impact the forensic science community by providing examples of disingenuous expert testimony and prosecutorial misconduct and how it impacts all stakeholders in the administration of criminal justice.

Disingenuous is defined in the dictionary, as “lacking in candor; giving a false appearance of simple frankness.” It resembles a lawyer’s or police officer’s use of subterfuge (claiming the existence of evidence that does not really exist). An appropriate definition of candor, from the same source is “freedom from prejudice or malice, i.e., fairness.” When an expert witness testifies regarding his or her opinions about the significance of the scientific evidence, the rules of evidence provide a framework to ensure that such testimony is relevant and reliable. Matters not covered by the rules of evidence may be addressed by the case law or may be left up to the judge’s discretion. According to FRE 702, the purpose of expert testimony is to help the jurors understand scientific matters that would typically be beyond their ken. Pursuant to FRE403, even relevant evidence may be suppressed or limited, when such evidence confuses the issues, misleads the jury or is substantially prejudicial.

**The Oath:** Before testifying, every witness takes the oath. The oath asks witnesses to testify to “the truth, the whole truth and nothing but the truth...” In breaking down these three portions of the oath, “the truth” means, the truth as opposed to a lie. “The whole truth” means, the whole truth as opposed to a half-truth. And, “nothing but the truth” instructs us not

to add or subtract any disingenuous statements that might cause the truth to be misperceived by the jury (Gutheil et al 2003). Not adulterating your testimony pursuant to FRE 403 is what you have sworn not to do, under the last part of the oath.

**Why Laws Regarding *per se* Drug Levels Contradict Criminal Jurisprudence:** In a Driving Under The Influence of Drugs (DUIDs) the prosecution's evidence of impairment usually consists of: an admission made by the driver, the appearance of the driver, and the way the driver performs standardized field sobriety tests (sFSTs). In an attempt to increase DUID enforcements, many states have taken to passing *per se* laws, developing numeric concentrations for drugs in the blood plasma, or urine above which a citizen is automatically assumed to be the DUID. In these instances, impairment does not have to be proven. The analogy is a *per se* level of 0.08% for blood alcohol. The scientific problems are: the blood levels of drugs and the onset and duration of impairment are not precisely related; people metabolize drugs at different rates causing blood levels to vary widely; people have individual sensitivities to drugs; people develop tolerance to drugs; urine concentrations do not correlate with current impairment; only prior ingestion; and, people produce anywhere from 1200 to 2500 ml of urine a day. Ultimately two identical twins could take the same 25 mg dose of diphenhydramine, if one drank an extra quart of fluid, that person's urine would be twice as dilute as his twin's, meaning that the other twin could be arrested for DUID, because his urine concentration was twice as high as his sibling, even though they both took the same dose. Inter-subject variability is so great that the error rate of offering an opinion is merely speculation and the degree of certainty less than reasonable certainty. If such a "zero tolerance" policy stands, many sober people will be prosecuted oppressively. Impairment must be proven beyond a reasonable doubt, not inferred merely from the presence of a drug.

**Prosecutorial Misconduct:** 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 man 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 judiciary must take a harder look at prosecutorial misconduct and not permit oppressive prosecution to continue, under the guise of zealous representation. Perpetrating a fraud on the court, suborning perjury, and deliberately withholding exculpatory evidence are unethical and illegal, not zealous advocacy.

**Drug Laws, Disingenuous Testimony, Prosecutorial Misconduct**

## E34 Ethical Implications for the Trial Judge Faced With Disingenuous Expert Testimony

*Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of Pennsylvania, Erie County Court House, 140 West 6th Street, Room 223, Erie, PA 16501*

After attending this presentation, attendees will learn the ethical implications for the trial judge when encountering disingenuous testimony. Judicial sanctions may result from the judge having an ethical responsibility to report the lawyers and their experts providing disingenuous testimony. Case examples will be shared.

This presentation will impact the forensic science community by alerting forensic experts that trial judges are active gatekeepers of scientific evidence whether in a *Frye* or *Daubert* jurisdiction and must take action in the case at hand and report the lawyers and experts who provide disingenuous testimony when appearing before the court.

The United State judicial system relies on reliable and relevant expert testimony to reach the truth of the matter before the courts. In the United States, we have state trial court jurisdictions which vary as to whether a state trial court is a *Frye* or *Daubert* jurisdiction or some version of both. The gatekeeper function of the judge differs as to what admissibility standards exist for admitting scientific evidence which is relevant and reliable. Most experts provide valuable testimony and assistance to the courts with

honesty and integrity; however, there are always exceptions unfortunately with experts who are disingenuous before the court. Disingenuous experts provide reports and testimony lacking in candor and by doing so give a false appearance of frankness in a calculating fashion. Lawyers have numerous ethical requirements with respect to the experts they bring before the court. State trial court judges in the United States have ethical responsibilities as evidenced in the Judicial Code of Ethics or Judicial Code of Conduct in each of their respective states. Each state has had the benefit of reviewing the American Bar Association's Model Code of Judicial Conduct and adopting a version of this model code with modifications tailored to the needs of their own state jurisdictions. Judicial ethical responsibilities include a disciplinary responsibility to report misconduct from the professional parties appearing before the court including lawyers and experts. Lawyers have a responsibility to ensure that the experts appearing before the court are properly credentialed and their reports do not include false testimony. If a lawyer discovers that an expert has falsified information or has been disingenuous, what duty, if any, does a lawyer have to disclose that new information to the tribunal he or she is appearing before? What is the duty, if any, to opposing counsel? What is the ethical duty of the judge to report lawyers who provide experts having disingenuous testimony? How does a judge handle this situation if the judge finds the expert is not properly qualified to render such a report but has been accepted as qualified for years by fellow judicial colleagues within the same jurisdiction? Examples of cases will be shared where these ethical dilemmas have appeared and how judges have approached these situations.

**Judicial Expectations, Ethical Responsibilities, Expert Testimony**

## E35 The Impact of Gunshot Residue in Military Investigations and Legal Proceedings

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After attending this presentation, attendees will have a general understanding of how gunshot residue (GSR) is used in military investigations and legal proceedings, the usefulness of gunshot residue evidence in criminal cases and suicide investigations, and an understanding of some of the caveats that come along with gunshot residue.

This presentation will impact the forensic science community by providing a solid foundation as to the use of gunshot residue in military investigations and trials, and providing a catalyst for more research in GSR analysis.

According to Michael Trimpe, from the *FBI Law Enforcement Bulletin* in 2011, gunshot residue (GSR), like most trace evidence, is not conclusive but supportive and circumstantial.<sup>1</sup> Supportive evidence can only take an investigation so far and the continuing controversy on the usefulness of GSR examinations brings about the debate of how important GSR examinations are to an investigation and/or legal proceedings. Garcia suggested in *The American Journal of Forensic Medicine and Pathology* in 2007 that the results of GSR examinations were crucial to medical examiners but very little has been done to show what it means to investigating agents.<sup>2</sup> Since the initiation of the Innocence Project, there have been several outcome studies that try to identify links and commonalities in criminal investigations/judicial proceedings in an effort to reduce the number of convicted innocent people.<sup>3</sup> Based on the effectiveness of these outcome studies and the circumstantial nature of GSR examination, it is necessary to determine the overall effect that these examinations have in the military system.

This retroactive outcome study started with 154 cases involving a gunshot residue kit that had been sent into the United States Army Criminal Investigation Laboratory (USACIL) since 2008. Cases were collected from all of the military branches. Of those, 68% of the cases were made up of

\* Presenting Author

suicide investigations. The remaining 32% were criminal investigation that involved murders, assaults, and negligent discharge crimes. Even fewer of these ever make it to a judicial proceeding. The forensic information for each case was drawn from the laboratory information management system (LIMS). This information included GSR examination results, case synopses and other pertinent data (rank, location, weapon type, contact numbers). Once all the applicable data was obtained, investigators, defense, and trial counsel were questioned regarding cases that had GSR kits submitted for examination. The questions asked of the agents and attorneys were used to gauge the overall impact of gunshot residue in their investigations and legal proceedings. A rating scale was developed at the end of the study to group responses based on how they used the examination reports. This placed a quantitative value on qualitative data. Statistical data was also determined using a Chi-Square model including items such as interviews before and after the results were released and sentencing outcomes for criminal cases.

The conclusion drawn from the investigative study was broken into two categories: suicide investigations and criminal investigations. Using the rating scale developed from the responses of the investigators, it was inferred that GSR results in suicide investigations had minimal to no impact 84% of the time. The impact of gunshot residue in criminal investigations was contrary to suicide investigations. The results showed that 87% of criminal investigations were impacted in some manner from gunshot residue results. The GSR examination results came back inconclusive on 13% of cases in which the investigators stated there was no impact from gunshot residue.

Most of the attorneys that utilized GSR results in legal proceedings stated that it was a part of the package that was used to either get a conviction or exoneration. There were two cases where the attorneys stated they didn't even look at the results and a few others saying they looked but decided against using it. One attorney said that the results were a big piece in negotiating a plea agreement.

Based on the research thus far, the impact of gunshot residue in military investigations is still circumstantial. However, based on the trends identified in this study, gunshot residue has significantly more impact in criminal investigations than in suicide investigations. Analyzing the data for legal cases reveals that most of the attorneys would like to see more research into the subject or at the very least, see it continue to be used with better training provided to investigators on how to collect GSR kits leaving less room for error in analysis. A decision cannot be made based solely on this study due to the quantity of data collected, but the trend shows that it would be worth looking into the possibilities of making GSR exams more specific before determining if it is still needed.

\*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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1. Trimpe, Michael. "The Current Status of GSR Examinations." *FBI Law Enforcement Bulletin* May 2011: 24-32. Print.
2. Molina, D. Kimberley, Michael Martinez, James Garcia, and Vincent J. M. DiMaio. "Gunshot Residue Testing in Suicides." *The American Journal of Forensic Medicine and Pathology* 28.3 (2007): 187-90. Print.
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#### Gunshot Residue, Military Investigation, Legal Proceedings



## F1 Forensic Dental Analysis of Degraded, Fragmented, and Commingled Human Remains

*Diana Aparicio, DDS\*, Servicio Medico Legal, Avenida La Paz 1012 Departamento de Tanatologia, I, Santiago, CHILE; and Soledad Martinez, MD, Servicio Médico Legal, Avenida La Paz N°1012, Independencia, Santiago, CHILE*

After attending this presentation, attendees will be able to recognize the morphological, anatomical and pathological criteria used in forensic dentistry and its application as an important tool in identifying commingled remains of skeletonized bodies in a mass grave intentionally disturbed

This presentation will impact the forensic science community by demonstrating that an accurate forensic dental analysis of degraded, fragmented, and commingled human remains can be essential for individual identification even when premortem records are not available.

During the military regime in Chile, thousands of people were killed. In many cases they were caught and killed in groups and illegally buried in mass grave sites. After a period of five to ten years, when some of the graves were discovered, the military carried out an operation to remove the bodies from the mass graves to make evidence disappear. At that time, bodies were skeletonized so they could not remove all the remains, leaving in place bone fragments, teeth and other small evidence. With democracy, judicial investigation of these cases started and the state forensic institution "Servicio Medico Legal" (SML) was called to perform exhumations of remains in different disturbed burial sites.

This particular case, involves a group of farmers killed in a creek and buried in a mass grave that was disturbed from removal five years later. Despite this, the SML recovered from this site human bone fragments, dental remains, ballistic evidence, personal objects, and non-human skeletal remains, all commingled, incomplete, and degraded.

Forensic dentistry is a useful tool in human identification, which achieves its goal through the comparison of antemortem and postmortem information. The postmortem examination is affected in this case by the admixture of remains as well as postmortem teeth loss caused by the intentional relocation process. Additional difficulties derived from the long interim from burial to judicial exhumation (thirty years). Performing a thorough dental macroscopic study using morphological, anatomical, and pathological criteria, evidence was organized in skeletal and dental clusters, partially or completely reconstructing the dental arches and providing the minimum number of individuals present in the mass grave.

One hundred twenty seven human remains good for dental analysis were recovered: 111 (87%) consisted of isolated teeth (including crown fragments), 10 (8%) were mandibular fragments (four with *in situ* teeth), five (4%) were maxillary fragments (three with *in situ* teeth) and one consisted of a removable upper dental prosthesis. All specimens presented taphonomic erosion with the teeth being the least affected due to dental enamel resistance.

Odontological analysis classified 87 of 127 specimens into twenty-one groups obtaining two complete upper arches, one complete lower arch, two partial upper arches and one partial lower arches, and other smaller parts. This led to a minimum of nine individuals for the burial site, considering only the odontological point of view.

The lack of adequate antemortem dental records ruled out postmortem comparison for identification purposes, thus genetic testing appeared as the only possibility to get individual identities. Teeth from some of these 21 groups and eight ungrouped teeth were subjected to nuclear DNA sampling; one to three samples from each group were sent separately. A

consistency up to 100% was obtained between odontological analysis and DNA testing: identical genetic profiles in all loci typed for teeth coming from the same group. The genetic testing results confirmed the odontological clusters and also provided other associations between upper and lower arcades and ungrouped teeth.

The dental analysis methodology and evidence organization allowed grouping and adequate selection of samples for genetic analysis that lead to successful identification of individuals, preserving dental remains suitable to be returned individually to relatives and terminate the grieving process. Multidisciplinary approach integrating judicial information, forensic archaeology, forensic anthropology, forensic odontology, forensic pathology, and forensic genetic was essential for this goal.

**Commingled, Odontology, Identification**

## F2 Digital Enhancement of Dental Radiographs to Facilitate Identifications

*Richard M. Weledniger, DDS\*, 931 Walt Whitman Road, Melville, NY 11747-2297*

After attending this presentation, attendees will understand the difficulties that poor quality antemortem or postmortem dental radiographic images can make impeding victim identification and how readily available simple digital enhancement tools can reduce these challenges in the identification process.

This presentation will impact the forensic science community by giving an overview of image quality issues and the digital enhancement tools that are available to make acceptable and non-acceptable corrections to these images without affecting the evidentiary value of the image.

Whether it is a multiple fatality incident or single person identification, the use of dental radiographs as a tool for biometric comparison and identification is well documented. Dental features can withstand severe conditions and resist the degradation that affects other body tissues. Dental radiographs not only reveal dental restorations but tooth morphology, bony trabeculation patterns, root and crown morphology, tooth size, rotations, spacing between the teeth, and sinus patterns. As dental caries rates decrease, more odontological identification decisions will be based on inherent dental features.<sup>1</sup> Dental radiographs often suffer from poor image quality which can affect the identification process. Development of a stringent protocol of acceptable image enhancement techniques is essential for the forensic odontological community.

Acquisition of diagnostic images requires the use of either a flatbed/transparency adaptor digital scanner to digitize analog dental radiographs or a digital sensor to obtain images directly. Numerous issues exist that can be detrimental to image quality. Equipment issues such as exposure time, MA/KV, film age, film processing chemical quality, and/or the actual storage of the image or images can lead to poor image quality that can increase the challenges of the identification process. If working with analog radiographs, efforts should be made to acquire the original radiographs, as scanned second or third generation images of poor quality can impede the identification process.

By either rescanning analog x-rays with different scanning parameters or by incorporating the enhancement tools available for digital films, many of the difficulties for making identification can be overcome. Current forensic dental standards such as the ANSI/ADA Specification 1058 advocate scanning resolution sizes of 96 DPI for screen viewing, 150 DPI for lower resolution viewing and printing, 300-600 DPI for higher

resolution viewing and laser quality printing and greater than 600 DPI for photographic viewing and printing.<sup>2</sup> This presentation will demonstrate support of these settings as well as color/grey scale depths to aid the odontologist in the understanding of the relevancy of these guidelines. In addition, this presentation will show the effects of additional scanning software settings to show how they can also enhance an image at the time of scanning.

The ANSI/ADA Specification 1058 specifies that a radiographic image should be of sufficient quality to ensure that an enlargement of any section will not result in an unacceptable image.<sup>1</sup> In addition to image resolution, the type of file format used to store the image can also affect image quality. This presentation will expand on the types of file formats that meet the standards criteria while promote ease of data access through the use of standard interoperable file formats.<sup>2</sup>

Essentially, the enhancement of a dental radiograph is the process of producing an improved quality image out of a degraded quality input while preserving the evidentiary value of the radiograph.<sup>2</sup> Although the enhancement process improves the visibility of objects of interest and thereby increases an image's diagnostic yield, it is vital that it does not alter the image in an unacceptable manner to enhance artifacts to the level that they are considered an anatomical feature.

This presentation will show various images reflecting several of the image management tools such as: contrast and brightness, sharpness and clarification, inversion, colorization, relief, subtraction, stereo, and invert logic that can help assist the forensic odontologist in the identification process.

#### References:

1. American National Standard/American Dental Association Specification #1058, **Forensic Data Set, 2010**, Radiographic Data Set, P56, P58.
2. EyadHaj Said Gamal Fahmy, Diaa Nassar, and Hany Ammar. Lane Department of Computer Science and Electrical Engineering West Virginia University, **Dental X-ray Image Segmentation**, research is supported in part by the U.S. National Science Foundation under Award number EIA-0131079, the research is also supported under Award number 2001-RC-CX-K013 from the Office of Justice Programs, National Institute of Justice, U.S. Department of Justice.

#### **Dental Radiography Enhancements, Forensic Odontology, Unidentified Persons**

### **F3 The Perfect Storm II: The Strengths, Weaknesses of Bitemark Analysis, and Answers to Inquiries Requesting Fact-Based Protocols for This Forensic Discipline**

*C. Michael Bowers, DDS, JD\*, Forensic Services, 2284 South Victoria Avenue, Suite 1G, Ventura, CA 93003*

The goal of this presentation is to discuss the current status of bitemark identification opinions in three specific areas: (1) strengths; (2) weaknesses; and, (3) discussion of the future uses of bitemark comparison in criminal investigation.

This presentation will impact the forensic science community by clearly describing the issues regarding the necessity to substitute DNA analysis in cases involving human bitemark evidence.

Three years since the 2009 National Academy of Sciences Report (NAS Report) was published, things have been quiet regarding any revision of the methods and protocols allowed by the recognized forensic bitemark certification organizations. The American Academy of Forensic Sciences has issued a general recognition of the need for improvements in forensic application of certain disciplines. The ABFO has argued that bitemark

comparisons still qualify as acceptable forensic science. The available literature on the foundations of bitemark comparisons is currently being reviewed by the ABFO in response to direct questioning by the Congressional Judiciary Sub-Committee tasked to establish the breadth, substance, and gaps in this area of forensic investigation. Reviewers outside this organization have completed their own literature investigation. Do these summaries tell us anything regarding a common ground of proof supporting bitemark comparisons? As of August 1, 2011 the answers have yet to be revealed. The content and status of these reviews will form the core of this presentation.

#### **1. STRENGTHS**

- Cases involving biting activity occur during assaults and homicides. Victims can be either children or adults. Sometimes an assailant may be bitten by the victim. In all of these cases, the identity of the biter is the foremost challenge. Currently available DNA technologies have recently changed the hierarchy of investigation of bitemarks. The penultimate resource to biter identification is via collection and processing of DNA containing saliva from both the region of the skin injury and also from clothing worn at the time of the attack. Well informed jurisdictions have adopted this protocol as a standard operating procedure. Nevertheless, this type of evidence must be collected in a timely manner due to its susceptibility to environmental degradation. This makes the identification of the skin injury as a bitemark the paramount task of the initial medico-legal investigation. Forensic dentists are dedicated to educating law enforcement and hospital staff to the appearance of human bitemarks and the proper collection, documentation and preservation of this biological evidence.

#### **2. WEAKNESSES OF BITEMARK COMPARISONS**

- Poor to non-existent scientific support regarding scientific issues published in NAS report and questions posed by the Congressional Judicial subcommittee to the ABFO in 2011.
- Multiple analysis methodologies and unrealistic expectation of the validity and judicial presentations of bitemark pattern analysis. Unopposed research now available indicates skin injury patterns are unreliable for comparison purposes.
- General lack of scientific determination of levels of certainty used by dental experts.
- History of expert disagreement in court regarding fundamental levels of bitemark pattern analysis.
- Resistance of odontology organizations to consider the basis of the numerous United States exonerations which overturned opinions of experienced board-certified forensic dentists.

#### **3. ISSUES AND SOLUTIONS**

- The weaknesses in Section B are currently unresolved.
- The default solution is to determine that DNA analysis is the best method for bite identification and that bitemark identification opinions should only be used for extra-judicial investigation. It is mandatory to refocus U.S. odontologists towards better training and education of law enforcement, hospital, dental and medical professionals about the basis of bitemark identification and biological evidence collection. The increased use of DNA analysis as a substitute for dependence on bitemark comparison is evident in the numerous cases in the United States and is the hallmark for well informed medical-legal agencies throughout this country and abroad.

#### **Bitemark Research, Bitemark Unreliability, DNA**

## **F4 Exploration of Bitemark Distortion in Human Skin: Effects of Size and Shape Deformation**

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The goal of this presentation is to describe the extent of distortion, relative to size, in a series of bitemarks with consideration of both maxillary and mandibular teeth. In addition, the ability to predict distortion in one arch relative to the other will also be discussed.

This presentation will impact the forensic science community by exploring one of the basic premises of bitemark analysis, that of reproducible transfer of dental shape to the skin.

The biomechanical nature of skin dictates that distortion will be inevitable, as skin behaves in anisotropic, visco-elastic manner. Given these properties the question then becomes *what is the extent of distortion possible in a bitemark and is distortion with respect to one dental arch predictive of the other?*

In order to explore this deformation of skin, an approach that can statistically evaluate the range and extent of shape change associated with distortion is necessary. A well-developed method to describe shape variation between biological specimens is Landmark based Geometric Morphometric analysis (GM).

In GM, landmarks are placed on digital images. These are preserved as coordinates that describe and preserve spatial information. The landmarks can then be extracted and used to describe shape changes between specimens in a quantitative and statistical manner. Traditionally, GM methods remove scale; however, GM methods can be extended to preserve size information. In forensic studies, information about size is generally important, so it was necessary to explore an approach that preserves size. Thus, a size-preserving Procrustes approach (Procrustes S-P) was used in this study, as well as the size independent approaches.

Human Subject Review Board Exemption (HSRIB) was granted for this project. Impressions of the maxillary and mandibular dentition were taken from a single volunteer. The impressions were poured in resin and mounted on a hand held vice grip instrumented with a load cell to monitor force application.

The apparatus was used to inflict 49 bites on unembalmed human cadavers. The cadavers were acquired based on availability and thus gender and age were not controlled factors in this study. Bites were made on the upper arm, lower arm, lateral thoracic wall, and upper thigh. The same examiner created all of the bites.

The resulting bites were digitally photographed with an ABFO scale in place. All photography occurred within two minutes of infliction. In order to avoid photographic distortion, the maxillary and mandibular arches were photographed separately as needed.

Landmarks were placed on the digital images with tpsDig freeware. Landmarks were placed on the mesial and distal extensions of the six anterior teeth as well as the center point of the canines. This resulted in a placement of 14 landmarks. Two additional landmarks were placed on the ABFO scale for size reference. The maxillary and mandibular dentitions were recorded separately.

The landmarks were then extracted from the images and analyzed with IMP freeware. Landmarks were also placed on digitally scanned images of the biter's maxillary and mandibular dental models as described for the bitemarks. Concurrently, a sample population of 297 paired maxillary and mandibular dental models were acquired from the University at Buffalo School of Dental Medicine. This was a sample of convenience. The models were placed on a flat bed scanner and digitally scanned and landmark placement performed. These models were used for comparison

purposes to the inflicted bitemarks. Error rates were calculated by repeated measures.

Results show that scale changes appear in the upper and lower arch in a relatively independent way and are not highly correlated. This result is not surprising given the anisotropic nature of the skin. A change in arch width was the largest factor seen with regard to distortion. Variation in arch width caused by distortion in the bitemarks was roughly ten times the measurement error. Arch width variation in the bitemarks from a single dentition was compared to the population of 297 models. The bitemarks span 42% of the population range of arch widths in the mandibular dentition, and 53% of the range in the maxillary.

It was concluded that substantial size variation exists in bitemarks produced by one dentition, as characterized by arch width, as well as by more complex geometric morphometric measurements. Scale changes appear in the upper and lower in a relatively independent way and are not highly correlated. To summarize, cadaver skin produced extensive and rather unpredictable distortions in arch width for the dentition used in this study.

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**Forensic Science, Bitemarks, Distortion**

## **F5 Ethical Considerations in the Use of Live Human Subjects in Bitemark Research**

*Kenneth P. Hermsen, DDS\*, Creighton University, School of Dentistry, 2500 California Plaza, Omaha, NE 68178*

After attending this presentation, attendees will be aware of the ethical considerations that should be taken into account when conducting bitemark research on live humans, including a brief history of human experimentation, the Institutional Review Board process, and the protection of vulnerable populations.

This presentation will impact the forensic science community by laying the ground work for the participants to consider the ethical ramifications in utilizing live human subjects in bitemark research and to move the discussion forward on prohibitions and/or guidelines if bitemark research is to be conducted on live human subjects.

Giving consideration to human research subjects goes back to the days following World War II when 26 Nazi scientists were held accountable for performing experiments on prisoners of war. Their trial at Nuremberg resulted in the first internationally recognized code of research ethics authored by the Nazi War Crimes Tribunal. The "Nuremberg Code" established the principles of voluntary consent which included: consideration of the capacity of subjects to consent, freedom of subjects from coercion, a comprehensive analysis of the risks and benefits of the research, minimization of risk and harm to the subjects, experimentation by qualified investigators, appropriate research designs, and the freedom of the subjects to withdraw at any time.

In 1974, the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research was established and the National Research Act was passed by Congress. The Act established the Institutional Review Board (IRB) and required all federally funded research projects involving human subjects be reviewed and approved by the IRB. In 1979, the Commission issued the Belmont Report which revised the principles to include all human research. The principles identified by the Commission fell into three basic categories: autonomy, beneficence, and justice. The Report provided guidelines to ensure research subjects' safety, total freedom of choice, and protection of vulnerable populations from coercion.

Much of the research on bitemarks has been done using live, anesthetized animals, and human cadavers. There has been significant insight gained from this research but there may be a limit to the extrapolation of the results from these models to live human victims. At some point consideration of using live human subjects will likely need to be considered. Currently, opinions by forensic experts on the ethics of this

endeavor appear to be varied. The risks associated with bitemark research falls far short of the risks associated with many other human research projects. However, most would agree that producing a bitemark in a live human subject would require inflicting a brief but significant amount of discomfort. So the question becomes: is bitemark research on live human subjects ethical and if so, what ethical considerations are involved?

One ethical consideration is research design. Will the design provide useful, reproducible results? Have specific parameters been identified and can they be measured? Will it provide a favorable benefit- to-risk ratio? Have sufficient measures been taken to minimize potential harm to the participants? All of these need to be answered to the satisfaction of the IRB overseeing the research. The other, and for most researchers the most important consideration, is the recruitment and selection of volunteers. Great care must be given to this process to ensure absolute autonomy for the participants. This involves recruitment without pressure or coercion, disclosure and explanation of the research design including adverse effects (i.e., pain and bruising), a signed informed consent that includes complete disclosure of the risks, the ability to withdraw at any time without prejudice, and extreme care in the use of potentially vulnerable populations. Vulnerable populations are populations that can be more susceptible to pressure or coercion. These populations include children, the elderly, prisoners, the mentally or socially impaired, and, in the case of research by university faculty, students. IRB oversight is extremely important in any research involving live humans to prevent under-compliance of these critical ethical considerations.

The ethics of using live human subjects for bitemark research is a complex issue that will need to be decided by the forensic dental community. Discussion should be held in an attempt to arrive at a consensus on the ethics of this type of research.

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**Research, Institutional Review Board, Ethics**

## **F6 Bitemarks in Perishables: 3D Laser Scanner Analysis**

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After attending this presentation, attendees will understand how new technologies, especially laser scanner 3D modeling of dental casts, can enhance the study of bitemarks. The sectioning performed allows an approach to further development in the study of bitemarks to help investigators and magistrates to identify the perpetrators of such injuries.

This presentation will impact the forensic science community by showing how this photographic technique provides a reliable system to use in the field or laboratory. It has been tested within the Forensic Science Institute of the French Gendarmerie (I.R.C.G.N). The practical applications presented can be used as a guideline to forensic odontologists.

In 1997, C. Georget and W. Baston compared experimentally produced bitemarks left in food with the dental casts of two suspects.

The pieces subjected to analysis were: (1) a piece of cheese with bitemarks; and, (2) study models of maxillary and mandible dental arches of two suspects.

The first step is to make a molding to duplicate the shape of the bitten substrate. The cast of the impression is then made of resin (resin INTERIM CD / SPAD) and is duplicated.

The observation of cast supporting bitemarks objectives evidence of marks indentations, the traces left on the edge of the food, tearing the material. The cast also specifies the dynamics of the bite (lateral movement and slippage) specific to the individual who has bitten. The meeting point between the upper and lower teeth are visible in the form of a bead. This

observation also helps to identify the visible teeth on the food. The shape of the teeth can give the orientation of the object in the mouth.

In 2011, Georget and Conigliaro repeat the previous studies.

The contribution of new technologies and especially the use of laser scanners assist in the production of fast and easy 3D models of the dental casts and perishable substance.

The use of a 3D laser scanner has advantages. The print is done without any risk of deformation due to the pressure of a conventional impression material on the perishable substance. Furthermore, as no impression material is used there is not risks of the material tearing. The average ambient temperature of the enclosure of the scanner is 22°C (74°F). This temperature does not alter the sample to be analyzed during the impression taking. In extreme cases, a fragile sample can be placed on a refrigerated pedestal. The shape of the food is preserved.

This non-destructive method enables the production of sections according to the needs of the forensic odontologist. The digitalized dental cast is available for further examination due to the non-destructive nature of the methodology.

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**Bitemark, Perishable, Noninvasive Analyze**

## **F7 The Uniqueness of the Human Dentition: Fact or Fiction?**

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After attending this presentation, attendees will be aware of both the difficulty in attempting to prove the proposition that the spatial arrangement of the human dentition is unique as it applies to bitemark analysis as well as the ultimate irrelevance of such a proposition to both forensic scientists and the law.

This presentation will impact the forensic science community by presenting attendees with examples from not only the forensic field, but general mathematical and philosophical principles that support the concept that uniqueness is an unattainable, irrelevant, and unprovable concept in bitemark analysis and other forensic areas. This will serve to highlight the fact that there is no need to resort to uniqueness principles in order to bolster claims of identification in courts of law or elsewhere. Such claims do not strengthen the position of odontologists as expert witnesses and do nothing to increase the reliability of the discipline as required under current evidence law mandates.

Fingerprint and handwriting analysts, firearms and tool mark examiners, and forensic odontologists often rely on the uniqueness proposition in order to support their theory of identification. In forensic odontology, several articles are commonly cited as providing evidence for the geometric uniqueness of the anterior dentition. These articles were reviewed in order to assess this claim, and benchmarked against more than thirty articles claiming to prove the uniqueness of other forensic traits. The literature providing support for uniqueness in forensic odontology is comparatively weak and archaic in its methodology; however, articles in all forensic disciplines suffer flaws that negate the conclusion that any forensic feature is unique. The sources cited as contributing towards the evidence for uniqueness include the anecdotal and experiential, biological, and mathematical, yet all of these approaches suffer disadvantages that result in little faith being able to be afforded to their conclusions. These included the employment of unrealistic assumptions, erroneous mathematics, and the drawing of illogical conclusions from experimental data. The finding of uniqueness in any study appears to be an overstatement of the significance of the results, and in several instances, this claim is made despite contrary data being presented. Recently, studies have been published regarding the uniqueness of the dentition, and these have definitively falsified the notion that the spatial arrangement of the dentition is quantifiably unique.

Both the mathematical and philosophical viewpoint regarding uniqueness is that obtaining definitive proof of uniqueness is considered impossible by modern scientific methods. More importantly, there appears to be no logical reason to pursue such research, as commentators have convincingly established that uniqueness is not a necessary requirement for individualization or identification by the forensic expert. In fact, such questions broach the scope of the expert witness, and should properly be left to the trier of fact. The courts have accepted this in several recent cases in the United States, and have dismissed the concept of uniqueness as irrelevant to more fundamental questions asked of forensic expert witnesses. Current concepts in evidence law mandate that the expert witness's testimony must be found to be relevant and reliable, and neither of these concepts are supported by statements of uniqueness. Odontologists would be better to focus their efforts in both research and the courtroom elsewhere in order to truly improve the reliability of the discipline.

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**Odontology, Uniqueness, Forensic Science**

## **F8 A Bitemark Classification That Makes Sense**

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After attending this presentation, attendees will learn classifications for logical and simple bitemark categories. Attendees will also learn the difference in each of four distinct types of human bites on human skin. The application of a classification in the practice of forensic odontology should help to standardize and better organize the science.

This presentation will impact the forensic science community by simplifying and categorizing bitemarks. When forensic odontologists can all speak the same language in bitemark analysis it will make this science more understandable. By applying a logical classification to bitemark analysis, errors in interpretation should be greatly reduced. Other disciplines all use classifications for logical separation and for impact in identification of specific procedures or categories.

In the literature, bitemarks are usually not referred to by a classification but are described as good, poor, limited evidentiary value, distorted bitemark, high evidentiary value, diffused, healed, etc. How much more professional would it be to group the bitemarks into a logical, simple, and understandable group. In most areas of science similar items are classified. For example; in dentistry, there are classifications of fillings, i.e., Class I, II, III, IV, and V. All dentists understand what falls into these classifications. They are used for the insurance industry and all speak the same languages. The orthodontist classifies occlusion into Class I, II, and III. All those in the discipline understand what the occlusion will look like in these classifications. So why have the forensic odontologists not used classifications for bitemarks? In the 1980's Dr. Ray Rawson proposed a classification for bitemarks which consisted of a total of six classes. To my knowledge it has never been used. Why? Because it was too specific, complicated, and not user friendly. However, bitemarks can be classified into four simple, clear, and logical classifications. If used by all forensic odontologists everyone would for once be speaking the same language. These classifications would make it clear to other disciplines what is being referring to; specifically for the legal profession, police, and medical examiners.

The most logical classification for human bitemarks on human skin should be based on appearance or lack thereof, of the pattern injury. The following are examples that have proven to be effective in practical application:

**Class I:** This is the diffused bitemark. The one that lacks individual characteristics and has limited class characteristics, sometimes referred to as a bruise, diffused bitemark, a smoking ring or, a faint bitemark.

**Class II:** This pattern injury has some individual tooth characteristics, some class characteristics, sometimes referred to as the "single arch bite" or the partial bitemark. This type of bite can be seen in human bites through

clothing or where several teeth are recorded such as teeth marks with a fist blow to the face or where the individual has an object over one arch when the bite is inflicted when only the opposing arch would mark.

**Class III:** In this classification, there are both individual tooth characteristics and class characteristics present. This type of bite is one that is used most often for comparison purposes and has great evidentiary value. This type of bite is usually found on the body part that has the least amount of tissue distortion such as buttocks, shoulder, an upper arm, or the chest. In this classification of bites, the pressure is often held for a long enough period of time and with a deep enough penetration of tissue to record the lingual surfaces of the anterior teeth.

**Class IV-** In this classification, the bite has caused avulsion or laceration of the tissue. Usually there are no class or individual tooth characteristics present. This type of bite is most common where scarring occurs or where there is avulsion of an ear or finger. This bite is used to demonstrate that in a bitemark, one has a permanent injury which will elevate a charge of battery which is a misdemeanor to a third degree felony aggravated battery.

If one avoids complications by adding sub classifications to the classes then this method of classification of bitemarks is one that all forensic odontologists should be able to live with and more importantly use as part of their analysis and report documentation.

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**Bitemark, Odontology, Classification**

## **F9 Developing the Evidence Base for Forensic Science – The Systematic Review**

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After attending this presentation attendees will: (1) understand the nature of evidence hierarchy; (2) understand the principles of systematic reviews; (3) be able to assess a forest plot; and, (4) be aware of the need for protocol development in reviews.

This presentation will impact the forensic science community by beginning the process of defining topics for systematic review in the forensic sciences to develop a robust research base.

With a rapidly expanding number of journals and publications it is becoming impossible for health care professionals to assimilate new research findings, assess their value and determine if their conclusions should impact on clinical care and practice. In response to this, literature reviews have always been popular methods of presenting summary findings to readers; however, narrative reviews are prone to bias and often reflect the opinions and philosophies of the authors. Out of this concern was developed the systematic review – a review with a robust protocol, inclusion and exclusion criteria for publications, and a statistical approach to meta analysis of results. Leading the field in systematic reviews is the Cochrane Collaboration, an international network which assesses the impact of randomized clinical trials on patient care.

Forensic science, and its application within judicial systems, can have a significant impact on the lives of those it touches and therefore should be held to no lesser a level of scientific scrutiny than medical interventions and therapies. The National Academy of Sciences Report has clearly identified concerns over the quality of research in forensic science and there is a need to take a systematic approach to define the literature, consider meta analyses of findings, and report recommendations. Cochrane reviews have changed the way medicine is practiced and a similar approach to forensic science seems timely.

While peer reviewed scientific evidence in the form of research publications can easily be rated using Sackett's hierarchy of evidence (with

systematic reviews being considered the highest level) forensic science is executed in two arenas – the peer reviewed journals and the judicial system. The opinions, judgments, and reviews that are conducted within the court system cannot be ignored nor can any positive or negative outcomes produced as a result of using a particular forensic science be ignored. One would not adopt a medical intervention that worked in the laboratory but that killed patients in the operating theatre.

Forensic science is rarely appropriate for testing using randomized controlled trials. As such, the Cochrane methodology is inappropriate. However, a sister organization, the Campbell Collaboration offers a review framework that is ideally suited to assessing forensic sciences and providing guidance, recommendations, and future research paradigms that will help strengthen the individual disciplines.

Part of a robust systematic review is the development of a protocol. In this presentation the nature of systematic reviews will be described along with their impact in the medical space and how homogenous evidence is combined in meta analyses. The protocol for a systematic review of bitemark evidence will be presented demonstrating the search protocol, the inclusion and exclusion criteria and the literature areas to be covered. Forensic science needs to meet the highest level of scientific evidence and the development of a suite of systematic reviews, with robust and agreed protocols, is one such way of achieving this.

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**Odontology, Review, Systematic**

## **F10 Disaster Victim Identification (Dental) Following the Christchurch Earthquake in February 2011**

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After attending this presentation, attendees will gain a brief overview of the devastating magnitude 6.3 earthquake in New Zealand, on February 22, 2011, which shook the city of Christchurch to its core, causing death and destruction. Attendees will gain understanding of the successes and difficulties with identification (focusing on dental identification) encountered following this mass fatality incident that claimed the lives of 181 people and injured many more.

This presentation aims to give a brief overview of the disaster and to share with the attendees the difficulties and successes encountered with the dental identifications. It will show the problems facing Christchurch for the future and the importance of having Disaster Victim Identification response plans and personnel in place to respond rapidly and effectively.

This years Academy theme is based around the global reach of the forensic sciences. Odontology's contribution to international forensic efforts is never clearer than when forensic dentists work in teams to assist the identification of victims of mass fatality events. While personnel may be local to the disaster, there are many instances when international assistance is gained. Irrespective of the nationality of forensic dentists, all are influenced by the development of standards, protocols, and IT systems that have been developed and enhanced through the efforts of those attending previous DVIs. Indeed, this interactive process of development is essential – some good must come from these devastating events.

Within a few moments Christchurch would be reduced to rubble with hundreds of aftershocks during the following months causing further distress and damage. The emotional, structural, and financial devastation will leave its mark on New Zealand for years to come. The collapse of the Canterbury Television Building and subsequent fire would claim the most lives - mainly young people and many from other countries.

New Zealand declared a state of national emergency and teams rapidly deployed to assist in whatever way they could. Recovery teams began the grim task of locating and transportation of the deceased. A temporary mortuary was established at Burnham Military Camp in preparation for the postmortem gathering of information and relatives were

approached for antemortem information. The different forensic disciplines worked long hours to establish identification of the victims.

Despite incineration, co-mingling, and trauma of the remains, forensic odontology would prove to be a useful identification method alongside fingerprints and DNA. To date only four persons have not been identified, but the work goes on to return every victim to their families. A devastated city fights to return to a semblance of order from the chaos of that ill-fated morning earlier this year.

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**Disaster Victim Identification (Dental), Christchurch Earthquake, February 2011**

## **F11 Classification of Human Remains — A New Classification That Describes the Condition of Human Remains in Simple Form**

*William E. Silver, DDS\*, 10 Edgewater Drive, #5G, Coral Gables, FL 33133*

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The goal of this presentation is to provide a simple method of describing the condition of human remains so that this information may be communicated effectively to other persons in the same or different disciplines. This is particularly applicable within the facial area. The interrelationship between the jaws will also be discussed.

This presentation will impact the forensic science community by allowing professional forensic operators from different areas of interest to describe the condition of human remains within the identifiable facial area by a simple method with the use of a numerical system that would describe those remains in terms that would not require the use of a particular language. This also allows for the entry of descriptive terms for computer entry by using numbers instead of phraseology that might otherwise be confusing to those who speak a different language. Examples of each classification and the dental relationship between the jaws will be demonstrated.

Acceptance of a standard of classification for human remains is essential to communicating among persons who speak different languages as well as those who speak the same language. The reduction of an object's description to a numerical evaluation simplifies and also allows for ready entry into the language of computers.

Orthodontic classification has long been the standard using Class I, Class II, and Class III to describe the anterior-posterior relationship of the maxilla and mandible. This classification system may also be helpful in describing the dental condition for identification purposes as well. When examining the dentition of an individual, the relative position of the upper and lower jaws will be reflected in the facial features, which may assist in the identification process and should be noted in the examination of human remains. In a Class I, dental relationship the upper first molar is slightly distal to the lower first molar. This is considered to be the normal relationship between the jaws and may result in a regular, spaced or crowded alignment of the teeth. In a Class II, dental relationship the upper first molar is mesial to the lower first molar, usually resulting in a maxillary protrusion or mandibular retrusion—except when the upper central incisors are retrusive and the upper lateral incisors are protruded together with a deep vertical overbite. In a Class III, dental relationship the lower first molar is completely mesial to the upper first molar. This condition is referred to as mandibular prognathism and may result in an anterior crossbite when the lower anterior teeth are in front of the uppers.

Class I, Class II, and Class III was used as the basic classification of human remains with particular reference to the dental and facial condition.

**Class I:** Refers to a fresh condition of the tissues when the facial structures are in good condition and could be readily observed and possibly used for visual identification. Any incision or removal of the facial tissues that would impact the ability to make an identification is contraindicated.

**Class II:** Would include facial features that had been appreciably lost due to severe decomposition over time, fire, or extended immersion in water. It would also include remains from severe facial trauma as a result

of a free fall, high speed automobile accident, or plane crash. These remains would not be in a viewable condition and would require surgical removal of tissue and debris as well as possible jaw removal procedures for examination of the dentition and possible identification.

**Class III:** Would be skeletal remains that were no longer covered by soft tissue and the dentition present in its entirety or fragmented.

**Classification, Dental, Remains**

## F12 Dental Ethnic Mutilation

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After attending this presentation, attendees will understand how dental mutilation may help identify a victim. They are a sign of identification between the tribes and even within a tribe. For this purpose, a software location of different types of mutilation still practiced today was established.

This presentation will impact the forensic science community by demonstrating how to identify a victim outside the conventional methods of identification based on data characteristics of the different ethnic groups still existing in the world.

The tooth is a particularly powerful symbol. Its exceptional hardness makes it possible to resist the vagaries of time, even over many hundreds of years. Thereby, it has become a symbol of strength, power, and even eternity. The use of intentional dental mutilations in humans only constitutes one chapter of a larger history, the one of body mutilations on the skin (tattoos, scarifications...), the genitals (castrations, excisions...), the feet (deformities, toe cutting...), the neck (the giraffe women), or the head (deformity of the skull...). Dental mutilation can be described as a voluntary partial or full amputation of one or several teeth mainly of the maxillary or mandibular incisivo-canine teeth.

Although customary dental mutilations are disappearing, they can still be observed in many ethnic groups in Africa and throughout the world. These mutilations, under very various forms from strategic dental extractions to modifications of the shape of the tooth or covering with diverse materials, are highly varied and are of great interest. There is no doubt that these mutilations have several characteristics: standards of beauty, initiation rites, warrior symbols, cultural, and religious character, in a wide meaning or symbolism. Distinguishing signs for some, religious or ethnic for others, the dental mutilations and sometimes the dental adornments can assist in the identification of an individual when one has no dental record or ante radiographs (which unfortunately happens in many countries).

The originality of this topic is in the presentation of software specifically created to associate a dental mutilation to an ethnic group, in relation to a country in order to identify the dental mutilation(s) with the goal to the identification of the individual.

As examples, there are different shapes of dental cuts existing in Africa and Indonesia:

- The shape of a saw with two separate tips: ethnic group of the Bakougnis in Gabon
- The shape of a saw with 2 or 3 contiguous tips: Congo, Togo
- The shape of an axe: Tchad
- The shape of a tip: the bantou and pigmy ethnic groups of Congo, the Mossi ethnic group of Burkina Faso
- The shape of niches: the Dzems ethnic group of Gabon
- The straight shape: Bali (Indonesia)
- Creation of inter incisive artificial diastema by avulsion between the maxillary and mandibular central incisors: the Massais ethnic group in Kenya, the Wollof ethnic group in Senegal, and the Hereros ethnic group in Namibie.

These multiple dental mutilations have been observed for centuries and in all areas of the world. In this presentation, dental mutilations still occurring today have been regrouped by classifying them according to their type (additive or subtractive dental mutilations) and according to their geographic location. The goal for the odontologist is to determine the ethnic origin of the human with the observed mutilation. For this, odontologists will be able to use the described computer program by completing the different sections describing the mutilation to determine the corresponding ethnic group as well as its geographic location.

**Teeth Mutilation, Ethnic, Dental Identification**

## F13 The Trabecular Bone in Identification — Part 2

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After attending this presentation, attendees will acquire new information regarding the use of trabecular patterns in the mandible for the establishment of positive identification.

This presentation will impact the forensic science community by providing new scientific evidence regarding the positive identification by trabecular patterns taking into consideration their variations in morphology and a method of calculating its significance.

According to Berkeley's Orthopaedic Biomechanics Laboratory, the trabecular bone can be classified as a porous cellular solid, consisting of an irregular three-dimensional array of bony rods and plates, called trabeculae, which are composed of a calcified matrix. Bone marrow fills the spaces of the pores. In addition, because all free bone surfaces are covered with bone cells, bone is a living tissue that is self-healing and has the ability to adjust its morphology in response to changes in its mechanical environment, the so-called but poorly understood phenomenon of bone remodeling. As such, the mechanical complexity of this two-phase biological tissue surpasses any engineering material making it a fascinating subject of study regardless of clinical applications.

Dental identification compares postmortem to antemortem records. It involves the analysis of different factors such as: the presence and the absence of teeth; crown and root morphology and their interrelationships; the evaluation of the periodontal status; the type and extent of restorative and endodontic materials; fixed, removable, and implanted prosthetics; tori and sinus configuration; anomalies and pathologies of teeth; and, bone as well as trabecular pattern morphology.

Few studies have been completed on the statistical reliability of trabecular bone patterns for identification purpose. Mann's research indicated that radiolucencies and radiodensities in the distal femur and proximal tibia are valid individualizing features for establishing a positive personal identification in human remains;<sup>1</sup> Hiss and Kahana used the densitometric analysis of the trabecular bone pattern as a sole means of identification that was confirmed later with two other methods;<sup>2</sup> Kahana, Hiss, and Smith's research concluded that the trabecular architecture is unique to each individual and stable enough to be used as a forensic marker for positive identification of human remains;<sup>3</sup> and, Couture, Whiting, Hildebolt, and Dixon studied the alveolar trabecular bone in radiographs.<sup>4</sup> On the other hand, other related studies discussing the radiographic recognition of dental implants,<sup>5</sup> the morphometric analysis of intra-oral radiographs of unrestored teeth,<sup>6</sup> the computer-aided dental identification,<sup>7</sup> the sensitivity, specificity and reliability of radiographic periapical diagnosis of posterior teeth,<sup>8</sup> the root morphology and anatomical patterns in forensic dental identification,<sup>9</sup> and the dentists' qualifications affecting the accuracy of radiographic identification have also been carried out.<sup>10</sup>

As a continuation of the preceding research, "The Trabecular Bone in Identification," the current research focuses on trabecular bone pattern

morphometric analysis and comparison as a viable and empirical method of positive identification. It involves the collection and analysis of panoramic, apical, and bitewing radiographs from the same patient over a number of years as well as from different patients. Locating, identifying, marking, and measuring common sets of trabecular patterns for each patient's radiographs, and determining whether trabecular patterns are unique to that patient, and if and how they are affected by the turnover rate in bone remodeling.

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**Forensic Odontology, Positive Identification, Bone Trabeculae**

## F14 Forensic Dental Data Standardization for Law Enforcement Applications

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After attending this presentation, attendees will be introduced to the various standardization activities currently underway to specify forensic dental data interchange and to cover the differences, similarities, and proposed harmonization of efforts in this area.

This presentation will impact the forensic science community by highlighting the numerous forums working to develop a standard for forensic dental information exchange: INTERPOL; the American Dental Association; the DVI community; and, the National Institute of Standards and Technology Information Technology Laboratory (NIST-ITL). Although the business users involved in each of these efforts are different, their data needs are similar. The creation of a common dataset among these various bodies would not only coordinate work efforts, but would benefit the forensic dental community by creating a common definition for data and a common way of specifying and defining that data while still allowing different encoding formats. This would lead to greater inter-operability of systems and streamline law enforcement and forensic efforts involving the exchange of dental information.

The American National Standard Institute/National Institute of Standards and Technology-Information Technology Laboratory (ANSI/NIST-ITL) publishes a biometrics-oriented standard known as the NIST Special Publication 500-271 ANSI/NIST-ITL 1-2011 American National Standard for Information Systems—Data Format for the Interchange of Fingerprint, Facial & Other Biometric Information that is used by Law Enforcement Agencies in over 100 countries. This standard defines not only the dataset for forensic information, but the formats and the data specifications to exchange biometric information on such metrics as friction ridge data for fingerprints, palm print, plantar prints, as well as facial metrics, and iris recognition data. It also covers the transmission of scar, needle mark and tattoo images, and related data, as well as DNA information for automated recognition and forensic use. Domestically, it is used by the Federal Bureau of Investigation, the Department of Defense, the Department of Homeland Security, the Department of State, and all state governments, as well as most local police departments to transfer biometric information amongst law enforcement agencies. It also forms the basis for fingerprint submissions to INTERPOL and its member countries.

Following the 2011 update of the standard, ANSI/NIST-ITL would like to address the next biometric modalities such as voice and dental records, as well as define new metrics for describing bitemarks that are submitted for analysis. ANSI/NIST-ITL has established a working group that is currently chaired by a representative of the Government of Argentina, and involves specialists from around the world. The ADA Specification 1058 has served as a starting point for forensics data standardization, but the hope of ANSI/NIST-ITL is to not only define the dataset, but the electronic protocol in which to transfer the information.

The goal of this presentation is to call for the development of a unified set of electronic specifications to submit to INTERPOL and ANSI/NIST-ITL by bringing together all business users to form a common foundation for forensic dental data exchange and to attempt to unify the DVI needs with the needs of law enforcement and government. This will involve several different stakeholders: forensic dental specialists, information technology specialists, standard development organizations, law enforcement, disaster recovery organization representatives, and others in order to ensure that all of the various needs and requirements are met.

**Pub. 500-271 ANSI/NIST-ITL 1-2011, ANSI/ADA Specification No. 1058, Forensic Dental Data Set**

## F15 Contribution of Photographic Techniques in Automated Dental Identification

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After attending this presentation, attendees will be able to understand the use of automated photographic techniques of mandibles and maxillas in mass disaster identification.

This presentation will impact the forensic science community by exploring a photographic technique that provides a reliable system for use in the field or laboratory. It is currently being tested within the Forensic Science Institute of the French Gendarmerie (I.R.C.G.N). The practical applications presented can be used as a guideline available to forensic odontologists.

Photography is an important element of dental forensic examination. It is often required by magistrates and investigators because pictures are able to show the mandibular and the maxillary teeth, their characteristics, their pathologies, and dental treatments.

**Materials and methods:** The digital SLR camera with a 60 mm lens is mounted on a stand equipped with a light source. This device is coupled to a laptop computer equipped with a distance shooting software package. A mouse is activated with the foot. This provides a means of capturing an image. The operator takes the photo hands-free and frames by visualizing on the screen.

**Results:** The testing environment enabled the identification of some key points.

*Automation:* On the camera the function, AF coupled with the function S treats the sharpness of the image. Thus, for each shot, the auto focus and sharpness is achieved without any manual intervention from the operator.

*Handling:* Holding the object to be photographed in their hands, the operator chooses easily the exact plane of the desired image.

*Ergonomics:* There is no need to use two operators, a clean hand operator and a dirty hand operator, or to continually change gloves between dirty and clean interventions. An operator can easily work alone.

*Framing:* Direct vision on the screen enables the observation of a greatly enlarged scale image. Details can be highlighted.

*Speed:* The reduced need for focusing decreases the time taken considerably.

*Repeatability:* The foot control of the camera permits the repeated shots with ease.

*Cleanliness:* The issue of the cleanliness of the collection of images and contamination of the photographic material is resolved because at no point does the operator touch the equipment.

*Flexibility:* The final results can be verified immediately on the screen and the ability to correct mistakes is possible – simply by acquiring another image.

**Discussion:** The use of photographic equipment and shooting a predetermined four orientations can establish a standardized photographic record.

Thus for each mandible and maxilla an occlusal view, buccal anterior view and two lateral views are taken. Photographs of specific details are made if necessary. A photographic record for each victim is always produced and it contains all the photographs of the case.

When producing a report, the photographs of the occlusal surfaces are preferably framed and each tooth is identified according to the classifications used in countries where the expertise is conducted (FDI nomenclature, Universal system; Army system...).

Other photographs (front view, left and right side views, and photographs of details) are subject to a layout on a specific page.

The entire photographic record illustrates the dental status of the victim. It will support the comparison, along with radiographs and antemortem records.

**Conclusion:** A question emerges from this presentation – if sound photographic records can be produced – is a pictorial odontogram required?  
**Odontology, Photographic Technique, Computer**

## F16 A Novel Forensic Use of Clinical Odontological Imaging: Geometric Morphometric Analysis of Sexual Dimorphism in the Mandible From Panoramic Scanning X-Ray Images

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After attending this presentation, attendees will have a better understanding of the sex determination during the skull assessment.

This presentation will impact the forensic science community by introducing a novel method of sex determination in the process of human identification investigations.

The human mandible is routinely utilized as part of sex assessment in forensic odontological and anthropological practice. Various studies have pointed to the utility of morphological and metrical traits in the mandible, such as symphyseal shape, gonial angle and eversion, and ramus flexure among others in the assessment of biological sex. The research here presented utilizes geometric morphometric techniques to investigate and quantify shape and size variation in the morphology of the mandibular corpus and ascending ramus, and consequently highlights the potential for forensic human identification. The results of a novel morphometric study are presented using clinical panoramic scanning x-radiography, the goal of which was to develop a methodologically and statistically robust means of investigating biological variation in lower jaw morphology from a commonly acquired clinical data source.

As part of proof-of-concept, clinical digital orthopantomogram images (OPG's) were acquired from 50 male and 50 female adult participants from a modern Italian sample population. Ten type I and type II landmarks were applied to the symphyseal region and the condylar and coronoid processes of the resulting 2D images in order to anchor a framework of semi-landmark curves. One-hundred equidistant semi-landmarks were established along the inferior border of the corpus, and the posterior border of the ascending ramus, thus encompassing the symphyseal region, gonial region, and posterior ramus – all of which are isolated anatomical regions which have been demonstrated to exhibit significant expression of sexual dimorphism in previous studies. The resulting landmark and semi-landmark configurations were subjected to Generalized Procrustes Analysis (GPA) with Full Tangent Space Projection. Principal Component Analysis (PCA) was applied in order to assess populational variation. Factor loadings were subject to Canonical Variates Analysis with stepwise and leave-one-out classification in order to assess the effects of sexual dimorphism on mandibular shape. The preliminary results showed individuals to be correctly classified for sex in 89.6% of cases (males were correctly classified in 90.1% of cases, and females in 85.6%).

A partial least squares (2-block PLS) method was further applied, in order to examine patterns of covariation between shape variables and the exploration of patterns of functional modularity. In this case, functional modules are assumed to be units within which there is a high degree of integration from many and/or strong interactions, but which are relatively independent of other such units. The nature of the interactions can be, for instance, developmental, functional, or genetic, depending on the context. Most interestingly the results indicate the greatest level of individual and sex-specific variation is found in the shape-curve and pattern of the inferior corpus, in contrast to that of ramal flexure. However, a moderate degree of modular integration between the corporal and ramal regions suggests that functional ties between the units are correlated in influencing sex-based morphological trait expression. Consequently such units may be studied together or in isolation, and this may allow for the development of identification criteria based on modular unit shape variables which may be

applicable for both whole specimens and fragmented remains depending on the forensic situation. Overall, the results are strongly significant and suggest dependently and independently that the shape relationship between the mandibular corpus and the ascending ramus offers significant power for forensic identification purposes.

This investigation was designed to introduce a more standardized method of sex determination in the process of human identification within the field of forensic dental radiology. Orthopantomogram images allow an objective and reproducible 2D images reducing observer bias especially when the analysis of the mandible utilizes geometric morphometric techniques. This study confirms that the mandible exhibits significant sexual dimorphism and that skull assessment of unidentified cadavers cannot leave aside the odontological investigation with the benefit of stored radiological images. Nevertheless, further assessment on a wider sample of OPG's should be carried out in order to increase the predictive accuracy of this novel methodology.

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**Geometric Morphometrics, Sex Assessment, Forensic Odontology**

## **F17 Comparing Cone-Beam CT With Conventional Digital Dental Imaging for Forensic Dental Identification**

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After attending this presentation, attendees will gain knowledge of the use of cone beam CT in forensic identifications.

This presentation will impact the forensic science community by understanding how the use of cone beam CT derived images in forensic identifications is a viable time saving and a resource sparing technique.

Studies have previously presented evidence showing the ease of use of cone beam computed tomography (CBCT) derived images for dental identifications. The data gained from CBCT can be formatted into images that appear similar to periapical, bitewing, and panoramic radiographs. To further test the hypothesis that CBCT derived images are similar enough to conventional digital images to allow use in forensic identification, an IRB approved survey was developed.

The survey presented sets of images, each with a CBCT-derived periapical or bitewing image ("postmortem") as an unknown as well as five conventional radiographs (either bitewing or periapical, "antemortem") which may or may not be a "match" to the unknown, all on a one page sheet of paper. The five radiographs reviewed for each case are from the same anatomic location, but may be from a different individual; or taken from a different angle; or include fewer or more teeth; or be a bitewing instead of a periapical. The survey will be administered to multiple groups of dental practitioners, including dental students, post doctoral residents, practicing dentists and forensically trained dentists.

Five human jaw dissection specimens were scanned using an i-CAT cone-beam CT system and digital periapical and bitewing radiographs were taken of all dentate alveolar bone areas of the specimens. For each conventional radiograph, an image representing the same anatomical area was generated from the CBCT data set, as described previously. CBCT-derived images were cropped to the field of view of a conventional dental radiograph and to an aspect ratio of 4:3. CBCT-derived and conventional images of same anatomic areas of the specimens are presented in a survey alongside de-identified conventional radiographs of the same general region from patients with similar clinical dental situations (distracter images).

The results of the survey study will be presented and show the matching accuracy of the CBCT-derived image with the correct conventional image of the same specimen. In addition, each subgroup will be evaluated against the others to determine if the ability to correctly identify the "match" image with the unknown is a factor of years of experience in dentistry, experience as a forensic dentist or if a dental knowledge base is not required.

It is believed that CBCT derived images are similar enough to the conventional digital radiographs to allow their effective use in forensic identifications. Furthermore, it is believed that in situations such as mass disasters, the use of CBCT derived images would allow for rapid imaging of the deceased, allow reviewers to be located at a distance from the disaster site and allow for adjusting the viewing angle of the CBCT-derived ("postmortem") images to approximate that of the comparison ("antemortem") images.

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**Cone-Beam CT, Dental Identification, Radiographs**

## **F18 Posthumous DNA Analysis Proves Equivocal Bitemark Analysis**

*Roger D. Metcalf, DDS, JD\*, Tarrant County Medical Examiner's District, 200 Feliks Gwozdz Place, Fort Worth, TX 76104*

The goal of this presentation is to remind attendees that not all high-profile post-conviction DNA analyses result in exoneration of the convicted person.

This presentation will impact the forensic science community by serving as a cautionary tale by recalling the facts around a case where the bitemark and palm print evidence used to secure a conviction in a capital murder trial might be considered equivocal, but, ultimately, post-conviction and posthumous DNA analysis showed the conviction was not improper.

In July 1989, Jesse Joe Patrick broke into the home of a neighbor, 80-year-old Nina Rutherford Redd, through a bathroom window. Redd was sexually assaulted before having her throat slashed. Patrick ransacked the home before leaving and was later arrested in Mississippi. A blood-soaked sock was found in the home of Patrick. DNA matched the DNA in Redd's blood sample. Patrick's live-in girlfriend identified the knife found at the scene as theirs. Patrick confessed to the crime shortly after his arrest, but later recanted. Patrick had previously been convicted of aggravated assault in 1986 and sentenced to four years probation, which was later revoked.

Patrick was convicted of capital murder in the 282nd District Court of Dallas County and sentenced to death on April 16, 1990. His conviction and sentence were affirmed by the Texas Court of Criminal Appeals on June 28, 1995, and his petition for *writ of certiorari* was denied by the United States Supreme Court on March 25, 1996. Patrick also filed a state *habeas corpus* application, which the Court of Criminal Appeals denied on April 22, 1998.

Patrick then filed a federal *habeas* petition, which the district court denied on Aug. 23, 2000. After the district court disposed of several post-judgment motions filed by Patrick, he attempted to appeal to the United States Court of Appeals for the Fifth Circuit, but both the district court and the Fifth Circuit denied him a certificate of appealability to do so. He then filed a petition for *writ of certiorari*, which was denied by the Supreme Court on September 12, 2002. Patrick's motion for a stay of execution was also denied on Sept. 12, 2002.

In addition to his appeal and *habeas corpus* proceedings, Patrick filed a motion for DNA testing in the state trial court. Although sperm had been found on the victim, a DNA analysis had not been performed. Following a hearing, the trial court ruled that Patrick was not entitled to testing under Chapter 64 of the Texas Code of Criminal Procedure because there was no reasonable probability that favorable DNA results would have led to an acquittal—Patrick had not been charged with a sexual assault.

Since Patrick was willing to pay the costs of DNA testing, the court did rule he could have testing at his expense. The State appealed that ruling

to the Court of Criminal Appeals and also filed a petition for *writ of mandamus* to force the trial judge to rescind her order. On September 11, 2002, the Court of Criminal Appeals dismissed the State's appeal but also granted the requested *mandamus* relief. The court held that because Patrick did not meet the statute's requirements, he was not entitled to testing regardless of whether he was willing to bear the costs. Jesse Joe Patrick was executed by lethal injection on Sept. 17, 2002.

Craig Watkins was elected Criminal District Attorney for Dallas County in 2006. Mr. Watkins promptly established the Conviction Integrity Unit at the Dallas DA's office in 2007, the first of its kind in the country. This Unit is responsible for the post-conviction review of more than 400 Dallas County cases in conjunction with the Innocence Project of Texas (IPOT) and in accordance with the Texas Code of Criminal Procedure, Chapter 64 (Motion for Forensic DNA Testing). In addition to the IPOT project, the Conviction Integrity Unit investigates and prosecutes old cases (DNA and non-DNA related) where evidence identifies different or additional perpetrators.

Dallas County has been at the forefront of testing and retesting DNA evidence from old criminal cases. One important reason for this, it is believed, is that the Southwest Institute of Forensic Sciences in Dallas has historically been very diligent about retaining and properly storing evidence—evidence that might well have been discarded in other jurisdictions.

Patrick's case was one of the initial 400 Dallas cases selected for review for potential DNA testing/retesting of biological evidence. The review was by IPOT student volunteers supervised and directed by the Conviction Integrity Unit's attorneys. The initial IPOT volunteers participating in this project were primarily from Texas Wesleyan University's School of Law. Even though Patrick had been executed in 2002, his case was nonetheless included in the initial 400 cases because it met a threshold screening criteria of apparently having suitable biological evidence still available for testing. As his case was being investigated by the law students, it was discovered that the physical evidence that had been admitted at trial—a palm print analysis and a bitemark analysis—might be considered equivocal by some, and it was decided to proceed with a DNA analysis of the remaining biological evidence (*please note: the original bitemark analysis was not performed by any current Diplomat of the ABFO or member of AAFS*).

Ultimately, results of DNA analysis of the sperm found on the victim's body "could not exclude" Patrick as a potential DNA contributor. It is left to wonder "in the end, did we arrive at the correct result, but by the wrong route?"

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**Bitemark, DNA Analysis, Postconviction**

## **F19 Guide of International Dental Chartings Translated to English – Decoding International Antemortem Dental Chartings for INTERPOL Disaster Victim Identification Forms (F2)**

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*Scheila Manica, MSc\*, University of Glamorgan Wales, 53 Street Dunstons Avenue, London, W3 6QD, UNITED KINGDOM*

After attending this presentation, attendees will be aware of a wide variety of symbols and abbreviations in different languages present in various dental chartings from INTERPOL member countries.

This presentation will impact the forensic science community by presenting a guide that could be a source of consultation for different needs such as: dental identification on foreign patient in mortuaries (when previous dental records are not written in English); and, additionally by aiding dental teams working for the antemortem dental records section in disaster victim identification in international scenarios while decoding international dental records.

The INTERPOL Disaster Victim Identification forms have a global standard of application for mass disasters and the collection of international antemortem dental records could be optimized by a guide of dental terminology applied on dental chartings from several languages to English.

The goal of this study was to analyze the tooth numbering system, symbols, and abbreviations used on dental charting worldwide. The countries studied are composed of the 188 INTERPOL member countries.

The logical approach to obtain current dental chartings from those countries was contacting national dental associations through the FDI (*Fédération Dentaire Internationale*) website. The letter addressed the goals of the project and requirements of samples of dental chartings. They were sent to the national dental associations email addresses and additionally by mail. In order to complement the amount of information, the letter was also sent to the twenty-one IOFOS (The International Organization for Forensic Odonto-Stomatology) members.

A total of 50 countries replied to the query. There was a wide variety of dental notations applied in a same country or the absence of those in other countries. Some of the samples were of little value; however, a fair amount of information and detail was present in most of them.

A literature review summarized the spoken language and density of dentists in each country as well as all the received information from the replies. This pointed out the two most used tooth numbering system in use namely: the FDI and the Palmer notations.

Focusing on the gathering of information for the filling of the INTERPOL DVI form F2 where the most important dental information is.

Twenty-four common dental alterations were selected such as: decay, fillings and prosthesis, and their symbols and/or abbreviations were summarized in ten languages. Some findings were very surprising, for instance: one determined symbol has two different meanings in different countries. Another example is the tooth surface nomenclature named "vestibular" which is not widely used in Europe.

This report also compares most of the historical tooth notations from Zsigmond (1861) to FDI two-digit (1971). After the results, it is also shown a percentage of the tooth numbering system most commonly used today.

This guide could be useful when the handwriting, symbols, and abbreviations on the antemortem dental chartings are not clear and this is paramount when antemortem x-rays and casts are not available.

**Disaster Victim Identification, Antemortem Dental Record, INTERPOL DVI Form**

## **F20 Protocol for a Systematic Review of Human Dental Age Estimation Studies**

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After attending this presentation, attendees will be informed step by step how a review of human dental age estimation studies can be performed and reproduced in an unbiased way.

This presentation will impact the forensic science community with a guideline classifying the different age estimation studies and related methods. It will reveal tools to compare and evaluate within each category the age predicting accuracy of the included study outcomes.

Teeth are assessed for age estimation because their changes in development, morphology, and biochemical structure can be related to the chronological age of an individual. Divers dental age predictors can be registered in a tooth conserving or tooth destructive way. The sampled data are statistically approached and analyzed to develop specific age estimation methods. Supplementary information related to the gender, ethnical or geographical origin, medical history and living condition of the sampled

individuals influences with variable weight the applied dental age estimation methodology. The different dental age estimation methods can be divided in specific groups: (1) methods based on developing teeth can be divided on the one hand in methods based on growth of all teeth except third molars and on the other hand methods based on third molar maturation; (2), methods based on mature teeth, contain a group of methods analyzing intact teeth and methods destructing teeth for age estimation examinations; and, (3), age can be predicted comparing a presented dental variable with a corresponding variable listed in common age related tables or atlases. In each of previous groups age is predicted following a particular method. This protocol aims to describe step by step the procedures to perform a systematic review of dental age estimation method studies in an attempt to classify the studies in defined groups enabling to evaluate the diagnostic accuracy of the included dental age estimation methods.

The systematic review will include studies presented to the scientific community by publication in a peer-reviewed journal, a book or in a doctoral thesis. All included studies should be written in English. They should describe the development, the evaluation or the comparison of dental age estimation methods, or they should report relations between dental variables and chronological age by means of common tables or a (n) atlas(es).

Two reviewers will screen, independently, in a first stage the titles and abstracts of all collected records and select papers for inclusion. In a second stage, full text of the studies selected in stage one will be screened and a lists of detected inclusion and exclusion criteria will be composed. Disagreements between reviewers will be resolved by mutual discussion.

The quality of each accepted study will be assessed, independently by two reviewers, using the Quadas tool. An initial search of Pubmed will be undertaken followed by an analysis of the text words in the title, abstract and the index terms used to describe the articles. Next, a search term will be established and used across the considered digital archives.

Out of each included study following characteristics related to the used dental age estimation method, together with related specific criteria will be extracted: the sample size; the considered teeth; the used dental variables; the dental variable outcomes; and, the study outcomes.

Specific outcome analyses will be established for each of the obtained dental age estimation study group in an attempt to compare the accuracy of age predictions between studies within a group and possibly between groups.

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**Forensic Odontology, Age Estimation, Systematic Review**

## **F21 Dental Age Determination – A New Software Tool**

*Kenneth W. Aschheim, DDS\*, 44 East 67th Street, New York, NY 10065*

After attending this presentation, attendees will become familiar with a flexible new software tool that automates the process of determining dental age from birth through the completion of third molar eruption.

This presentation will impact the forensic science community by exploring the next generation of dental forensic software for prototype programs that are being developed for proof of concept testing. One area of interest is the ability to automate the dental aging process. The goal of this presentation is to present a proposed dental age determination software prototype system for possible inclusion in a future version of morgue management software that is being developed.

The use of dental radiographs for age determination has been well documented. Tables, by Moorrees, Fanning and Hunt (1963) and Demirjian, Goldstein and Tanner (1973), have been a common references for the forensic odontologist in determining dental age. However, they are not routinely used in daily morgue operations because a simple software package to aid in visualizing tooth bud development does not exist, and the mathematical calculations are often tedious. Previous software for age determination analysis based on third molar development is available;

however, it does not do calculations based on succedaneous teeth in individuals with either a deciduous or mixed dentition.

The need for a software tool that was designed to automate the dental age determination process, as well as accommodate a greater range of ages, has led to the development of the Dental Age Determination (DAD) prototype software. Designed as a proof of concept project, it is hoped that it will ultimately be incorporated into currently available full forensic management software packages.

The software is divided into four sections: the demographic information section, the image manipulation section, the current dental assessment section, and the dental age calculation section. In addition, the software allows for user supplied “age-tables” based on ethnicity, sex, or any other custom metric.

The demographic information section utilizes standard fields for specimen familial data. It currently supports the Familial Data Set of ANSI/ ADA Specification No. 1058, the Forensic Dental Data Set. By allowing a one-to-one relationship with recognized data fields, future data transfer to other software products will be supported.

The image manipulation section allows for importation of multiple radiographic images and contains standardized tools to allow for magnification as well as scrolling for better visualization of the tooth bud stage determination. Importation of multiple images of standardized image formats (jpg, bmp, etc.) is currently supported. Future DICOM importation support is anticipated.

The key section for age determination is the algorithmic or current dental assessment section. Because recent studies have questioned the accuracy of the standard dental age estimation charts for children of different ethnic groups, DAD allows for the importation of user supplied multiple data sets which may be more accurate. The current prototype also displays both line drawings and radiographic samples of individual tooth bud development stages to assist in dental stage determination. This allows for the visual comparison of tooth bud stages to known standards.

The dental age calculation section is the final section of the software. This section performs an average dental age determination based on the forensic odontologist’s assessment of tooth bud development. In addition, it calculates simple statistical analysis of the data based on individual tooth bud development stage estimates. A final output screen displays the final calculation.

At the time of submission, the current version of DAD only contains a rudimentary reporting output, although a more robust system is anticipated as prototype development continues.

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**Dental Age Determination, Computer Software, Dental Informatics**

## **F22 Dental Age Estimation Combining Developmental and Morphological Age Predictors**

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After attending this presentation, attendees will be informed on the age predicting effect combining age related dental variables based on third molar development and on tooth morphology.

This presentation will impact the forensic science community by providing knowledge that, observed on orthopantomograms, data combining developmental variables of third molars and morphological changes of other teeth is not improving age predictions based only on third molar development.

Most frequently asked questions from judicial authorities regarding age estimations in living individuals is whether or not the age of majority

is reached. In most countries this age threshold is set at 18-years-old. For this sub-adult age category dental age estimations are most commonly based on developmental changes of the third molars. Compared to all other maturing teeth, third molars have the highest human variability, resulting in age estimations with wide prediction intervals. For the adult age category, dental age can be estimated examining diverse morphological tooth variables. One of them, apposition of secondary dentine on the walls of the pulp cavity can be evaluated in living individuals using radiographs. This technique was developed on periapical radiographs by Kvaal et al. (1995). Diverse authors studied the feasibility of the Kvaal technique on orthopantomograms. The goal of this study was to detect on panoramic radiographs if additional tooth morphological measurements according to the Kvaal technique ameliorate the accuracy of age estimations based on scores of third molar development.

Retrospectively, 450 digital orthopantomograms from individuals with a Belgian nationality and Caucasian origin were collected from the dental clinic files of the Katholieke Universiteit Leuven. In the age range between 15 and 23 years, 25 radiographs were selected in each age category of one year for both genders. All available third molars were classified and scored according to the ten stage system proposed by Gleiser et al. (1955) and modified by Köhler et al. (1994). The Kvaal technique was applied on the upper central and lateral incisor and second premolar, as well as on the lower lateral incisor, the canine, and the first premolar from the left side. On the obtained measurements, the mean of length and width ratios (M), and the difference of mean width- and mean length ratios (W-L) were calculated separately for each tooth, for all upper, for all lower, and for all six teeth. After one month, twenty randomly selected orthopantomograms were rescored and measured again by the same and another observer to detect a high intra- and inter-observer reliability. Linear regression models with age as response and third molar scores as explanatory variables were established for the whole sample and for males and females separately. To these models M and W-L measurements were added for each of the six measured teeth, for all upper, all lower and all six teeth together. From the models the determination coefficient ( $R^2$ ) and the root mean squared error (RMSE) were calculated.

The regression models calculated on the whole sample and including only third molar scores information revealed a  $R^2$  of 0.60 and RMSE of 1.63 years. Adding the Kvaal information (M, W-L) maximally increased  $R^2$  with 1% and decreased RMSE with maximally 0.02 years (for FDI tooth #22). The regression analyses performed separately for males showed comparable results. In the regression analyses based on females less accurate predictions were detected, most supplementary Kvaal information was observed adding the measurements of all six teeth.

Adding radiologically observed morphological secondary dentine apposition information to developmental third molar information was not providing more accurate age predictions. Moreover, the Kvaal technique was time consuming and not applicable on all panoramic radiographs.

#### **Forensic Odontology, Age Estimation, Third Molar Development and Kvaal Technique**

## **F23 Anthropological Measurement of the Juvenile Mandible and Dental Assessment Using Multi-Detector Computed Tomography**

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After attending this presentation, attendees will understand the principles of odontological and anthropological identification of juvenile remains, multi-detector computed tomography (MDCT), three-dimensional imaging, and the necessary research required for the implementation of a virtual anthropological examination for juvenile age determination.

This presentation will impact the forensic science community by providing vital data which supports the implementation of a virtual/near virtual anthropological examination for the identification of juvenile remains. As the primary demographic feature used in the identification of juvenile remains is age estimation, determining the accuracy and repeatability of measurements derived by traditional and MDCT measurements would be a vital preliminary step in validating the utility of MDCT analysis in special situations such as forensic and disaster victim investigations (DVI). This would accelerate the process of anthropological assessment and remove the necessity to deflesh remains, which may also be more ethically and morally acceptable, particularly when dealing with young victims and offers significant health and safety benefits for practitioners.

Anthropological examination of defleshed bones is routinely undertaken in medico-legal investigations to establish an individual's biological profile (age, sex, stature and ethnicity). However, when dealing with the recently deceased the removal of soft tissue from bone can be an extremely time consuming procedure that requires the presence of a trained anthropologist. In addition, due to its invasive nature, in some disaster victim identification (DVI) scenarios the maceration of bones is discouraged by religious practices and beliefs, or even prohibited by national laws and regulations. Radiological imaging modalities have been used in forensic practice as an adjunct to traditional anthropological techniques for many years. Currently, three different radiological techniques may be used in the investigative process; plain x-ray, dental x-ray, and fluoroscopy. However, recent advances in multi-detector computed tomography (MDCT) means that it is now possible to acquire morphological skeletal information from high resolution images, reducing the necessity for invasive autopsy procedures. Currently there is no standardised protocol for MDCT measurement of juvenile remains. In order for this "virtual approach" to be implemented and accepted internationally as part of a forensic investigation, accuracies must be shown to be comparable to that of traditional anthropological techniques. A series of studies conducted by the Developing Human Research Group, based at the University of Leicester, utilises skeletal material from the Scheuer Juvenile collection in order to construct a protocol for the measurement and age assessment of the entire immature human skeleton.

In this study, twenty juvenile mandibles were scanned using a truck mounted 16-detector CT scanner. This simple was used to construct a protocol for the measurement and age assessment of the immature human dentition. The results of this study illustrate that there is no significant difference between the measurements taken by MDCT and those by direct osteometric methods. MDCT had greater flexibility of measurements and offered the opportunity to take measurements not easily made on dry bone.

This research assesses the value of these new measurements in addition to considering the limitations and the potential applications of this virtual approach.

**Forensic Anthropology, Multi-Detector Computed-Tomography, Imaging**

## F24 Technological Advances in Forensic Odontology

*Irena Dawidson, DDS, PhD\*, The National Board of Forensic Medicine, Retzius v. 5, Solna, 17165, SWEDEN*

The goal of this presentation is to enhance the performance of the postmortem odontological investigation.

This presentation will impact the forensic science community by improving the equipment standards resulting in a swifter and safer forensic odontology investigation.

DMORT teams that exist in the United States are an exception that has no real counterpart in other parts of the world. In many countries the disaster victim identification organization is put together *ad hoc* when the fatal event occurs. Among the disciplines involved in the identification effort, the forensic odontology seems to have the least official affiliation in spite of its fundamental contributions to the establishing of identity. Subsequently, there's often little involvement of the proper agencies in the developing and modernization of the equipment and facilities to suit and enhance the forensic odontologists' working environment. That is ultimately left to the individual forensic odontologist with whatever means that are available, presenting financial problems as the forensic odontology quite often is a sideline occupation for dental professionals in academia or in private or community practice. Since their services are required infrequently it has not been possible to consistently develop the technology needed in the identification work, especially when large efforts are necessary in cases of mass disasters. The odontological identification work requires quite a lot of instrumentation, including x-ray machines as well as the radiographic reception media and very good illumination. All these devices have been traditionally gathered and/or built by the forensic odontologist, comprising among other things "home-made" portable x-ray machines on tripods, which would fall and break all too easily, portable "dark-rooms" for developing analogue radiographs, headlamps, and flashlights. All these things were heavy, cumbersome, and generally difficult to haul around as well as unreliable.

During the last decade the technology of the forensic odontology has gone through a dramatic progress diminishing the amount of equipment that has to be moved by about 75% in volume as well as in weight. Since the combined international effort in identification of the December 26, 2004 Bengal Bay Tsunami victims, there has been a swift innovation in the field of the forensic odontology.

The portable x-ray machine has become a handheld device that works on batteries. Also, replacing the analogue radiographs with digital radiography has reduced both the time needed for obtaining good quality radiographs as well as the quantity of machinery. A battery operated intraoral lamp that can be placed inside the oral cavity will provide bright lighting. Another common feature of these devices is that they can run on batteries for several hours making the forensic odontologist independent of external power supplies for at least a working day at a time. There is however, the drawback of the costs of these devices prohibiting many of the forensic odontologists from acquiring them. Neither the policemen nor the pathologists have to pay for their own equipment.

**Conclusion:** In order to improve the ability of the forensic odontologists to carry out their part of the identification process the concerned national authorities should be encouraged to obtain the modern equipment needed. Another advantage would be that compatible pieces of equipment can be assembled.

**Forensic, Odontology, Equipment**

## F25 Quality Assurance in an International Perspective and IOFOS Recommendations

*Tore T. Solheim\*, University of Oslo, Blindern, Box 1052, Oslo, 0316, NORWAY*

After attending this presentation, attendees will understand the importance of quality assurance in forensic odontology. Also the difficulties of quality assurance on an international level will be appreciated and knowledge of the IOFOS' work and recommendations will be acquired.

This presentation will impact the forensic science community by increasing awareness of quality assurance and systems for such assurance. Also the IOFOS recommendations may not be so well known in the United States, but could be of use also for American forensic odontologists.

According to the law in many countries, medical and dental work should be subject to quality assurance. Thus also forensic medicine and forensic odontology has a duty to implement systems for quality assurance. What quality assurance actually is may be considered differently and it also may consist of many different elements. All efforts to make sure the quality fulfill a minimum requirement and also effort to improve the quality may be called quality assurance. Most important may be descriptions of procedures to be followed in the work; however, also important is the quality of how the presentation is, for instance in the form of the quality of the written report.

Other aspects of quality assurance are quality improvement, registration of deviation from normal, and registration of accidents. Education of the expert and level of competency is part of quality assurance. Quality assurance may be a so called "paper tiger" if it is not implemented and followed up in some way. Quality assurance is the responsibility of leaders. Except for within an ID-commission, forensic odontologists operate on an individual basis. Thus there is no authority to control if procedures are followed.

To agree upon procedures on a national level is difficult. Therefore, in most countries each dentist must have his own procedure descriptions. It is even more difficult to establish procedures on an international level that people may agree upon to the extent that they are willing to follow them. Except for ethical rules there have been no known attempts to establish international systems of quality assurance. From the beginning, it was clear it would be difficult when the IOFOS decided to come up with recommendation for procedure descriptions in the various fields of forensic odontology in 2003.

In 2004, an international meeting was held in Norway with participating forensic odontologist from a number of countries representing all continents except America and Australia. It showed that they could agree upon very little and some would thing one step was important others would thing a different step was important. To reach some degree of agreement it was decided that only procedure steps should suggested on an international level, not how each step should be carried out. We felt that should be left to quality assurance in each country or society.

There was a great deal of disagreement about the steps. Only those where there was full agreement is a stage recommended as obligatory. Others, although reasonable enough, are only recommended and may be omitted. To implement the recommendations we advise each forensic odontologist to write in their report that they are following the IOFOS recommendations for quality assurance. Also we advise each society to see if they may accept these recommendations for their members and thus sign a contract with IOFOS.

**Quality Assurance, International, IOFOS**

## F26 A Twenty-Two Year Old Cold Case Homicide With Bitemarks

*William T. Lichon, DDS\*, 4028 State Street, Suite A, Saginaw, MI 48603*

After attending this presentation, attendees will realize that even a 22-year-old cold case can contain bitemark evidence which can be processed.

This presentation will impact the forensic science community by examining how even after many years, a cold case homicide can contain forensic odontology evidence that may be useful in the conviction of a long time suspect.

The year was 1989. Kim Currington was found by two of her children bludgeoned in her bedroom. Earlier that morning, the children, nine-year-old, seven-years-old, and a six-year-old, related they saw the boyfriend of their mother, stab her in the right leg during an argument. She screamed to the children to run to school. When two of the children returned from school near noon, they found their mother lifeless in the bedroom. They ran across the street for help and police were notified. The Saginaw Police Department arrived and secured the scene. They confirmed the fact a homicide had occurred. The crime scene investigators arrived and began to process the scene. Photos were taken of the exterior of the house and surrounding area. Following that, the interior of the house was processed. There were two entrances to the home. The entrance on the driveway side of the house revealed the door was locked. The front porch entrance door had a full size sofa placed directly in front of the door. The first responding officer related they had to push the sofa away from the door to gain entrance. There was no sign of forced entry on this door, plus the other house door was locked. Photographs were taken of the complete interior of the home. The windows in the home were all secure and no signs of a forced entry. There were two bedrooms; one for three children and a master bedroom. The children's bedroom appeared to be orderly and undisturbed. The master bedroom doorway was partially covered with a thin curtain. Upon entering, the bed was to the right with the victim lying face up on the bed with her feet near the floor. On examination of the victim, there were two large openings in the right lateral forehead, with other visible lacerations to the mid and left forehead. There was a large pool of blood around her head with blood splatter on the wall directly in the back. The victim was dressed in thin pink top and black dyed jeans. After crime scene photographs were completed the victim was transported to the hospital for an autopsy. After the clothing was removed, the pathologist noted a bitemark on the right upper arm and on the left breast. Swabs were obtained from both bitemarks. Photographs of both of the suspect bitemarks were taken with the ruler in place. Also noted was a stab wound on the right leg. There were multiple impacts to the head.

At the time, living in the house was the victim, the victim's three children, along with the victim's boyfriend. The boyfriend was questioned by the police and a signed search warrant was obtained for dental information. A local dentist at the time, who is now deceased, took upper and lower impressions of the suspect's teeth along with wax registrations. Photographs were also taken of the suspect's teeth. Later the photographs and dental models were taken to a forensic dentist in the Pontiac, Michigan area for evaluation. This dentist is now in poor health and has left the state. This was done on February 17, 1989. The case for a number of reasons was put on hold and not litigated. In 2009, the case was activated by the Michigan State Police Violent Crime task force, Cold Case Unit. The bitemarks evidence which had been stored in the evidence locker for over twenty years was examined. The dental models were wrapped in bubble wrap and in excellent condition. This interesting case was litigated in April of 2011 and concluded with a verdict of first degree murder.

**Cold Case, Homicide, Bitemarks**

## F27 The Joplin Missouri Tornado Disaster

*John E. Filippi, DDS\*, 1325 North 127th Avenue, Omaha, NE 68154; and Peter W. Loomis, DDS\*, New Mexico Office of the Medical Investigator, 700 Ranchitos Road Northwest, Los Ranchos de Albuquerque, NM 87114*

After attending this presentation, attendees will better understand how the forensic sciences supported the identification process in this particular mass fatality Incident (Joplin Tornado). The main focus of the presentation will be directed to the utilization of forensic dentistry in the human identification process.

This presentation will impact the forensic science community by enhancing a forensic team's knowledge (specific to odontologists) and the ability to better prepare and respond to future mass fatality Incident events.

The goals of this presentation are to familiarize and help the attendees understand the following:

- The destruction an EF5 (Enhanced Fujita Scale) Tornado can produce to a community and utilizing a short video.
- Discussion regarding who directs a forensic operation of this magnitude, via the Stafford Act, ESF #8 (Emergency Support Function), federal team assistance, and the assets that were deployed to Joplin.
- The timeline of the disaster, logistics and morgue operations, forensic science work stations, with special attention to the dental comparison analysis, utilization of the WINID3/DEXIS system and final identification results.
- How the federal disaster team supported the local coroner system with forensic services, family assistance, and multi-forensic science supported victim identifications.

**Background:** The 2011 Joplin tornado was a devastating EF5 multiple-vortex tornado that struck Joplin, Missouri late in the afternoon of Sunday, May 22, 2011. It was part of a larger late-May tornado outbreak sequence and reached a maximum width in excess of one mile (1.6 km) during its path through the southern part of the city of Joplin Missouri.

The Joplin tornado ranks as one of America's deadliest tornadoes and is likely to be the costliest, with an estimate of \$3 billion to rebuild Joplin. It was the first F5 or EF5 tornado in Missouri since the Ruskin Heights tornado struck south of Kansas City in 1957. It is also only the second F5 or EF5 tornado in Missouri history dating back to 1950. The May 2011 tornado was the deadliest tornado to hit the United States since 1947—the seventh-deadliest single tornado in U.S. history, and 27th-deadliest in world history. As of July 8, officials reported that 158 people died from the tornado, with another killed by a lightning strike during cleanup operations the next day.

The tornado intensified greatly as it entered a densely populated portion of the city at about 5:41 p.m. (C.S.T). Damage was widespread and catastrophic in residential subdivisions in the southwest portion of Joplin, which included the St. John's Regional Medical Center. Virtually every house in that area (near McClelland Boulevard and 26th Street) was destroyed and some were literally blown away in the area as well. Trees sustained severe debarking, a nursing home and a church school in southwest Joplin were also destroyed, and several other schools were heavily damaged along with numerous dental and medical offices.

As the tornado traveled across the southern portion of the city, heavy objects, including concrete parking bumpers, and large trucks, were tossed a significant distance, as far as 1/8 mile (200 m) away from a parking lot and some tornado debris was recovered as far as 75 miles away from Joplin. The tornado dissipated east of Joplin at 6:12 p.m. (CST) after approximately twenty-five minutes on the ground and the tornado's total track length was at least 14 miles long.

**Conclusion:** The presentation will also review the organization of the dental team, including the postmortem, antemortem, and comparison sections and the methodology used by them.

The experience gained from this unfortunate mass fatality incident (MFI) has enhanced the forensic team's knowledge and the ability to better prepare and respond to future MFI events.

**Forensic Odontology, Mass Disasters, Human Identification**

## **F28 The Identification of Burned Human Remains**

*James McGivney, DMD, 346 Tulip Drive, Webster Groves, MO 63119; and Eric S. Wilson, DDS\*, PO Box 50, Cole Camp, MO 65325*

After attending this presentation, attendees will; (1) understand the steps necessary to fully document the dental features of burned human remains; and, (2) will appreciate the difficulties encountered when working with burned human remains.

This presentation will impact the forensic science community by demonstrating the importance of step by step documentation of burned human dental features.

The identification of burned human remains is one of the most difficult tasks faced by forensic odontologists. In cases where there is extensive burning, the skin is charred and flakes off, bones become discolored, brittle, and fracture, and the crowns of teeth may crack or at times explode.

At initial recovery, the thermal destruction makes retrieval of all biological fragments difficult. In many cases small, yet important portions are missed, disturbed, or even destroyed during scene processing. A systematic, conservative approach to recovery and examination is needed to prevent the loss of valuable dental information before a thorough dental charting, intra-oral photographs, and radiographs can be obtained.

The lips, tongue, and cheeks initially insulate the oral cavity and present a barrier to heat and fire. The teeth can remain relatively undamaged while the rest of the body shows signs of extensive fire damage. This pattern is seen in cases where an accelerant is used to burn a body in an attempt to hide a homicide.

When a fire burns long and hot, the insulating tissues are consumed by the fire and the damage to the oral structures will be extensive. Teeth will be completely consumed in a hot fire. While teeth do not survive the cremation process, certain dental restorative materials such as porcelain and stainless steel can be found in cremains.

Delattre has described a four stage process to follow when examining burned human remains. First photographs are taken of the undisturbed remains. Then soft tissue is removed to allow better visualization and additional photographs. The third step is to carefully obtain access to the oral cavity to allow exposure of radiographs and photographs. Lastly loose dental specimens are placed in suitable containers to avoid further damage and loss.

In the spring of 2011, an extensively burned set of human remains was recovered from a car fire. Investigation by medical examiner's personnel allowed the formation of a putative identity and contact was made with the suspected victim's mother who was able to provide an antemortem dental chart. The mother was also able to provide a decorative tooth grill and a dental model from which the grill was fabricated. In hip hop culture, a grill (to include front or golds) is a type of jewelry worn over the teeth. Grills are made of metal and are generally removable. Grills are fitted to a dental model of the wearer. The long term safety of grills is debatable, as is their effect on oral health and hygiene. Grills can be obtained online and from beauty and barber shops.

The body was examined, photographed, and digitally radiographed. Examination of the body disclosed a loose crown and a tooth grill.

A positive identification was confirmed by several methods. Antemortem and postmortem radiographs of a root canal filling in an anterior tooth compared favorably. The grill found on postmortem examination fit the dental model obtained from the mother. The tooth grill obtained from the mother fit the postmortem dentition; and a loose metal crown found postmortem, fit a tooth on the dental model.

Care used in the examination and documentation of severely burned human remains made possible a positive dental identification via several modalities.

**Odontology, Identification, Human Remains**

## **F29 Remains of Climber Identified After 21 Years on a Glacier: A Case Report With Discussion of the Need for an International Missing Persons Database That Includes Dental Records**

*Lowell B. Riemer, DDS\*, 8-25102 TWP RD 542A, Sturgeon County, AB T8T 0C4, CANADA*

After attending this presentation, attendees will understand the circumstances and factors involved in a climbing accident, which resulted in rescuers being unable to locate a missing mountain climber for 21 years. Positive identification of the recovered remains was possible because of dental records retained by the local police agency.

This presentation will impact the forensic science community by reinforcing the importance of retaining dental records of missing individuals for an indefinite period of time. Priority must also be placed upon consensus and implementation of a national and international missing persons database that includes accurate dental records.

The American Alpine Club states that between 1951 and 2005 there were 1,686 deaths in Canada and the United States related to climbing activities with 18 and 59 fatalities occurring each year. The report, Accidents in North American Mountaineering, provides statistics regarding injuries and fatalities in all reported climbing incidents and the factors involved.

This case report concerns a pair of experienced winter climbers in the Canadian Rocky Mountains. Upon reaching the summit of the mountain the climbers unclipped from their ropes. One climber proceeded to move about to locate their desired descent route. While venturing too far onto an unsupported snow/ice cornice, the climber disappeared from view as he fell 300 feet before likely striking the face of the mountain and another 1,800 feet before striking a snow slope, which then fanned out over a 160,000 square foot area of glacier. Due to deteriorating weather and snow conditions, the body could not be recovered. Dental records were acquired by the local police agency and retained on file in anticipation of a future recovery of the body of the deceased. Twenty-one years later, a body was observed partially embedded in glacial ice and was subsequently identified as the missing climber through comparison with the dental records.

The body, still clothed in climbing gear, was desiccated and mummified. The remains showed evidence of being trapped and compressed within the glacier for the 21 years. The right foot had been disarticulated at the level of the ankle and was located in close proximity to the rest of the body. Some bony fractures and adjacent soft tissue discoloration were suggestive of possible antemortem trauma. The cause of death has been attributed to multiple blunt injuries.

The head and climbing helmet were extensively compressed in a midline sagittal plane resulting in a compressed thickness of approximately 6.5 cm in width. Brown head hair and a moustache were still evident. The teeth were still in good condition and position with only one tooth displaced through avulsion despite the lateral compression of the dental arches. A considerable amount of previous dental work aided the comparison with antemortem dental records.

The Royal Canadian Mounted Police are to be acknowledged for their record keeping and readily accessible antemortem dental records for this individual. This case emphasizes the importance of comprehensive antemortem records, including dental, being compiled, stored, and being accessible for all missing persons cases.

**Dental Identification, Mountain Climbing Fatality, Glacial Compression**

### **F30 A Study of Bitemark Characteristics in Live Human Subjects**

*Kenneth P. Hermsen, DDS\*, Creighton University, School of Dentistry, 2500 California Plaza, Omaha, NE 68178; Eric S. Wilson, DDS\*, PO Box 50, Cole Camp, MO 65325*

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After attending this presentation, participants will understand the issues associated with performing bitemark research on live human subjects, including the Institutional Review Board process and the challenges encountered in doing research on live human subjects.

This presentation will impact the forensic science community by reporting on the latest attempt to move the study of bitemarks into the realm of research on live human subjects, providing insight into the issues encountered in the endeavor.

Thirty-two live human subjects volunteered for the project which was designed to compare the bitemarks that resulted from a bite. The bite was delivered by a device that was fitted with a set of denture teeth provided by the prosthetics laboratory at Creighton University School of Dentistry. The device was outfitted with a pressure sensor that could measure the amount of pressure being exerted by the bite. The intent of the project was to administer a bite with the same amount of pressure to each volunteer and compare the marks created. It was an attempt to determine if all the marks were similar in appearance or if there were significant variations that might be related to individual characteristics of the volunteers, like gender, stature, or muscle mass. During the IRB process, the project was altered to first determine the minimum amount of pressure that would be required to produce a mark in all the volunteers.

To accomplish this, fifteen volunteers from the original group of thirty-two were selected to participate. The forearm was selected for the site of the bite for this initial phase of the project. The volunteers were subjected to incremental increases in pressure until a mark was produced that lasted for a minimum of twenty-four hours. Based on the unexpected results of the initial phase, it became apparent that proceeding with the original research design would be extremely difficult and would bring into question some ethical issues that should be resolved before proceeding.

The results of the initial phase showed a significant variation in the amount of pressure required to produce a mark that lasted for twenty-four hours as well as significant variations in how the marks were manifest. The least amount of pressure to create a mark was in an Asian female who was the smallest in stature of the group. She exhibited a bruise at the site of the bite at a reading of 60 lbs. as indicated on the pressure sensor. It was not a pattern bruise but rather a round bruise approximately 10 mm in diameter in the area of the maxillary central incisors. The last two volunteers to mark were two males who did not exhibit a mark that lasted for twenty-four hours until the pressure sensor had indicated a pressure of 235 lbs. Neither of these marks were bruises, per se, but rather red marks corresponding to the incisal edges of the teeth. One of the males was the largest in stature of the group. The length of time the indentations made in the skin by the bite was also noted.

In all volunteers, the indentations left by the teeth had clinically disappeared within two hours of the administration of the bite with only one exception. In that exception, a female reported that the indentations were clinically detectable for approximately six hours. However, in spite of the persistence of the indentations, she did not exhibit bruising or marking of the bite for the required twenty-four hours. It should be noted that in previous and subsequent bites, her indentations disappeared within two hours. She reported having been working out prior to the application of the bite and suggested perhaps she was dehydrated. It is unknown whether this could be a factor. This study revealed a variation from 60 to 235 lbs in pressure required to produce a mark lasting twenty-four hours, it also showed a wide variation in the manifestations of the mark even though the volunteers were all young, healthy adults. Further study is required to draw any conclusions, but there are ethical issues that should be considered before moving ahead with further human studies.

**Bitemark, Human, Live**

### **F31 Bitemark Analysis in Hungary**

*Armin A. Farid, DDS\*, Avicenna Med & Dent Bt., Podmaniczky Utca 33, III Floor, 8, Budapest, 1067, HUNGARY*

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After attending this presentation, attendees will gain a clearer understanding of bitemark analysis in Europe as it is becoming more and more an integral and vital part of police investigation work.

This presentation will impact the forensic science community by raising awareness that violent biting happens all over the world despite not gaining the attention it deserves.

Hungary has a long history of bitemark recognition, study, and analysis. The Forensic Institute of Semmelweis University, one of the most traditional and renowned centers of medical education in Europe, undertook the work of bitemark analysis as early as 1904. Medical textbooks make reference to bitemark analysis conducted by Dr. Laszlo Harsanyi in 1968; however, due to the low rate of criminal activity in socialist countries such as Hungary, little attention was given to this topic in the past. Although animal bitemark analysis was conducted frequently, the occurrence of human bitemarks was considered rare or nonexistent.

Beginning in 2008 as a result of an aggressive media campaign, effort and a special educational program aimed at raising awareness among the Hungarian Crime Scene Technicians, the importance of bitemark analysis was brought to light among not only the police force but also the general public resulting in the reporting of several bitemark cases within a short period of time.

The most prominent case occurred in December 2010 in a suburb of Budapest and shocked the Hungarian nation. A two-month-old infant was beaten by her father and then bitten twice to be silenced. The police were called in once the infant was admitted into the hospital twelve days after the initial abuse had taken place. During the initial medical assessment it was apparent that additional abuse had taken place. Since this case involved a "closed population" suspect pool, a forensic odontologist was called in to analyze the dental evidence obtained from both parents of the infant. A thorough examination and analysis of the bitemarks ruled out the mother as a suspect and concluded that the father was the probable offender. The highly individual characteristic of the bites helped strengthen the father's conviction. The forensic dentist in Hungary involved in this case collaborated closely with his mentor in the United States who offered valuable advice and guidance during this process.

A few weeks later, another case was reported of a child bitten multiple times by a neighbor who was babysitting him while his mother was at work. Soon after this report reached police investigators, an elderly woman attacked in her home was reported to have bitten her attacker in self defense. In both of these cases, bitemark analysis served as strong evidence in the respective criminal investigations.

It is believed that the education of not only the police force and agencies concerned with children and women's welfare, safety, and protection, but also that of the general public of the significance of bitemark analysis, which will result in an increase of reported cases ultimately serving to protect the general population as well as providing the justice system with critical evidence in solving crime.

**Hungary, Bitemark, Police Investigation**

### **F32 Bitemark Analysis — The First Step Utilizing Bitemarks as Evidence**

*Richard R. Souviron, DDS\*, Medical Examiner's Office, Miami-Dade County, Number One on Bob Hope Road, Miami, FL 33136*

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After attending this presentation, the attendees should be able to implement a systematic and logical approach to analyzing a human bitemark with the objective of providing useful information to the authorities.

The presentation will impact the forensic science community by providing a comprehensive and useful description of a human bitemark. The proper analysis of a bitemark will impact the use of this evidence for later comparisons.

The terminology used in describing bitemark evidence is confusing at best and misleading in many cases. The confusion has to do with the term analysis which means "separation into component parts, qualitative, and quantitative." The term comparison means to examine for similarities or differences.

In real time, the odontologist is often presented with a photograph of a pattern injury. He may or may not have scene photos, the photos may or may not have a ruler or scale present. He may have no information as to the circumstances of the event. If it is a Class I bite, the odontologist may give an opinion such as "diffuse bitemark with no evidentiary value in bitemark analysis." However, there is valuable evidence and analysis should be conducted of a Class I bite. The analysis consists of breaking the bitemark down into logical parts. The most important factor to decide is whether it is a human Bitemark; and if so, is this a child or adult dentition? Further analysis may be able to determine when it was made in relation to time and the circumstances of the event, i.e., an old healing bite, peri-mortem bite, postmortem bite or a fresh bite. If possible to locate and differentiate upper and lower arches so one may be able to determine the position of the biter in relation to the victim. Analyzing the bite may also determine whether the bite was made through clothing. Evaluation of the bite should help to determine whether or not pain was inflicted on the victim. In Class II and Class III bites more definitive opinions can be given. These opinions may or could include a profile of the biter, positions of the biter to the victim, arch form identification, adult versus child bite, or more. Transillumination of the bite may give more details of the individual and class characteristics of the teeth.

All of this analysis does not take place in a vacuum but with knowledge of the circumstances of the event, scene photographs, orientation photographs, scaled photographs, etc. The analysis phase of a bitemark has nothing to do with a comparison to anyone or to the ID of a biter.

The comparison phase may rule in or rule out (exclude) a potential biter and is separate and apart from the analysis of the injury pattern. In the case of a good bitemark (a class III) with both arches and distinct individual tooth characteristics present and with a closed population, an odontologist can state "within reasonable medical/scientific certainty of a match." However, "scientist may have a high level of confidence if there is abundance evidence but they won't ever claim absolute truth or absolute certainty" (GLY.UGA.EDU).

#### **Analysis, Comparison, Odontology**

### **F33 Patterns of Variation and Match Rates of the Anterior Biting Dentition With Regard to Size and Shape: Characteristics of a Database of 3D Scanned Dentitions**

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The goal of this presentation is to describe dental shape variation and shape matches in a large population of three-dimensional (3D) dental models.

This presentation will impact the forensic science community by addressing one fundamental premise of bitemark analysis, that of "uniqueness" of the human anterior dentition.

Critics of the concept of "uniqueness" claim that individuality cannot be proven since examination of every individual that exists, and has

existed, is not possible. To this end, it might best to describe "uniqueness" as "sufficiently similar" such that two objects cannot be measurably distinguished once impressed in a substrate.

To state that two objects are indistinguishable requires a definition of measurement resolution and error. In any given system, these parameters may be determined by repeated measures of an object in question. Any two (or more) objects that fall below this defined error threshold, when compared with each other, may be said to be indistinguishable.

An established means to study size variation in shape in biological systems is landmark based Geometric Morphometric analysis (GM). It is a multivariate approach and allows statistical comparison of the change in shape in large datasets. With this approach, shape information can be visualized by plotting landmark positions in Procrustes superimposition, a method of optimally matching one shape to another. This technique can be performed with or without scale and for this study both approaches were used. Procrustes distances can be used to summarize variations in populations, to express the degree of similarity of individual specimens, means of populations, or to search for matches between specimens.

Another statistical tool available in the GM framework is Principal Component Analysis (PCA). This allows for determination of which shape aspect is responsible for the most variation. Finally Partial Least Square (PLS) was utilized to observe patterns of covariation in maxillary and mandibular data.

In this study, the anterior human dentition was examined in 3D data using size-preserving Procrustes (Procrustes-SP) methods, as well as scale-independent Procrustes methods.

A collection of 1,099 distinct paired sets of 3D scanned maxillary and mandibular dentitions were obtained from a commercial dental laboratory, which produced these scans for the production of occlusal guards (night guards). Of these, 497 pairs were taken from an earlier data set (Bush, Bush, and Sheets, 2010) and 602 pairs were newly measured for this study. The individuals involved were private practice patients from dental practices across the United States. All patient identifying information was stripped from the data prior to any additional processing. This was a sample of convenience, containing a wide range of alignment patterns, from relatively straight to fairly mal-aligned. All necessary Human Subject Institutional Review Board protocols were completed for this project and exemption was granted.

Curves were placed which delineated the incisal edges of the six anterior teeth in both uppers and lowers. Each curve contained 10 landmark data points on each incisal edge. A total of 60 data points in 3D were obtained for each arch.

Following landmark data point extraction, statistical analysis was completed to describe the variation in these human biting dentitions and to determine match rates in the population studied. The statistical tools, as described above, were used in conjunction with LM methods to produce a summary of the variation present in this large data set, as an initial approach to understanding the types of variation present in the population of dentitions.

Results indicate that, measurably indistinguishable maxillary and mandibular dentitions could be found (defined as a match rate). The paired dentitions were also evaluated as a set. Two specimens (one pair) with just shape information, and four specimens (two pairs) with size and shape were found to be indistinguishable in the dataset. PCA revealed definite patterns of common mal-alignment, allowing exploration of the variation that exists in this population.

The sensitivity of match rate on measurement error was also determined by searching for matches as a function of assumed error level, indicating rapid increases in match rate as measurement error increased. The nature of the relationship of arch width and centroid size among and between the maxilla and mandible was examined. Lastly, Principle Components Analysis and Partial Least Squares revealed readily interpretable patterns of variation and covariation in the data, with both dominated by the variation in relative arch width.

It was concluded that arch width was the principal factor in variation. A number of specimens were found to be measurably indistinguishable

when investigating the maxillary and mandibular arches separately. When investigated as a set, incorporating size caused an increase in the number of specimens that were indistinguishable.

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**Forensic Science, Dental Uniqueness, Bitemarks**

### **F34 Couplers for Scale Combination and Orientation**

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After attending this presentation, attendees will be able to better use currently accepted scales for forensic documentation.

This presentation will impact the forensic science community by allowing better use of forensic scales while demonstrating photographic documentation more accurately in a court of law.

For many years, the field of forensic Odontology has had the luxury of using scales (rulers) in the forensic community that have been proven to be of high accuracy. This is undoubtedly of utmost importance when portraying evidence in a court of law, and obviously important when one studies and interprets a photographed patterned injury such as a bitemark.

However, until now, it has been difficult to professionally “surround” a patterned injury for accurate measurement recording. In the past, it has been advised that when one wished to photograph a patterned injury from multiple sides concurrently, two or more scales could be laid end-to-end or corner-to-corner. For a number of reasons, it is recommended that this technique no longer be used. When one places multiple scales end to end, or corner-to-corner, there is no assurance the scales will be in the same plane. Further, there is no assurance the scales will meet in an end-to-end relationship with no space between. And further yet, there is no assurance the scales will not overlap or be at an improper angle.

By using the scale couplers, the above-mentioned problems are solved. It has already been noted that the forensic community uses scales that have been proven to be of high accuracy, and are accepted by the legal and judicial communities. The use of the scale couplers enables existing scales to be used in a better and more professional manner. These couplers eliminate the need for the fabrication of other scales by putting to better use our already proven and most accurate scales.

The scale couplers have been fabricated in a variety of shapes with which to orient two or more scales; however, the two most common used couplers are the “corner” or 90° coupler, and the “straight” or 180° coupler.

To use, existing straight or right angle “L” shaped scales are simply inserted into the couplers that firmly hold the scales oriented. By doing so, a patterned injury, or other such displays needing to be photographed can be completely encircled. Thus, easier and more accurate measurements can be made. As one may imagine, a multitude of patterns can be photographed this way, but not limited to tire patterns, shoe prints, blood, and other body fluid spatter, gun shot residue etc.

These couplers allow scales to be oriented in the same plane and brought together to an end-to-end or corner-to-corner relationship. These couplers maintain the accuracy of the “joint” or “joints” of two or more scales for accurate measurement. These scale couplers allow the forensic community to use scales already proven accurate in yet a better manner.

Simple, and yet fabricated of high quality and standards, these scale couplers are already being used in many locations. As an advantage to photographic departments, these scales further allow the use of iridescent scales to be oriented for ultra violet imaging.

In conclusion, the scale couplers are easy to use, reusable, and enable the forensic community to better use scales already trusted.

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**Scale Couplers, Couplers, Photographic Couplers**

### **F35 Bitemark Analysis in an Italian Judicial Case: Dentists – Do They Bite Off More Than They Can Chew?**

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After this presentation, attendees will have a better understanding of the expert witness testimony in the Italian legal system.

This presentation will impact the forensic science community by raising awareness of the importance of the involvement of trained and experienced odontologists when a life sentence is at stake.

The purpose of bitemark analysis is to retrieve both distinct individual and class characteristics of teeth on the bitten skin. The odontologist compares the dentition of the suspect’s with the injury pattern and draws a conclusion as to whether or not they were compatible using a variety of methods. The expert witness assessment of a bitemark pattern injury commissioned by the defendant is presented. The framework is a twenty-year-old homicide cold case, where only one suspect has recently been found guilty.

The purpose of the presentation is to promote a discussion among experts about who should perform a bitemark analysis, thus reducing potential errors and observer bias. At this point dentists and odontologists cannot be considered of equal forensic ability, thus suggesting two separate expert witness categories in civil cases related to professional litigation and/or dental traumatology and judicial cases related to crimes.

The case presented is suggestive of a partial bitemark where the forensic significance is crucial. This provides the basis for a controversial comparison with only one suspect, although it is well understood that, except specific cases, the comparison should be carried out within a sample of suspected biters. Poor quality of evidence, primary and secondary distortion of the bitten skin, combined with the lack of a direct assessment on the human specimen, plus absence of a proper metric scale, make it impossible to decide upon the correct orientation of the biting teeth. In view of this, the results of the forensic evaluation should be restricted to exclusion. The analysis of the bitemark injury by the forensic odontologist, in conjunction with the forensic pathologist, remains of unquestionable value for the investigation of this criminal case.

The case presented is not designed to provide an account of how a bitemark comparison should be undertaken but raises concerns of the validity of bitemark evidence that is dramatically connected to those involved in the technical analysis of a bitemark. This case confirms the importance of involving a forensic odontologist, rather than a general dentist, in the analysis of a bitemark injury pattern to reach a technical conclusion and reduce to the minimum the risk of subjective conclusions. The need of education, training, and updating in forensic odontology, which goes beyond everyday dentistry and dental training, suggests the need of a periodical review of those professionals registered in court for expert testimony. In the Italian legal system, as in other European countries, it may be worth considering the institution of a subcategory (or a separate one) of experts in forensic odontology and legal dentistry, within the actual single category board of court experts/consultants who only have a degree in dentistry. Many dentists involved in the judicial system in Italy are still not sufficiently aware of the extent of forensic odontology as an autonomous discipline within the forensic science field, least of all how a bitemark assessment should be performed.

The use of non-peer reviewer techniques, the lack of formal training in forensic odontology, poor quality evidence, and prosecutorial interference are some of the possible pitfalls that the review of this cold case reveals.

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**Bitemark Analysis, Forensic Odontology, Cold Case**

### F36 Lens Correction, White Balance, and Other Photographic Prerequisites to Bitemark Interpretation

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After attending this presentation, attendees will be briefed on new scientific evidence regarding the requirement for lens correction caused by optical aberrations of a particular lens and the necessity for “white balance” and methods used to accomplish the tasks as well issues surrounding different file formats.

The theme of this year’s Annual Meeting is *Global Research: The Forensic Science Edge*. This presentation will impact the forensic science community by providing forensic odontologists with the leading edge of knowledge on the subject of bitemark evidence.

Bitemark analysis is by far the most complex and difficult subject matter in forensic dentistry.<sup>1</sup> Following bitemark recognition, the analysis involves a series of noninvasive procedures that includes photography regardless of whether the bitemark recipient is alive or deceased. The conclusions are, at times, solely based on this analysis. It is therefore paramount to understand the factors that influence photographic evidence and which may ultimately contribute to erroneous conclusions.

A search of the Journal of Forensic Sciences between 1972-2010 reveals not a single article dealing with the issue of lens correction or “white balance” in forensic photography.

Lens aberrations are deviations from a norm of an optical system that leads to image blurring. Even if the image is sharp, it may be distorted. Ideally, the magnification of an object is inversely proportional to its distance to the camera. Distortion can result from non-uniform stretching of an image. The most prominent form of distortion is referred to as “barrel distortion” where the center of the image is magnified more than the perimeter.

“White balance” in a photo reflects the lighting condition under which it was taken. It is influenced by daylight, cloudiness, shading, tungsten, fluorescent and flash conditions, and so on.

This presentation will focus, in part, on methods of correcting lens distortion and “white balance” using Adobe® Photoshop® CS5 Extended.

RAW file format is a class of formats and each manufacturer creates its own version of a RAW file format for each camera model. This data block contains unprocessed pixel readings from the camera metadata and sensor chip. The standard for RAW image format is ISO 12234-2, TIFF/EP. Adobe’s Digital Negative (DNG) specification is an attempt to standardize a RAW image format for cameras.

File compression is the process of reducing the size of a file to facilitate storage and transfer. The resulting file may retain all of the data or there may be data loss. Compression algorithms that retain all of the original data are referred to as “lossless” compression, and those with a loss of data as “lossy.” When using the former, the compressed file uses fewer bits to represent the information. The original data when re-opened is reconstructed despite a compression ratio of approximately 2:1. Digital files such as ZIP and LZW (Lempel-Ziv-Welch) are examples of lossless compression files. There are various types of compression encoding including Quantization, Run-length and Lexicographic.

This presentation will concentrate, in part, in comparing various file formats and its influence on photographic quality and ultimately on bitemark interpretation.

#### Reference:

<sup>1</sup> Dorion RBJ, editor. Bitemark Evidence. 2nd edition, Boca Raton, FL., CRC Press, 2011.

#### Bitemark, Lens Correction, White Balance

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### F37 Bite Wafers for Additional Analysis of Patterned Injury Bitemarks

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After attending the presentation, attendees will understand an additional method to use with patterned injury analysis.

This presentation will impact the forensic science community by giving the investigator of the patterned injury another tool to use to interpret the injury.

In the attempt to better analyze and interpret patterned injuries, in particular bitemarks forensics depended on various methods to aid in the collecting and presentation of data.

The purpose of this presentation is to provide the attendee with additional method to be used. This is not a method intended to be used in place of currently used methods, but rather to be used as an adjunct. Thus, when one is required to present in a court of law, he or she will now have yet an additional display to aid in the explanation to those of the nonscientific community. Further, this method will help the forensic odontologist to interpret the patterned injury bitemark.

**Methods:** This method uses a standard sheet of aluminum wax which is spray painted with white latex paint. It does not matter if the paint is gloss or flat. What matters is that the paint “blocks out” one side of the wax. Next a sheet of white impression wax is sprayed on one side with contact cement. This side is then adhered to the already dry painted side of the aluminum wax to create a “Bite Wafer.” The impression wax is then trimmed and luted to the circumference of the aluminum wax. Once dental models have been made of the suspected biter, the models are then impressed on the impression wax side of the wafer. By impressing a model in the softened wafer, a pattern will occur which will resemble a pattern capable of being made by said subject. The incisal edges and cusp tips will be denoted as darker areas. The layer of paint prevents bleaching or burn through of pressure areas not caused by the dentition.

Similar to fingerprint analysis, this technique compares an image of a bitemark to an image of a possible pattern created by a suspected dentition. This technique does not require any flipping or transposing of images. This is obviously easier for the non-scientifically educated person to understand and trust. Though untrue, people sometimes believe an image has been altered when it is demonstrated to them that transposition has taken place in order to complete the analysis and subsequent demonstration.

As with other similar methods, one to one overlays can be made and simply placed on images of the pattern injury and images of the wafer.

This method is already being used and has been used with success in the courtroom. As stated above, this method is not recommended as a substitution for currently used techniques, but rather as an adjunct. This technique allows the forensic odontologist yet another tool for use in his/her ability to better interpret and explain a patterned injury bitemark in question.

#### Bite Wafers, Wafers, Bite Sheets

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### F38 Suggested Use of a Hand-Held Scanning Device to Produce Accurate Evidentiary Measurements in Bitemark Analysis

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After attending this presentation, attendees will learn how the forensic odontologist must be capable of presenting evidentiary accurate measurements and documenting such measurement by photographic and/or digital images. This presentation will explore the use of a hand-held scanning device to facilitate a more accurate means of documenting bitemark evidence.

This presentation will impact the forensic science community by exploring how the utilizing of a hand-held scanner in bitemark analysis is examined.

Previous studies discussed how the forensic odontologist relies on highly accurate measurements to facilitate evidentiary quality bitemark analysis. Reasonably accurate Alginate or the more stable and accurate polyether or polyvinyl siloxane impression materials are capable of producing measurement friendly dental stone study models. All these measurements are usually taken in a flat plane linear environment. For example the inter-canine cusp measurement is accomplished by simply placing the standard ABFO #2 ruler across the model and recording the appropriate dimension. Such data accurately translates to photographs (both film and/or digital) through specialized scanning techniques and photo processing software. The resultant images are generally accepted as evidence in litigation. Analysis of the bitemark is more problematic.

Bitemarks by their very nature are subject to either *in vivo* healing or postmortem decomposition. Elastomeric impressions, methacrylate tissue excision techniques, and specialized 1:1 close-up photographs or digital images all serve to preserve the bitemark as evidence. Measurement problems occur because bitemarks are rarely made in a truly flat plane environment. It is the natural curves of the human body that lends itself to exhibiting a bitemark that has been made around a curved surface. If one should photograph the bitemark with the #2 ruler in view all objects are in a two dimensional posture and all measurements taken of a curved surface with a straight ruler will have some inherent inaccuracies. It was determined that such inaccuracies could be as high as 36.31% if one considered a bitemark on a perfectly round body part. It is the recording of analytical and subsequent evidentiary documentation that this paper will address.

Methodology for this analysis will be based on comparisons of measurements taken from digital photographic images produced by a standard professional close-up camera and lens and a digital image produced by a hand held scanning device with measurements taken *in vivo* on a test subject.

Several measurable marks were made on a volunteer utilizing a non-permanent marking device. Digital photographs were taken with a single lens reflex digital camera fitted with a 105mm macro lens and close-up strobe lighting apparatus. An ABFO #2 ruler was placed in some of the photographs and a self-adhesive flexible measuring tape was used in other photos. An eight and one-half inch hand-held scanning device was used to obtain similar images. All photographs taken with the digital camera were in color and at a high resolution. The scanner was also set for color scanning at a resolution of 600 DPI (dots per inch). Results showed a comparison of measurement differences and similarities by the various recording modalities. It is noteworthy that under certain circumstances such as a curved flat-plane area the measurements elicited by the scanner and the adhesive tape were more accurate than the camera with the ABFO #2 ruler.

This analysis suggests that measurements along a curved surface should be made by rotating the ABFO #2 ruler along the arc, by using some flexible measuring device, or by using a hand-held scanner.

#### **Measurement, Scanner, Bitemarks**

### **F39 Validation of Three Methods of Estimating Age-at-Death From the Dentition of Modern Colombians**

*Clara I. Valderrama, DDS\*, and Edna M. Buitrago, DDS\*, National Institute of Legal Medicine and Forensic, Bogota, COLOMBIA*

After attending this presentation, attendees will understand the principles and application of research in dental age determination by validating the original method proposed by Lamendin et. al (1992), as well

as updates proposed by Prince and Ubelaker (2002) and González-Colmenares et al. (2007), on a modern Colombian sample of documented individuals.

This presentation will impact the forensic science community by demonstrating that the dentition can be used with some degree of success in modern Colombian populations. In addition it demonstrates that the methodology of Prince and Ubelaker (2002) performs with slightly more accuracy than the technique of González-Colmenares et al. (2007).

In the identification process, forensic practitioners are often required to estimate age-at-death from skeletal and dental tissues. While numerous methods utilize morphological structures of the post-cranial skeleton, various methodologies utilizing the dentition estimate age based on dental development, time of eruption, dental attrition, and root translucency. These methods have been developed for different populations on the basis of various morphological features and have been modified and/or applied and validated for other populations over time; however, with the exception of González-Colmenares et al. (2007), few have been specifically developed or tested on modern Colombians.

Therefore, this study validated three techniques that each utilize periodontosis and root translucency to estimate age-at-death. The techniques included Lamendin et. al (1992), Prince and Ubelaker (2002), and González-Colmenares et al. (2007). The sample used was the Collection of Modern Colombian Skeletal Remains curated by the National Institute of Legal Medicine and Forensic Sciences in Bogotá, Colombia. Both male and female individuals were utilized and age-at-death ranged from 19 – 93 years with a mean age of 47.5 years. Although 133 individuals were examined, only 88 were included in this study, due to either poorly defined root translucency or entirely edentulous individuals.

All data were collected blind to real age and the teeth utilized included maxillary and mandibular incisors and canines which met the requirements of root translucency, level of periodontosis, and root length. The usable dental structures were cleaned with a 0.05% sodium hypochlorite solution for 60 seconds, dried with gauze, analyzed on a light box with electronic caliper, and finally packaged in plastic bags, labeled, and filed with the case file. A subset of the original sample was measured twice by each author so that intra and inter-observer could be assessed.

Correlation coefficients were calculated for root translucency, periodontosis, and root height and age-at-death was calculated with each method. Not surprisingly, root translucency and periodontosis were significantly correlated with age ( $r_s = 0.515$  and  $0.612$ , respectively;  $p < 0.001$ ) but root height was not ( $r_s = -0.025$ ;  $p = 0.820$ ). Such results mirror those of González-Colmenares et al. (2007) who also found that root height did not increase with age.

The mean difference between real age and estimated age were as follows: Lamendin (+0.25 years, standard deviation 15.01 years), González-Colmenares et al. (+0.17 years, standard deviation 14.84 years) and Prince and Ubelaker (+3.52 years, standard deviation 13.82 years). Simple scatterplots which plotted estimated age against real age generated the following  $R^2$  coefficients: Lamendin (0.406), González-Colmenares et al. (0.413), and Prince and Ubelaker (0.532). Moreover, overall results fit a consistent pattern of over-aging young adults and under-aging old adults. These results indicate that dental estimation methods may inform overall age-at-death estimates, particularly in younger adults, and that population-specific standards should continue to be developed for Colombia.

#### **Dental Age Estimation, Colombian Population Standards, Teeth**

### **F40 Odonto-Listics**

*Diane T. Penola, MA\*, 54 Fayson Lakes Road, Kinnelon, NJ 07405*

After attending this presentation, the attendees will become familiar with the similarities between victim identification and criminalistics.

This presentation will impact the forensic science community by drawing attention to the fact that many of the steps taken by the forensic odontologist in the process of making a dental ID are the same as those taken by a crime scene investigator.

During the 2011 proceedings of the American Society of Forensic Odontology, AAFS President Joe Bono commented that an elementary program in dental identification would be a good thing. He indicated that there is interest, by the other members of the Academy, in the subject.

There is a level of research and innovation that defines the odontology section presentations. The goal of this research is to provide new information, support previous research, and explore current initiatives. These presentations are wonderful for the members of the section and those who choose to follow new developments. However, they do not educate the members of other sections on the most basic responsibilities.

Last year one presentation, quantified the various duties of the forensic odontologist. By far, the most frequent task performed is the identification of unknown human remains. This being the case, it seems incumbent to provide meaningful information for the non-dental professionals who are attendees and Academy members.

Of all the sections in the American Academy of Forensic Sciences, the one which has the most in common with Odontology is Criminalistics. The sequence and rationale behind the procedural steps is actually identical, in general terms. Both sets of professionals are expected to recognize material of evidentiary value. They preserve and collect that evidence, taking care that in transport it is protected and remains in the condition in which it was found. They document that evidence with photography, radiography, and other means. The material is analyzed for significant findings. The findings are compared with known samples.

For example, a bullet is removed from a murder victim. The bullet is carefully collected, with attention being paid to the entrance and exit wounds and the position and damage to any clothing the victim may have been wearing. The forensic pathologist will have dictated, or in some other way, recorded these findings. The suspected murder weapon will be test fired in such a way as to protect the projectile from damage. The two bullets can then be examined side by side with a comparison microscope. In a best case scenario, the striations on the outside of the bullets will match, confirming the identification of the murder weapon.

The scene in which the forensic odontologist is called might involve the collision and subsequent explosion of a motor vehicle. The driver is burned beyond recognition and a dental identification is necessary. The dentist must recognize what is of evidentiary value, as the impact and explosive forces may have dislodged dental material. All dental structures will be carefully examined and radiographed after being collected and transported. Please note, in most cases of this type, the dentition will usually be preserved within the oral cavity.

The dental findings will be analyzed for characteristics that have particular significance. The findings will then be compared to known records provided by the dentist who rendered treatment. This can actually be a difficult or impossible step if there is no information available regarding the name of the treating dentist.

Members of both the odontology and criminalistics sections should expect to come away with a greater appreciation for the challenges that face their colleagues across the hall.

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**Odontology, Criminalistics, Comparison**

## **F41 Human Skeletal Remains Found During Excavation of Rufisque's Town Hall Annex in Senegal**

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After attending this presentation, attendees will understand how to utilize multiple methodological approaches for identifying human skeletal remains.

This presentation will impact the forensic science community by establishing that the reliability of scientific evidence based opinions may be overlapped by experts from various disciplines.

The construction of an annex to the Rufisque Town Hall building necessitated excavation and removal of earth to allow for the annex's foundation. Once underway, the removal unearthed a large stone that halted progress until it could be broken up. Underneath the stone, human remains were found. On the second day of the project, the Attorney General of Senegal ordered excavation to continue where a second pit revealed additional human bones and four bottles of dubious liquid. The third day of excavation unearthed a third pit containing an entire human skeleton along with the smell of putrefaction.

The commissioned forensic pathologist, anthropologist, and odontologist were assigned to determine race, age, and sex, as well as the manner and likely date of death for those interred in the pits. Photographs of bone fragments, teeth, a skull, and a black, cord-like substance were cataloged for study. The teeth were x-rayed and sand samples were extracted from the spaces between teeth and the eye orbits to cross reference with soil samples from the immediate area.

In this investigation, each discipline is trying to determine the sex, race, and age of the skeletal remains. The odontologist will examine the skull, teeth, and postmortem radiographs that will reveal the status of the person's oral health and can be compared to antemortem dental records if they can be located. The odontologist is one of the best person to work up skeletonized, decomposed, and macerated bodies to arrive at a confirmation or unknown identification.

The anthropologist would arrange and study the skeletal remains to also make an analysis as to age, sex, race, and height. All human are unique and an analysis will make the compared groups smaller. The more unique the characteristics the smaller the group become by eliminating other groups.

The pathologist is concern with race, sex, disease, DNA, trauma, and cause of death.

In conclusion, the first body found in pit one was Black in race from Africa. The upper canine appeared to be 50 years of age according to Gustafson's analysis. In the second pit, there were only human bones and four bottles of liquid substance. The race, sex, and origin are undetermined. In the third pit the human remains is that of a Black African male. The determination of average age according to Gustafson is 30 years of age. A longitudinal section of an upper premolar was use to determine the age.

This is not a case of murder but a case of some mystic practices.

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**Excavation, Human Remains, Forensic**

## **F42 Contributions of a Forensic Odontologist in a Criminal Child Abuse Dog Bite Case**

*Kenneth F. Cohrn, DDS\*, 422 Teague Trail, Lady Lake, FL 32159*

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Forensic odontologists are often a part of a team effort to investigate criminal bitemark cases. Cases can involve human-human, animal-animal, or animal-human abuse situations.

After attending this presentation, attendees will have an appreciation of the contribution of a forensic odontologist in a criminal child abuse dog bite case spanning three years involving multiple agencies, the law enforcement officer suspect, the infant victim, and a dog.

This presentation will impact the forensic science community by adding to the knowledge of case investigation and trial results.

In 2007, the State's Attorney requested the examination of injuries on a two-month-old female who was admitted to the hospital with life threatening injuries. The infant was in the care of her father, a police officer, at the time of the incident. He indicated that the family dog had injured his daughter and he drove her in the family car to the hospital for emergency care. The emergency room physician noted numerous superficial scratches, linear red marks, bruising, and a number of superficial

circular lesions on the trunk of the infant as well as severe life threatening internal injuries. The child protection team was alerted.

There were a number of red flags associated with the parent's version of the events. First, the child was seriously injured and the father, a police officer, indicated that he "did not trust EMS" and decided he would drive the near death child to the hospital rather than use emergency services. The visual injuries were superficial yet the child had multiple posterior rib fractures, a lacerated spleen and liver, and was in respiratory distress. The emergency room physician indicated that her external examination was inconsistent with the parent's history that she sustained her injuries as a result of an attack by the family dog. The CTP medical examination suggested physical abuse.

This case evolved over three years involving multiple agencies, experts, including an animal psychologist and bite mark expert, culminating in a trial with unexpected results. The prosecution's case alleged child abuse by the father blaming the dog. The defense position was that the dog was entirely responsible. There was contentious testimony from the experts, including several medical examiners, about the cause and nature of the injuries. Were the deep crushing injuries the result of the dog or the father squeezing the torso out of anger and frustration? Could the dog have applied enough force to lacerate organs and break pliable ribs yet leave only very superficial marks with virtually no bleeding or puncture wounds? How did the one-zee pajamas end up off the infant almost unscathed if the dog viciously attacked the infant? With the child in extreme distress, why did the father elect to drive the infant to the ER rather than call 911? Were the circular marks of the skin from the dog's teeth or another source? Certainly the demeanor of the parents was inconsistent with the severity of the situation. The entire emergency room staff felt that the parents were hiding something. This case had a number of difficult issues that were difficult to prove with certainty that ultimately led to a controversial result.

**Forensic Odontology, Animal Bite Marks, Child Abuse**

## F43 The Case of the Frustrating Floater

*Thomas V. Brady, DMD\*, 1823 Boston Post Road, PO Box 622, Westbrook, CT 06498*

After attending this presentation, attendees will gain an appreciation of the multiple avenues of investigation in missing/found persons. This case involves an extensive array of medical, dental, communicative, and investigatory procedures in an attempt to identify a found unidentified dead body.

This presentation will impact the forensic science community by stressing the multiple ways an investigation of an unidentified dead body can be conducted. It will introduce the attendee to little known resources and will stress the value of persistence in an investigation.

The Connecticut River runs north to south separating the eastern third of Connecticut from the western two thirds. The Town of Old Saybrook is at the mouth of the Connecticut River where it runs into Long Island sound. It is about 45 miles southeast of Hartford, Connecticut. It has a population of approximately 11,000 people. The police department has 21 sworn officers. In late March 1998, the Hartford police informed the Old Saybrook police that they had a report of a body floating in the river. Hartford reported they were unable to find the body after a "cursory search." The normal current and tides would indicate that the body, unimpeded, would arrive in the Old Saybrook area in three or four days.

On March 31, 1998, the Old Saybrook police received a phone call that a fisherman spotted the body 1.1 miles north of the mouth of the river. Police responded to the scene to find a badly decomposed body floating face up in the river. With the help of the Coast Guard, DEP police, and a local towboat operator the body was brought to shore. The body appeared to be male, wearing a tan jacket, dark pants, and black sneakers. There was no wallet, jewelry, or other means of identification on the body. The local medical examiner spotted two holes on the back of the skull possibly indicating bullet holes but stated the sites needed further examination.

While the body was on the dock a silver cigarette lighter fell out of the coat pocket.

The body was sent to the medical examiner's office in Farmington, Connecticut for autopsy. The autopsy revealed that the victim was probably a white/Hispanic male, approximately 68 or 69 inches tall, and weighing approximately 200 lbs. A local oral pathologist completed the dental charting and took a full mouth set of x-rays. Evaluation of the skull by a forensic anthropologist confirmed the sex but also confirmed the holes to be anatomically normal orifices for large veins. The body was determined to have been the water for several years. Marsh grass in and around the body indicated it had been hung up in swampy ground before floating free. Due to the badly decomposed state of the body, a cause of death could not be determined. Fingerprints were also not possible.

The time and ingenuity spent on this case by a small police department with limited resources is admirable.

An extensive type went out via NCIC to all police agencies regarding the found person. A request was made of the Department of Defense through Senator Leiberman's office for a review of the dental chart for possible identification. Copies of the dental charting were sent to many dentists in the Connecticut and Massachusetts area asking for help. There were no matches found. The investigators focused on the cigarette lighter. Several lighter manufacturers were contacted to no avail. There is a National Lighter Museum in Guthrie, Oklahoma, the museum was contacted and was very helpful. They determined that the lighter was of Chinese origin, most likely made between 1992 and 1995. It was a very cheap model most likely sold to young people at flea markets or at convenience stores. In effect it had no individual characteristics to make a source for narrowing down an investigation.

The police then contacted the Center for Missing and Exploited Children in Alexandria, Virginia who agreed to do a facial reconstruction of the skull. The results now gave a face to the victim - giving him a persona. The facial reconstruction picture and pertinent information was placed on the America's Most Wanted website. The case went cold.

In 2003 the investigation was reopened. The skull was brought to the office where the teeth were photographed and recharted to update NCIC records. The dental chart and a picture of the facial reconstruction was submitted to the Connecticut State Dental Association newsletter requesting information from any dentist who might know the victim. A similar request was sent to all the New England state dental associations and the American Dental Association for publication. To date there has been no response.

The victim's dental chart has been resubmitted to NCIC and NAMUS. There has been no successful matches found on the \$M reports as of this date but attempts will continue to be made. Everyone deserves a name.

**Floater, Cigarette Lighter, Communication**

## F44 ID – The Unknown – A Piece to the Puzzle

*Charles S. Mandell, DDS\*, 3220 Stirling Road, Hollywood, FL 33021*

After attending this presentation, attendees will understand how that the field of dental implantology can provide the dental forensics division an invaluable tool in the identification process of the unknown and missing.

This presentation will impact the forensic science community by pointing out the grossly overlooked possibility for a new identification tool in the field of dental forensics.

The design and production of dental implants began 34 years ago with Leon Shaw, one of the first producers of dental implants. Since this time, implants have undergone various transformations in relation to design, shape, architecture, and metallurgy or component consistency. Today, there are literally hundreds of different designs and components.

Dental implants were introduced in the U.S. in 1940 with Gustavo Dahl presenting the first dental implant – a subperiosteal design. Since its inception in 1940, the field has gone from subperiosteal to blade-type or endosteal implants invented by Dr. Leonard Linkow of New York City, mucosal inserts (Park Dental's Jack Wimmer). Endodontal stabilizers,

screw-type implants, mini implants, with each type promoting different restorative options in the field of dental implantology. All of these, when located in the unknown, can provide a useful tool for the forensic dentist to utilize as a possible piece of the puzzle in the identification process.

Serving on the IAPC (International Organization of Police Chiefs) Forensic Committee and the committee to ID the missing, one simple goal presented itself—the placement of ID numbers on all dental implants. This may sound simple; however, for six years after contacting congressional members to no avail (Markey, Boston; Schultz, Broward County, Florida), progress has not been made. Unfortunately, there is now 69 years of dental implants being placed in this country without any IDs located on them. The cost factor, with today's technology, would be insignificant, amounting to less than 50 cents per implant. The ID numbers are not visible to the naked eye and can only be read with a special reader, which many companies already have, and would not influence the success or failure of the implant, but would provide an invaluable tool in the ID process.

This past year approximately 600 million dollars were spent on dental implants in the U.S. The significance of this figure speaks volumes as to this piece to the puzzle. However, all is not lost. At this time dental implants without IDs to help ID unknowns can still be utilized.

Recently a mandible (lower jaw) was found on a beach in California. After x-rays were taken, it was found to have a dental implant and a referral was made for identification of the type and manufacturer of the implant. With the cooperation of several people in the industry, the identity the manufacturer, type, and approximate year it was produced were identified. This reduces the number of dentists in the U.S. who might be able to provide an ID in this case from 165,000 to 200 (only those customers on the list from the manufacturer); and reduces it even further down to the one customer who might have purchased this type of implant. If this implant had an ID number, this process would have been immediate.

At this time, the goal is to expand this process to all orthopedic implants inserted in a human body (hips, knees, shoulders, etc.) with hopefully someday a central bank – perhaps located in UCF under the direction of Carrie Whitcoff for the registration of all implants.

Resolutions from the IAPC and the committee to ID the missing have been granted regarding this matter, with both organizations giving their unconditional support to this ongoing process.

The goal is to provide any and all information regarding: (1) type; (2) manufacturer; (3) metallurgy; and, if possible, (4) year manufactured.

**Implantology, Missing, IDs**

## **F45 Challenging Odontological Identification of Severely Burned Remains**

*Glen A. Smith, DDS\*, 2136 North Cole Road, Boise, ID 83704*

After attending this presentation, attendees will illustrate the collaborative effort between forensic odontologist and death investigator to find the evidence that was needed to make a positive dental identification on a severely burned set of remains. Attendees should also learn some of the unique antemortem records available from an orthodontist, and how to clean cyanoacrylate cement off of teeth.

This presentation will impact the forensic science community by demonstrating how detective teamwork between the odontologist and death investigator can find the evidence needed to make an identification. It will demonstrate the use of orthodontic antemortem records in the identification process where there was insufficient postmortem material available to use common odontology techniques. The presentation will also demonstrate a technique on how to deal with cyanoacrylate residue on charred dental remains.

In a remote area in Southern Idaho, human remains were found burned beyond recognition in a vehicle fire. The coroner with primary jurisdiction in the case processed the remains and then transferred them to the Ada County Coroner's office in Boise, Idaho for a more in-depth work up by a forensic pathologist and forensic odontologist.

Coroner's office personnel collected and fixated dental structures with a cyanoacrylate product to help preserve those structures from further damage. Even with this effort, not all of the dental skeletal remains were recovered and only one intact tooth remained for odontological examination. This one tooth was an unrestored maxillary second molar and was difficult to examine and evaluate because it was covered with deposits of burned material sealed in place with cyanoacrylate.

Local law enforcement used the vehicle identification information to arrive at a likely identification of the victim who turned out to be a local resident. Detectives contacted the victim's family and they were able to provide information as to who the family's general dentist was. That general dentistry office was able to provide antemortem dental records, which local law enforcement then sent as "the dental record" to the forensic odontologist.

The antemortem dental record consisted of chart notes and two sets of bitewing radiographs. The postmortem dental evaluation included radiographic, photographic, alternate light, and visual examination. To be able to fully examine the sole surviving intact tooth, various materials were used in an attempt to clean the sealed burn material off of the tooth. In the end, standard hardware store acetone was the most effective solvent. An analytical comparison of antemortem and postmortem dental information showed that there were no significant inconsistencies between these records. Unfortunately, there was insufficient information available to be able to confirm the suspected identification of the victim.

The odontologist's review of the antemortem records discovered that third molars had been extracted at some point in time at another dental office, most probably by an oral maxillofacial surgeon. The odontologist contacted law enforcement with this information and they were able to identify the oral surgeon who had extracted the teeth, collect the surgeon's records, and send them on to the odontologist so that he would have "all of the victim's dental records." Within these records were copies of radiographs taken by a local orthodontist. Again, local area law enforcement was contacted, and the orthodontic written records, original radiographs, and plaster models were obtained for the odontologist. These orthodontic materials provided the record of a unique collection of intracranial anatomic structures and dental crown morphology by which a odontological identification was made.

**Odontological Identification, Orthodontic Records, Cyanoacrylate**

## **F46 Child Abuse Reporting by Pediatric Dentists in Texas**

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After attending this presentation, attendees will understand the attitudes, knowledge and practices of Texas pediatric dentists in reporting child abuse.

This presentation will impact the forensic science community by exploring how this research could help in the implementation and dissemination process of child abuse information for medical, dental and legal professionals.

**Objectives:** The data produced from this study identified: (1) the clinicians' training and management strategies for children with injuries suspected as related to unreported abuse; (2) the factors weighed by clinicians when deciding whether to report injuries suspected to be abuse; and, (3) how clinicians explained reasons for not reporting suspicious injuries. This research could help in the implementation and dissemination process of child abuse information for medical, dental, and legal professionals.

**Discussion:** One previous study reports that approximately 1% of general dentists had reported cases of suspected abuse while a larger percentage had suspected abuse. This suggests that the information gained from dental schools may be insufficient and contribute to the lack of knowledge of the signs of abuse and the legal responsibility to report.

The Prevent Abuse and Neglect through Dental Awareness (PANDA) coalition has trained dental personnel through continuing education. According to this program, “the extremely low reporting rate by dentists seems to be related to the lack of training dentists receive in how to recognize and report abuse and neglect, and concerns about the ramifications of becoming legally involved in such cases.”

**Methods:** Surveys were sent out to all 115 pediatric dentists in Texas who had provided an email address to the American Academy of Pediatric Dentistry (AAPD) 2010 directory. Fifteen questions were included in the survey that was conducted by email. The online service, Survey Monkey ([www.surveymonkey.com](http://www.surveymonkey.com)) was used to facilitate the survey. The interviews were analyzed for the rationale utilized for the decision to report suspected child abuse as well as management strategies, bitemark cases, most common signs observed, and the level of training of the dentist in identifying abuse.

**Results:** The response rate, distribution by gender, and professional associations will be reported in the oral presentation. The collection of data was ongoing at the time of submission of the abstract. Early responders indicated that the most common sign of abuse observed was bruising of the skin. Data on this and on the other signs of abuse listed are reserved for the presentation.

**Conclusions:** Dentists should consider all relevant factors when deciding whether or not to report cases of suspected child abuse. Pertinent factors include history and circumstances of the injury, knowledge of and experience with the family, consultation with others, and previous personal training and experiences with child abuse and bitemark cases. Data on bitemark reporting as a form of child abuse can provide valuable reference material for forensic odontologists to present to their local medical examiners, emergency room personnel, law enforcement agencies, and prosecutors.

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**Child Abuse, Odontology, Bitemarks**

At the time and even now, it is not exactly known how long the body was in the vat. The body was identified to be that of a woman. An interesting note to take into account is that the body and bones were somewhat “rubbery.” The skin and hair remained intact, but the teeth were missing, causing one to believe that the victim was edentulous.

X-ray analysis using film showed what appeared to be six fragments most probably from a fragmented bullet in the skull. These were noticed on a lateral skull X-ray film. Once the fragments were retrieved, they were found to actually be six separate dental restorations. The acid had dissolved the teeth but had not harmed the amalgam restorations or the composites, of which there were three of each.

The suspected victim’s family was notified and the victim’s dentist in Toronto was located.

Though unsure of exactly which teeth the restorations belonged to, it seemed evident that the three amalgams were all from maxillary teeth, two from molars and one from a pre-molar. This was deduced from the shape and size of the restorations. The three composites seemed to be of the shape and size that would be found in mandibular molars. When the dental chart was forwarded to the Cuyahoga County Coroner’s office (some of which was in French), it was discovered that the numbering system used had to be converted to our commonly used international system. Further, it was found that the suspected victim did in fact have six charted restorations, three amalgams and three composites. And lastly, the three maxillary amalgams were of two molars and one pre-molar, while the three mandibular composites were all from mandibular molars. Viewing of the restorations from the side indicated all six to match with the shaped of the bite-wing films also forwarded.

With DNA, fingerprinting, and visual identification not possible, this dental restoration identification indicated with a high degree of probability who the victim was.

As a conclusion to the story, the suspect was apprehended shortly after in the western United States with a receipt found in his glove compartment for—driveway cleaning acid.

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**Dental Identification, Identification, Restoration Analysis**

## **F47 A Dental Identification Without Teeth**

*James C. Schneider, DDS\*, Cuyahoga County, Coroners Office, 8660 Columbia Road, Olmsted Falls, OH 44138*

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After attending this presentation, attendees will learn how teeth are not always necessary to make a dental identification.

This presentation will impact the forensic science community by demonstrating how a dental identification can be made with the use of analysis of restorations.

A man and woman met in Toronto in the year 2000. After some time together, they relocated to a south eastern suburb of Cleveland, Ohio called Maple Heights. Prior to becoming involved with each other, the man had a list of charges including: domestic violence, drug use and possession, drug trafficking, and armed robbery.

On a day in 2001, a tenant who was living below the couple noticed a liquid dripping from their balcony to his. Upon reporting this to the landlord, and when the above apartment was notified, there was no answer. When the landlord entered the apartment, there was nobody present. While searching the balcony, a large plastic trash barrel was found with a soupy liquid and what looked to be a blanket or comforter of some type contained inside.

It had immediately become apparent that something was awry and the police were called. Upon inspection it was found that the contents of the barrel was some type of liquid mixed with what was most likely a human body. When retrieved, a body was in fact what was found. However, one arm, one leg, and the head had been severed off. These body parts were found in the barrel as well. After further analysis, it was noted that the liquid was an acid of some type; a form of hydro-flouric acid.



## G1 The Significance of Ventricular Volume Measurement in the Macroscopic Evaluation of a Postmortem Heart

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After attending this presentation, attendees will understand how measuring the ventricular volume at autopsy could be a new indicator used in forensic pathology, one that can complement the limitation of classical evaluation methods of the postmortem heart.

This presentation will impact the forensic science community by proposing a new indicator for evaluating a postmortem heart macroscopically.

A normal heart maintains its structure by being very well adapted to its role of supplying blood to the pulmonary and the systemic circulation systems. But, in various conditions, the heart goes through a remodeling process resulting in reduced cardiac contractility and ultimately cardiac failure. During this process, hemodynamic and morphological changes occur concurrently. The morphological changes can be clinically categorized into cardiac hypertrophy and cardiac dilatation. An accurate evaluation of the heart during autopsy is critical. The weight, shape, and consistency of the heart and the thickness of the ventricular wall are used as parameters for evaluation of the postmortem heart and diagnosis of cardiomyopathy at autopsy.

A total of 58 hearts were categorized into four groups: 13 cases in the control group; 14 cases in the dilated heart group; 9 cases in the hypertrophied heart group; and, 22 cases in the undetermined heart group by using conventional evaluation methods. In addition, the ventricular weight and volume were measured and analyzed.

The weights of male and female hearts in the control group were 329.2±30.2g and 277.9±30.7g, respectively, and the ventricular weights were 271.5±34.4g and 219.3±23.9g. Left ventricular and right ventricular volumes for male were 25.8±9.7ml and 34.3±13.2ml; and they were 15.7±8.2ml and 35.6±9.0ml for females. The thickness of the left ventricular free wall in males and females were 1.4±0.1cm and 1.3±0.1cm, respectively, and for the interventricular septum they were 1.3±0.3cm for both males and females.

In the group of dilated hearts, the ventricular weight, the ventricular volume, ventricular volume/ventricular weight, and left ventricular volume/right ventricular volume were increased and the thickness of the ventricular wall was decreased. Such a result is the consequence of excess increase of ventricular volume, particularly of the left ventricle. In the group of hypertrophied hearts, the ventricular weight, ventricular volume, the thickness of the ventricular wall were increased; but, ventricular volume/ventricular weight and left ventricular volume/right ventricular volume did not change significantly. Such a result is the consequence of ventricular hypertrophy exceeding ventricular dilation in the hypertrophied heart group. The increase in ventricular volume was thought to be mostly due to the increase in right ventricular volume. The most evident morphological characteristics that distinguish hypertrophied hearts from dilated hearts were a more obvious increase in ventricular weight than volume, and in right ventricular volume than left in the hypertrophied heart. Such characteristics were revealed using ventricular volume to weight ratios and left ventricular volume to right ventricular volume ratios in this study.

In the group of undetermined hearts, compared with the control group, ventricular volume showed only a slight increase, but the ventricular weight increased by approximately 400g. Additionally, the ventricular volume to weight ratio and the ratio of left and right ventricular volume remained relatively similar with those of the control group and hypertrophied heart group. It was thought that secondary myocardial changes in the undetermined heart group would have progressed to a dilated heart through cardiac hypertrophy. In the group of undetermined hearts, it was later found that four of the cases should have been included in the dilated heart group and another two cases in the hypertrophied heart group according to aforementioned characteristics.

This study concludes that measurement of the ventricular weight and volume may be an objective parameter that can aid in distinguishing between dilated and hypertrophied forms of secondary cardiomyopathies, as well as providing an objective indicator for evaluating the degree of change in cardiac remodeling at autopsy.

### Ventricular Volume, Postmortem Heart, Cardiac Remodeling

## G2 Acute Respiratory Insufficiency During Computed Tomography Procedure for Pituitary Adenoma

*Elizabeth Ventura, MD\**, Baylor University Medical Center, 3500 Gaston Avenue, Dallas, TX 75246; and *Joseph M. Guileyardo, MD, 2911 Turtle Creek Boulevard, Suite 300, Dallas, TX 75219*

After attending this presentation, attendees will understand that potentially fatal respiratory insufficiency can result from procedural sedation in patients with upper airway narrowing due to lymphoid hyperplasia.

This presentation will impact the forensic science community by demonstrating the relevance of autopsy examination to accurate certification in cases involving adverse events during medical therapy.

A case is presented of a 39-year-old man who underwent an outpatient CT scan for recent headaches. His past medical history included mental retardation due to a penicillin reaction during childhood. He also had recurrent viral infections and a seizure disorder which was previously treated with phenytoin. Currently, his seizures were controlled with oral levetiracetam (Keppra) 1,000mg twice daily, and 5mg diazepam, three times a day. Prior to his CT scan, he received 1,000mg IV levetiracetam over 15 minutes and 5mg of IV midazolam slowly; however, due to continued agitation in the scanner he was slowly given 75mcg of intravenous fentanyl. He was then administered IV contrast, but four to five minutes after contrast injection he appeared to have a "vagal" reaction which was treated with increased IV fluids. He did not respond, and full cardiopulmonary resuscitation was initiated. His mother informed caregivers of his "do not resuscitate" status; however, he had already regained spontaneous respiratory and cardiac activity. Unfortunately, he was found to have profound and irreversible hypoxic-ischemic encephalopathy, and he was discharged home to hospice care where he died the next day. The local Justice of the Peace declined to order an autopsy, and the family allowed the body to be embalmed; however, they later requested a private autopsy in hopes of obtaining a better understanding of the cause of death.

**Autopsy Findings:** The sella turcica was expanded by a friable and uniformly grey-tan, 2.0cm mass which compressed the adjacent optic chiasm. Microscopically, the normal pituitary histology was effaced by a monomorphic population of cells which were strongly positive for prolactin by immunohistochemistry. At the periphery of the tumor there was a compressed rim of normal pituitary tissue. Within the thalamus there were

scattered red neurons. The upper airways were markedly narrowed at the laryngeal inlet by thickening and induration of the epiglottis and aryepiglottic folds. The epiglottis was also folded towards the midline, resulting in transverse airway narrowing to approximately 4 millimeters. Microscopically, the epiglottis contained prominent lymphoid infiltrates with germinal centers containing numerous tingible bodies. Plasma cells were also increased within the subepithelial tissues.

It is the opinion that this man died of anoxic encephalopathy due to multifactorial acute respiratory insufficiency during computed tomography for evaluation of headaches due to pituitary adenoma. The respiratory insufficiency was due to the combined effects of upper airway narrowing associated with lymphoid hyperplasia and respiratory depression associated with procedural sedation.

The effects of phenytoin on lymphoid tissues have been known for some time and airway compromise due to lingual tonsil hyperplasia causing laryngeal obstruction has also been previously associated with phenytoin therapy. In addition, treatment-emergent adverse events reported with levetiracetam therapy have included pharyngitis and this patient was also reported to have recurrent viral infections. Therefore, multiple factors may have contributed to laryngeal narrowing in this case. Furthermore, studies indicate that safe procedural sedation is best ensured by careful presedation risk assessment and monitoring during the procedure. It is the opinion that "accident" is an appropriate manner of death in this case, since death was not solely due to natural disease; however, others may certify such deaths as "natural" (based on predictable or foreseeable consequences of treatment for a medical disorder). The "death certification and manner-of-death classification require judgment, and room must be allowed for discretion on a case-by-case basis." In conclusion, this autopsy provided helpful clarification regarding the multiple complex and inter-related factors which were contributory to this man's death.

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**Lymphoid Hyperplasia, Procedural Sedation, Airway Compromise**

### **G3 Case Report of a Death Involving Methylenedioxypropylvalerone (MDPV) From Bath Salt Use**

*Diane C. Peterson, MD\*, Office of the Jackson County Medical Examiner, 660 East 24th Street, Kansas City, MO 64108; C. Clinton Frazee III, MBA; and Uttam Garg, PhD, Children's Mercy Hospital, Department of Pathology, 2401 Gillham Road, Kansas City, MO 64108; and Mary H. Dudley, MD, Jackson County Medical Examiner's Office, 660 East 24th Street, Kansas City, MO 64108*

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After attending this presentation, attendees will be aware of the rise in use of bath salts as an alternative to methamphetamine use. Attendees will understand that one potential active drug in bath salts is methylenedioxypropylvalerone (MDPV) and how to include it in drug screens. Attendees will understand the effects of MDPV and its potential contribution to death.

This presentation will impact the forensic science community by alerting attendees to a relatively new designer drug in use across the nation and the world.

A 41-year-old white male was found unresponsive in his bed. He had a history of hypertension, anxiety, and bipolar disorder. He also had a history of chronic ethanol abuse and methamphetamine use. The decedent had recently stopped using methamphetamine and had begun using bath salts approximately two weeks prior to his death. According to his wife, he had been "high" and awake for the previous three days.

Autopsy revealed superficial ulcers of the mucosa of the upper and lower lips. Head and neck cyanosis was also observed. Linear discontinuous healing superficial excoriations as well as an apparent needle puncture site with adjacent ecchymosis were on the skin of the arms. The coronary arteries were mildly to markedly narrowed by atheroma. The lungs exhibited moderate edema. Mucosal erosions were also at the mid and lower aspects

of the esophagus. Histologically, no definitive contraction band necrosis was identified.

Femoral blood, heart blood, vitreous fluid, urine, liver tissue, brain tissue, gastric contents, and a packet labeled "Blue Magic 350 mg" were submitted for toxicological analysis. The femoral blood was used for all drug and volatile testing and the remaining biological samples were stored in a freezer at -20°C.

Using gas chromatography with a flame ionization detector, two separate aliquots of femoral blood tested negative for ethanol, acetone, isopropanol, and methanol. A 0.5mL aliquot of femoral blood was extracted with methanol and analyzed for nine drugs of abuse using enzyme immunoassay (EIA). A 1mL aliquot of femoral blood was extracted using bicarbonate buffer (pH 11.0) and n-butyl acetate. The aliquot was then analyzed by gas chromatography/mass spectrometry (GC/MS) for more than 150 drugs. Benzodiazepines, diphenhydramine, tramadol, and MDPV (methylenedioxypropylvalerone) were detected. Benzodiazepines analysis showed the presence of alprazolam. All of the drugs except diphenhydramine were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). O-Desmethyltramadol, an active metabolite of tramadol, was also quantified. Quantification of diphenhydramine was deemed clinically insignificant. Toxicological analysis of femoral blood revealed MDPV at a concentration of 130ng/mL. Tramadol and its metabolite, o-desmethyltramadol, were identified at concentrations of 9000ng/mL and 320ng/mL, respectively. Alprazolam was detected at a concentration of 26ng/mL.

The packet submitted, "Blue Magic 350mg," had a zip lock closure with the inscription "Bath Salts, Novelty Bath Salts, Not for human consumption" on the back aspect of the packet. The packet contained a broken blue capsule with an unknown white powder. Approximately 10mg of white powder was dissolved in deionized water and analyzed by EIA, thin layer chromatography (TLC) and GC/MS. MDPV was determined to be present.

The concentration of tramadol is markedly above the therapeutic range and is sufficient alone to cause death. At this concentration, tramadol causes respiratory depression, hypertension, tachycardia, and seizures. MDPV is a synthetic stimulant, or designer drug. It is sold in the form of bath salts and is known to cause insomnia, severe agitation, tachycardia, and hypertension. The final cause of death was ruled as tramadol overdose with MDPV intoxication as a contributing factor. The manner of death was accident.

Scene investigators should be aware that the use of bath salts is increasing as an alternative to methamphetamine. Any packets of bath salts should be collected with the body. Bath salts may be ingested, injected, or inhaled. In the case above, the exact method with which the decedent used the bath salts is unknown. He had evidence of ingestion and injection. Oral mucosal (aphthous) ulcers have not been previously reported as being a potential adverse effect of bath salt use. With evidence of bath salt use, the pathologist should notify the toxicology lab of the suspicion. MDPV may or may not be detected on typical drug screens. The bath salt packet may also be sent to toxicology for evaluation.

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**MDPV (methylenedioxypropylvalerone), Bath Salts, Intoxication**

## G4 Fatal Waterhouse-Friderichsen Syndrome: Crime Scene, Autopsy, Pathology, Bacteriology Microscopic, and Toxicology Features

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The goal of this presentation is to educate the attendees about the differential diagnosis between infectious disease syndrome and battered child syndrome.

This presentation will impact the forensic science community by the collection of information before performing an external examination or an autopsy.

**Introduction:** Waterhouse-Friderichsen syndrome is adrenal gland failure due to bleeding into the adrenal gland. It is caused by severe meningococcal infection or other severe bacterial infection. Symptoms include acute adrenal gland insufficiency and profound shock. It is deadly if not treated immediately. However, the infection leads to massive hemorrhage into one or (usually) both adrenal glands. It is characterized by overwhelming bacterial infection meningococemia, low blood pressure, and shock, disseminated intravascular coagulation (DIC) with widespread purpura, and rapidly developing adrenocortical insufficiency.

**Materials and Methods:** A fatal case of Waterhouse-Friderichsen syndrome resulting from bacterial infection in a 4-year-old boy is reported. The course is rapid and the clinical symptoms are serious (hyperthermia, *purpura fulminans*, dyspnea). The clinical symptoms are not known by the forensic pathologist during the external examination. The presence of purpura (numerous skins' petechial haemorrhages) evoked a potential "battered child syndrome."

**Results:** External examination: numerous ecchymosis are observed, principally located on thoracic and abdominal area. The anamnesis is incomplete and an autopsy is performed. Autopsy findings: included numerous petechial haemorrhages on the heart, the pancreas, the thymus gland, and the bowel. Macroscopic examination showed encephalic edematous, hemorrhagic and edematous lungs, and mainly a bilateral acute hemorrhagic necrosis of the adrenal glands. Toxicology: negative results. Pathology: showed fragments of pulmonary parenchyma with alveolar necrosis. Adrenal glands presented parenchyma complete apoplexy. Bacteriology: the origin of the WFS is pneumococcal meningitis.

**Discussion:** The autopsy findings (a bilateral acute haemorrhagic necrosis of the adrenal glands) are typical of Waterhouse-Friderichsen syndrome (WFS). Without complete anamnesis (hyperthermia, dyspnea, no traumatic lesions), an error of diagnosis is possible. Indeed, a "battered child syndrome" could be evoked. With numerous petechial hemorrhages on the body and infectious context, the forensic pathologist has to think about the WFS and rapidly perform a lumbar puncture to lead to the good diagnosis. The diagnosis of WFS as the cause of death will be established postmortem based on autopsy findings and additional tests (pathology, bacteriologic cultures). The death of a child with numerous ecchymosis has to be considered as suspicious and an autopsy should be systematically required and performed to confirm or invalidate the diagnosis of WFS. Moreover, the largest number of information must be collected by police (infectious context) before the external examination and the autopsy. Indeed, these important elements of context and anamnesis guide to the best diagnosis and avoid legal implications for the parents if "battered child syndrome" is kept. **Waterhouse-Friderichsen Syndrome, Adrenal Glands, Autopsy**

## G5 Diatom Analysis From Suspected Drowning Cases

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After attending this presentation, attendees will become familiar with the utility and value of the diatom test for the assistance of drowning diagnosis, including the quantitative and qualitative analysis of 105 suspected drowning cases.

This presentation will impact the forensic science community by demonstrating how diatom analysis from sphenoid sinus fluid, lung tissue, and bone marrow is valuable for the diagnosis of drowning.

Many rivers cross the crowded cities, and Taiwan is surrounded with sea. Thus, cadavers were commonly found in water or river banks. In Taiwan, 9.2% autopsy cases per year are found to be a result of drowning. Whether cause of death is actually drowning is the first question raised on these cases. The place where the corpse was discovered in water may not be the initial site of drowning, so it is a challenge to identify the cause and the manner of death for drowning victim. An autopsy conducted by a medical examiner is the essential method for the identification of drowning cases so far. Currently, there are various methods for drowning diagnosis. Diatom screening is one of the methods which can link the cadaver to the natural waters. The cell wall of diatom (also known as frustule) is made of silicon, which is acid-resistant and thermostable. A forensic investigator is capable of doing diatom test by using these characteristics. Diatoms found in sphenoid sinus fluid, lung tissue, and bone marrow can provide an excellent evidence to determine the cause of death. This study was also compared with the findings of autopsy and evaluated the applicability of diatom test for forensic use. There are 105 suspected drowning cases and 20 non-drowning cases collected from Institute of Forensic Medicine, Taipei, Taiwan. Sphenoid sinus fluid, 5g peripheral lung tissue, and rib/clavicle bone marrow were collected during autopsy for diatom analysis. After strong acid digestion, a quantitative and qualitative analysis was conducted by counting the number of diatoms under phase contrast microscope. Diatoms can be observed in most samples from drowning corpses. The percentages of positive diatom results from sphenoid sinus fluid, lung tissue, and rib/clavicle bone marrow were 84.7%, 69.2%, and 6.2%, respectively. The sensitivities of diatom analysis from the same tissues were 84.7%, 84.7%, and 6.2%; the specificities were 81%, 89%, and 100%, respectively. All non-drowning samples showed negative results in the diatom test. The environment of sea water and the seasons of winter and spring could cause the higher false positive rate of diatom analysis in lung samples. Five cases with positive diatom tests in rib/clavicle have the diatom density of lungs above 70 diatoms /per 5g lungs. The qualitative results showed *Nitzschia*, *Navicula*, *Cyclotella*, and *Thalassiosira* are most commonly genera in all samples. The results demonstrated that diatom analysis from sphenoid sinus fluid and lung tissue is valuable for drowning identification. The diatom test for suspected drowning cases is a routine screening in Taiwan. For accurate identification of the cause and the manner of death, the results of diatom test from suspected drowning have to be integrated with the investigation from the scene of death and other autopsy findings with careful consideration.

**Cause of Death, Drowning, Diatom**

## **G6 Fatal Pulmonary Fat Emboli Following First Trimester Elective Abortion: Is This Amniotic Fluid Embolism**

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After attending this presentation, attendees will understand the clinical presentation, pathophysiology, differential diagnosis, and histological diagnosis of fat and amniotic fluid embolism.

This presentation will impact the forensic science community by reminding attendees of the different approaches to post-abortion deaths and the steps needed to accurately determine the cause of death.

A healthy 28-year-old G5P1041 Haitian woman underwent an elective first trimester abortion at a licensed clinic. The procedure lasted four minutes and there were no complications. During the procedure, she was administered diazepam, meperidine, and propofol. In the recovery room, vital signs taken at five minute intervals were normal. At five minutes after the last vital sign check, 20 minutes into recovery, she was found in asystole. Cardiopulmonary resuscitation lasted 82 minutes with the patient in asystole throughout. At autopsy, the patient was not obese and there were no significant external findings. The right and left lungs were 590 and 530 grams respectively. No thromboemboli were seen grossly. The uterus was 190 grams and approximately 5 milliliters of blood and blood clot, were in the uterine cavity. There was no uterine perforation. A small laceration was on the ectocervix. Accompanying the decedent were two surgical specimens consisting fragmented placental and membranous tissue. The fetus was reported approximately eight weeks gestation. Osmium tetroxide staining of lung tissue revealed obstruction of pulmonary capillaries by fat globules. Mucin stains were negative and no intravascular squamous cells, trophoblasts, or bone marrow elements were identified. There was no polarizable material. The surgical specimens consisted of chorionic villi, gestational endometrium, and membranous tissue. Testing for drugs of abuse was negative, though diazepam, temazepam, and meperidine were detected. Postmortem hemoglobin electrophoresis revealed the decedent to have sickle cell trait. The cause of death in this case was non-traumatic pulmonary fat embolism associated with therapeutic abortion procedure and the manner of death is natural.

Fat embolism syndrome and amniotic fluid embolism syndrome (AFE) have similar clinical presentations though their causes are distinctly different. Fat embolism is usually associated with skeletal trauma but has been described in acute pancreatitis, extensive burns, liposuction, decompression sickness, orthopedic procedures, parenteral infusion of lipids, and sickle cell disease. AFE usually occurs in term childbirths or in the early postpartum period. Risk factors include cesarean section, vacuum and forceps delivery, increased maternal age, diabetes, fetal macrosomia, placenta previa or abruption, and cervical laceration or uterine rupture. Deaths from AFE have been reported following abortion in the first or second trimester of pregnancy but none have been reported in therapeutic abortions performed at as little as eight weeks gestation. In fact, advanced gestational age at the time of abortion is a powerful risk factor for AFE and no deaths have been reported to have occurred at or prior to 12 weeks gestation. Postmortem diagnosis of AFE is made microscopically. One can see amniotic debris in the pulmonary microvasculature including lanugo hair, epithelial squamous cells, granules of bile pigment, meconium, as well as abundant fatty globules. Special stains for mucin and cytokeratin may be of use. In this case abundant fat is seen in the pulmonary microcirculation in a patient who died after a therapeutic abortion at eight weeks gestation. The patient was found to carry sickle cell trait after postmortem hemoglobin-electrophoresis. Rarely have complications during pregnancy been described in patients with sickle cell trait. Case reports have described pulmonary thrombo-embolism following

delivery and probable peripartum cardiomyopathy aggravated by intravascular sickling. Sickle cell trait has also been reported to possibly be associated with acute chest syndrome which may be due to fat embolism while sickle cell anemia is definitely associated with fat embolism syndrome caused by bone marrow necrosis and infarction. This patient had diffuse multifocal fat in the pulmonary microcirculation without evidence of other amniotic fluid constituents and at present it is unknown whether the fat emboli are due to amniotic fluid or associated with her sickle cell hemoglobinopathy.

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**Amniotic Fluid Embolism, Fat Embolism, First Trimester Abortion**

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## **G7 Normal Isn't What It Used to Be — The Spectrum of Liver Abnormalities in an Australian Autopsy Population**

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After attending this presentation, attendees will gain an appreciation of the spectrum of liver normality and disease in an autopsy population.

This presentation will impact the forensic science community by providing updated information, including the range of disease normality, and normal and abnormal weights of relevant organs in the body.

The autopsy is an excellent method for cataloguing disease, whether minor or very serious, and whether incidental or directly related to the cause and manner of death. Autopsy reports should describe pathology in great detail, and even minor pathology is therefore often graced with a detailed description. However, what is normal and what is disease? Is normal the absence of any pathological change or is it what is found in the majority of the population who appear to be healthy? This paper examines the problem of what is a normal liver in a population where aetiological factors for liver abnormalities are present in more than half of all deaths reported for medicolegal autopsy in Sydney, with excessive alcohol consumption, obesity, and hepatitis B and C virus infection predominating. Many abnormalities of the liver are clinically silent, although they can have a significant role to play in the death. For example, a person with an enlarged fatty liver is much more likely to sustain a liver laceration in blunt abdominal trauma than a person with a morphologically normal liver.

This study evaluated the prevalence of liver abnormalities in an autopsy population at the Department of Forensic Medicine, Sydney. Data was extracted from 1,472 autopsy reports from the year 2008. Analysis was performed on the data collected, producing demographic information, cause of death, information on the nature and extent of liver abnormality at autopsy, and information on organ weights in this population.

In this analysis, structural liver abnormalities were very common, with the liver not described as normal in 83.4% of all reports examined. Almost one third of cases had histologically diagnosed steatosis and in the morbidly obese (body mass index greater than 35kg/m<sup>2</sup>) this approached 50% of cases. There was steatohepatitis in a further 4% and cirrhosis in 5.6%. In those cases where the history provided documented excessive alcohol consumption, there was steatosis in 36.4% of cases and progression to cirrhosis in a further 24%. There was hepatitis C positive serology in 5% of cases and cholelithiasis was reported in more than one in seven cases.

The mean liver weight for men was found to be 1,747 grams and 1,472 grams for women, with a minor increase in mean liver weight in those where pathology was identified. The mean spleen weight in men was 210 grams and 153 grams in women, with only a modest increase in splenic weight to a mean of 289 grams in cases with hepatic fibrosis and cirrhosis, both of which can be expected to cause portal hypertension with associated significant splenomegaly.

This study of liver pathology in a medicolegal autopsy population provides useful information on the extent and nature of liver abnormalities

which can be expected to be seen. Despite there being a very high prevalence of liver abnormalities in this series, only a small number of cases were considered to have directly or indirectly died as a result of their liver disease. Although this series is not representative of that expected in Australian deaths in general, given the understandably higher rates of death due to physical injuries, poisonings and other external factors than in the general population, this study nevertheless provides useful data which can also be extrapolated to the living general population. It is questioned whether more than four out of five cases in this series had liver disease, or whether in fact many of the changes seen in the liver at autopsy can be reasonably accepted as normal morphology.

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**Autopsy Pathology, Normal Range, Liver Pathology**

## **G8 Mesenteric Venous Thrombosis as a Cause of Rapid and Unexpected Death**

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After attending this presentation, attendees will learn about mesenteric vein thrombosis as a rare cause of abdominal pain and death. Attendees will review a case report of a middle-aged woman that presented to the emergency room with abdominal pain and died approximately three hours after admission.

This presentation will impact the forensic science community by discussing the underlying causes of mesenteric vein thrombosis and reviewing the general work up for thrombophilic states in the context of the postmortem examination.

Mesenteric venous thrombosis is an unusual cause of abdominal pain and bowel ischemia that may not be initially suspected because of its vague clinical presentation. In this presentation, a fatal case of a woman with extensive abdominal venous thrombosis who died within a few hours of her arrival to the emergency department is reported.

A 45-year-old Hispanic female was transported to the hospital for abdominal pain of three days duration. Earlier that day, she had presented to an outside clinic with the same complaint, where she was diagnosed with a urinary tract infection and given an intramuscular injection of ceftriaxone. Throughout the day, her abdominal pain became increasingly severe requiring hospitalization. On admission, she was oriented but complaining of severe abdominal pain and nausea. She was afebrile and tachycardic. Physical examination showed a pale, distressed female with diffuse abdominal tenderness and moderate distension, without guarding or rebound. The patient was nulliparous, took no medication, and had no past medical or surgical history. She denied tobacco, alcohol, or illicit drug use.

Laboratory testing showed an elevated white blood cell count (36.0 K/mm<sup>3</sup>) and anemia (hemoglobin - 6.9g/dL; hematocrit - 24.9%). Her platelet count was within normal range (246 K/mm<sup>3</sup>). Additional laboratory studies were unrevealing. She was given intravenous fluids and morphine for pain. Antibiotics were ordered. The patient refused blood products on religious grounds. A CT scan of the abdomen was ordered; however, she suddenly became distressed, bradycardic, and hypoxic. Following intubation she became pulseless. Despite resuscitative efforts, she was pronounced dead two hours and 50 minutes after admission.

An autopsy was performed at the county medical examiner's office. She weighed 133 pounds and had a body mass index of 28.8 kg/m<sup>2</sup>. Diffuse ischemia and infarction were evident in the small intestine, from the ligament of Treitz to the proximal ileum, and in the cecum, ascending colon, and the corresponding mesentery. The spleen and right lateral lobe of the liver were also infarcted. Thrombi occluded the superior mesenteric vein, the portal

vein, and its branches throughout the liver parenchyma, and the splenic vein. Thrombi were also noted in the smaller peripheral branches of the mesenteric vein. Additionally, the uterus was enlarged, weighing 625 grams, and contained multiple leiomyomata, the largest measuring 9cm. The left ovary was enlarged, weighing 550 grams and measuring 14cm in greatest dimension, and contained a benign cystic teratoma. The liver was not cirrhotic. Postmortem laboratory testing of hospital blood revealed mild to moderate prolongation of the prothrombin and partial thromboplastin time (PT - 14.9 seconds, PTT - 45.9 seconds) with an INR of 1.5. A lupus anticoagulant screen was negative. Upon further questioning of the family, the patient had no history of deep venous thromboses, abdominal trauma, oral contraceptive use, or prior malignancy.

Mesenteric venous thrombosis (MVT) has been described in the medical literature since 1895. It was first characterized as a clinical entity in a 1935 publication by Warren and Eberhard. Primary forms occur when there is no underlying etiology or associated condition. Secondary forms have an associated condition such as thrombophilia associated with antithrombin III deficiency, protein C or S deficiency, factor V Leiden or prothrombin gene mutations, and the antiphospholipid antibody syndrome. Other associated conditions include liver cirrhosis, prior abdominal trauma, asplenia, oral contraceptive use, malignancies, pancreatitis, intraabdominal infections, and congestive heart failure.

The signs and symptoms of MVT are nonspecific and mimic a large number of abdominal processes. Patients can present with diffuse abdominal pain of several days duration, abdominal distension, nausea and vomiting, and bloody diarrhea in cases that have already progressed to intestinal infarction. Laboratory studies are nonspecific; however, may reveal an elevated WBC count. Diagnosis of MVT is made by CT, MRI, or mesenteric angiography.

In this patient, the thrombosis was extensive, which led to visceral infarctions. No underlying etiology was established; however, coagulation studies were limited by postmortem samples. Venous stasis secondary to her enlarged uterus and left adnexal mass may have been factors. It has been suggested that MVT is a heterogeneous disease, whereby both hereditary and local factors may play a role in the development of the thrombosis. With an early diagnosis and appropriate medical and surgical intervention, the mortality of mesenteric venous thrombosis can be reduced.

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**Venous Thrombosis, Mesenteric Ischemia, Thrombophilia**

## **G9 Unexpected Death Due to Undiagnosed Medulloblastoma in Twin Pregnancy: A Case Report**

*Federica Portunato, MD\*, Manuela Botto, MD, Maria Celeste Landolfi, MD, and Enrico Pizzorno, MD, University of Genova, Department of Legal and Forensic Medicine, Via de Toni 12, Genova, I-16132, ITALY; and Francesco Ventura, MD, University of Genova, Department of Forensic Pathology, Via de' Toni, 12, Genova, I-6132, ITALY*

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After attending this presentation, attendees will learn about a peculiar case of unexpected death due to a brain tumor in a young pregnant woman.

This presentation will impact the forensic science community by stressing the importance of a complete forensic approach to ascertain the causes and means of death and to evaluate possible profiles of professional liability.

A case is presented of unexpected maternal death due to an undiagnosed medulloblastoma in a 28-year-old woman at the 33rd week of bigeminal pregnancy with neurologic symptoms which had been going on for the month prior to her death.

A woman in her 33rd week of twin gestation, presented at hospital with headache, nausea, and vomiting. She had a history of gastritis one month beforehand.

During the last hospitalization there was the onset of vertigo and nystagmus. Following a consultation with a neurologist and an

otolaryngologist; vestibular neuritis was diagnosed. She didn't undergo any instrumental examination since she was pregnant. Two weeks after the diagnosis while she was still hospitalized, she presented with acute frontal headache and died approximately three hours later.

An emergency cesarean section was attempted but it failed to save the twins. A forensic autopsy was performed four days later, which showed a non-encapsulated neoplastic mass between the left lobe of the cerebellum and the vermis (70 x 30mm) surrounded by massive edema. At histology the neoplasm was identified as a medulloblastoma with a IVth grade of malignancy (Louis D.N. et al, 2007).

Death was ascribed to acute cardio-respiratory failure caused by the compression of the brainstem carried out by the tumoral mass.

The twins didn't present with any pathology that could have possibly lead to death, so the cause of their death may be attributed to hypoxia following the maternal cardiac arrest.

Although pregnancy is considered a normal biologic process, it is associated with various physiologic and anatomic changes resulting in an increased risk of death.

The most common types of maternal death include vascular accidents, ranging from 10 to 20%, along with pulmonary embolism, cervical and coronary thrombosis, cerebral hemorrhage, as well as rare events such as rupture of the splenic artery aneurysm (Sharma BR et al, 2009 - He MX et al, 2010).

The incidence of unexpected death due to primary intracranial tumors in forensic practice is low. Particularly, medulloblastomas are relatively rare in adults accounting for only 2.4% of all intracranial tumor types (Merchant TE et al, 2010).

In an analysis of 10,995 medicolegal autopsies in only 19 (0.17%) resulted in an unexpected death due to an intracranial neoplasm of which only one (0.01%) was a medulloblastoma (Di Maio S et al., 1980).

Brain tumors tend to become larger and show accelerated growth during pregnancy due to fluid retention, increased blood volume or pregnancy hormones (Chang L. et al., 1999).

In conclusion, in this case the autopsy was paramount in order to ascertain the cause of death. It is questionable whether a prompt medical intervention with a cesarean section would have allowed the survival of the twins. However, considering the advanced stage of pregnancy, the absence of any sign of pathology regarding the two babies and the availability of perinatal intensive therapy care unit it is highly probable that the two would have survived.

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#### **Pregnancy, Medulloblastoma, Unexpected Death**

## **G10 Contribution of Histo-Pathological Examination in Electrocutions: Report of Two Cases and Review of Literature**

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After attending this presentation, attendees will learn the most specific features and the role of histo-pathological examination in the cases of high and low voltage electrocutions. Furthermore, each lesion will be compared with their main differential diagnoses, especially for the skin examination.

This presentation will impact the forensic science community by a clarification of the most specific features of histo-pathological examination and its medico-legal relevance in cases of electrocutions.

High-voltage electrocution is an uncommon cause of death. Few articles describe histo-pathological findings in deaths by electrocution and those that do usually focus on a single organ, without medico-legal relevance or interest, because of lack of specificity of histo-pathological signs.

Two cases of electrocutions observed in the Medico-Legal Institute of Toulouse Hospital, France are reported. For each case, this study reports autopsical and histo-pathological findings of viscera and skin. A review of the literature concerning the pathological examination of deaths secondary to electrocutions is performed, and establish the contribution of reported findings to the medico-legal purpose.

In the first case, the victim was found lying in a wet footpath near a 380 volts bare electric cable (low voltage), which was on the ground. The autopsy found several burns consistent with electric burns. The points of contact of electric current were on the abdomen and on the right upper limb. Notes was cyanosis of the head and neck and reddish foam in the airway suggesting asphyxia. Each internal organ appeared congested. The pathological examination found some features of electric current marks, particularly an iron deposition in the abdominal burn (identified by the Perl's Prussian Blue staining). This histological finding confirmed direct contact with the electric cable. The lungs presented some features of mechanical asphyxia suggestive of respiratory spasms in the context. The heart showed no signs of ischemia.

The second case concerned a man who was climbing on a metallic ladder to take down a hornet's nest in a tree. According to the eyewitness account, the ladder felled a power line carrying 20,000 volts (high voltage). The man was given emergency treatment and then was sent to the hospital, where he died. The autopsy found electric current marks on the upper and the lower limbs. The left-ventricular myocardium macroscopic examination revealed full-thickness diffuse circumferential hemorrhagic alterations. The pathological examination found a pseudo-asphyxic aspect of the lungs, an ischemic and hemorrhagic aspect of the heart with features of ventricular fibrillation, and distinctive ischemic liver injury. Since there was no doubt about the mechanism of the skin lesions, the electric current marks were not sampled.

A review of the literature had allowed the clarification of the role of the histo-pathological examination in the diagnosis and medico-legal classification of death by electric shock. The pathology of the skin (electric current marks), heart, lungs, and liver were studied in an effort to establish specific criteria defining death by electrocution. It appears that there is no pathognomonic sign of electrocution for each organ individually. However, we establish a list of arguments which, added to the accident investigation data, are highly suggestive of the cause and mechanism of death by electrocution shock. Pathological findings in high and low voltage electrocutions were compared, differences between direct contact with the electric source, and arcing injuries were discussed. Skin metallization is presented as a particularly useful histopathological feature for the diagnosis of electrical burns.

It appears that the histo-pathological examinations, in association with the investigation data and the autopsy findings, are helpful for clarification and determination of deaths when death secondary to electrocution is evocated.

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#### **Electrocution, Pathology, Medico-Legal Relevance**

## **G11 Sudden Unexpected Death Due to Acute Myeloid Leukemia: A Case Report**

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After attending this presentation, attendees will have learned about a case of unexpected sudden death due to undiagnosed acute myeloid leukemia.

This presentation will impact the forensic science community by stressing the importance of performing an accurate necropsy examination completed by a thorough histopathological study in all cases of unclear sudden death.

Acute myeloid leukemia (AML) is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia or anemia), with or without leukocytosis.

The clinical signs and symptoms of AML are diverse and nonspecific, but they are usually directly attributable to the leukemic infiltration of the bone marrow, with resultant cytopenia.

Typically, patients present with signs and symptoms of fatigue, hemorrhage, or infections and fever due to decreases in red cells, platelets, or white cells, respectively. Pallor, fatigue, and dyspnea on exertion are common. Leukemic infiltration of various tissues, including the liver (hepatomegaly), spleen (splenomegaly), skin (leukemia cutis), lymph nodes (lymphadenopathy), bone (bone pain), gingiva, and central nervous system, can produce a variety of other symptoms.

The primary diagnosis of AML rests on the morphologic identification of leukemic myeloblasts in preparations of peripheral blood and bone marrow stained with Wright-Giemsa. The presence of more than 30 percent leukemic blasts in a bone marrow aspirate is required for a definitive diagnosis of acute leukemia.

AML has typically been categorised with the FAB system, which is based on cytomorphology and cytochemistry. The current WHO classification system incorporates cytogenetic data and defines four major categories of AML. At least 20% of the blasts must have surface antigens associated with myeloid differentiation.

A case is presented of a woman of 67-years-old died in Hospital of Genoa's emergency room for unknown clinical causes. The day before his death, she had been visited at home by doctor for symptoms of diarrhea and vomiting which had persisted for several days. The doctor gave antiemetic and antiarrhoeal therapy and rehydration.

The body's autopsy was performed by Institute of Forensic Medicine of Genoa. External examination highlights numerous small purplish ecchymosis on the whole body surface. Small and diffuse hepatic nodules were also found at autopsy.

Histopathologic examination performed on the tissue samples, confirmed the presence of neoplastic cells in cerebral, heart, lungs, pancreas, kidney, and spleen blood vessels. Neoplastic elements at immunohistochemical examination showed weak expression of myeloperoxidase and molecule CD 34, absent expression of molecule CD 20 and CD 3. This immunohistochemical profile identified a neoplastic proliferation of immature granulocytic elements. The multiorgan neoplastic involvement was due to acute myeloid leukemia (FAB M1/M2).

The data obtained at autopsy and histopathology attributed the death due to multiple organ failure secondary to acute myeloid leukemia.

In the literature are other examples of unexpected death secondary due to complications of undiagnosed acute myeloid leukemia, but in these cases, the cause of death was hemorrhage secondary to disseminated intravascular coagulation.

In conclusion, the present case is interesting especially because unexpected death, came after non-specific symptoms, caused by a multiple organ failure (MOF) secondary to acute myeloid leukemia, stresses the importance of postmortem examination complemented by histopathological investigations, to define cause of death.

**Acute Myeloid Leukemia, Sudden Unexpected Death, Multiple Organ Failure**

## **G12 Homicide During Police Procedures: From Crime Scene Investigation to Reconstruction — The Long Way to the Truth**

*Francesco Vinci, MD\*, Maricla Marrone, MD, and Sabrina Leonardi, MD, Section of Legal Medicina - University of Bari, Piazza Giulio Cesare n. 11, Bari, 70124, ITALY*

After attending this presentation, attendees will understand that ballistics investigation is the prerogative of several professionals. Among them, forensic pathologists are essential to ensure a correct reconstruction of the dynamics of events in cases of murder or injury. In fact, a multidisciplinary approach combining the specific skills of different professionals will guarantee the best results.

This presentation will impact the forensic science community by showing how pathologists are irreplaceable in forensic ballistics. Their knowledge, enabling an objective assessment, can integrate and complete the conclusions of other ballistics experts, and assess the compatibility of the bodily injuries and the reconstructed dynamics.

A case is presented of a 47-year-old man who died of a gunshot wound during capture by the police. The victim was driving down a city street in the wrong direction when he was spotted by policemen. A chase by two police cars began and three policemen fired several shots at the victim's car, one of which killed the victim. During the crime scene investigation, a different military force found nine cartridge cases on the tarmac, several bullet fragments in the car (back and front right), and two bullets (a deformed bullet on the floor of the left front seat and an intact bullet on the right front seat). Fragments of glass were found on the tarmac at a distance of two to three meters from the car and the right front window was broken.

The agents reported the following dynamics: the man entered a dead-end street, so he tried to re-enter the main road probably intending to run over the three policemen. Two gunshots were fired against the back wheels and several shots toward the front right of the car. The victim's car started to move again and a policeman broke the front window with a gun. The car stopped immediately and when the policeman opened the front door where he found the victim slumped lifeless in his seat.

A team of forensic pathologists performed autopsy, genetic, and toxicological examinations as well as a ballistic investigation. Autopsy findings showed a single through wound with entry in the victim's left shoulder and exit on the right chest, then striking the arm. The gunshot course was from left to right and back to front at a downward angle of 18°. The bullet struck the left lung and the heart, causing massive hemothorax and slight hemopericardium. The victim also showed a bruise on the left cheek, consistent with a blow from the firearm magazine.

Examination of gunshot residues on the jersey sleeve revealed a shooting distance of 40-60cm. Ballistic examinations were made of the bullets, bullet fragments, cartridge cases, three guns, and on the victim's car: the findings were compared with the evidence markers at the crime scene. The two bullets in the car were 9mm caliber gun. Genetic investigations revealed the victim's DNA on the intact bullet. Firing tests identified the weapon which fired the fatal bullet and studied the decrease of  $V_0$  and gunshot residue after firing against the window of a similar car. The victim's car had been hit by at least seven bullets: five shots against the front left (one of which ended on the floor of the left front seat) and two shots against the back wheels.

The following event dynamics were reconstructed: during the capture, the policemen shot at the back wheels and front left of the victim's car. Then a policeman broke the car window with the gun muzzle; meanwhile, a bullet was fired from the same gun and hit the victim's shoulder. It is not known whether the shot was intentional. The car traveled on a few meters while the man died. The blunt injuries to the head probably occurred when the victim was forcibly removed from the vehicle.

In conclusion, serious discrepancies were highlighted between the crime scene reconstruction by the pathologists and what had been reported by the policemen: the man's death was not due to an accidental gunshot fired at

the car wheels, but to a gunshot fired directly at the victim, probably accidentally. Finally, ballistic investigations identified the weapon and the policeman responsible for the death.

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**Ballistic Findings, Forensic Pathology, Shooting Reconstruction**

### **G13 Minimal Submersion Period Estimation Using Freshwater Benthic Fauna and Wagner's Parsimony Method (WPM): Tools for Forensic Investigations in Different Lotic and Lentic Environments**

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After attending this presentation, attendees will learn information about a new method to estimate a minimal submersion period for a corpse found in freshwater.

This presentation will impact the forensic science community by possibly providing a PMI estimate in freshwater by studying fauna using Wagner's parsimony method.

The forensic investigations are generally very difficult when a body is discovered immersed for an undetermined period of time in freshwater. Due to long periods of immersion and a high level of putrefaction, the estimation of the time since death is problematic. Classical forensic entomology is based on existing links between necrophagous insects and the state of decomposition, in order of appearance of necrophagous insects on the cadaver. This science does not allow a pertinent evaluation of the time since death for a crime scene technician. It is indeed impossible to reach any conclusions based on the underwater colonization process once the cadaver has resurfaced. The purpose of this work was to analyze from February 2001 to October 2002, the different cases of cadaver discoveries in order to isolate pertinent bioindicators. The database consists of a medicolegal set, ecological, and judicial observations recorded on 30 freshwater. This study gathered the following data: freshwater loci (lotic or lentic), geographical location, first elements on discoveries, season, and the postmortem interval (PMI) recorded by pathologist during autopsy. In all cases, the PMI was estimated between half a day and three years and only involved adult victims.

Two types of mathematical methods can offer an ecological data processing: phenetic's methods and parsimony method. Phenetic' methods, based on the total inter-sites similitude, give calculations of distances and links between sites.

Because of the risk of confusion due to the infinite mathematical distances, number, and computational tools, phenetic' methods seem inappropriate for this type of investigations. So, Wagner parsimony method which does not authorize scenarizations but does allow a strict objectivity between diagram and data matrix was chosen.

Forensic application of WPM allows analyzing presence/absence of aquatic and terrestrial invertebrates on different crime scenes according to the method described and respectively applied by Masselot et al. to freshwater biomonitoring and Coiffard et al. to taxon lists of fossil plant assemblages of the Cretaceous. Leaves of the trees were considered as crime scene "localities." There were gathered according to their environmental or taphonomic states of characters (synapocoenoses sensu Nel et al.). Attributes are independent sets of information (e.g., "victims" parameters).

For corpses found after a one to three weeks period of partial submersion (floating) in a lotic or lentic freshwater environment, attributes required for colonization were the accessibility to the surface, the presence of clothing, a high decomposition level, and the presence of algae and mud. In the 30 cases studied (PMI from one day to three years), invertebrates were systematically absent on fresh (low PMI of under two to three days) and on

very decomposed cadavers (skeletal remains). Evidence of a colonization process had disappeared on unclothed cadaver or when postmortem changes occurred. In the laboratory, correlation was explained between the development rate reached by each arthropod specimen and the stage of decomposition recorded at the autopsy. Study of the parsimonious biocenogram demonstrated different gradients associated with increasing postmortem delays:

- Specimens of the order of Gasteropoda were the first colonizers on a cadaver submersed for one week or more. Gasteropoda sampling is particularly congruent with a lotic environment, partial submergence, and presence of clothing. First gastropods were collected from carcasses just after five days of immersion.
- Second contributors to cadaver colonization were immature forms of Diptera. They were mainly collected from the muddy clothing on the cadavers. At the same time, the presence of species of Diptera was found during Spring on submerged cadavers (PMSI from three weeks to two months). Totally submerged corpses were mainly attractive to aquatic species of Chironomidae or Simuliidae, and floating cadavers had a concomitant colonization of aerial Diptera and aquatic fauna.

With regard to the colonization by aerial Diptera, it appears wrong to use necrophagous insects alone for the estimation of PMI. In fact, the aquatic environment was able to defer oviposition (egg-laying), to reduce larval density on emergent parts of the corpse and to slow down the development rate of insects (influence of lower water temperatures and washing effect of the corpse).

A simple but effective sampling protocol to get a real solution to the problem of estimating the postmortem submersion interval (PMSI) of a cadaver found in lotic or lentic water was created.

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**Postmortem Interval, Freshwater Invertebrates, Wagner's Parsimony Method**

### **G14 Insect Timing and Succession on Buried Carrion**

*Emily C. Pastula, BS\*, 3434 Schlee Street, Lansing, MI 48910*

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After attending this presentation, attendees will have a better understanding of how insect succession is used in forensic work, which insects are present on buried carrion as opposed to exposed, and how long it takes insects to arrive on buried carrion.

This presentation will impact the forensic science community by enabling forensic entomologists to better understand the interaction between insects, carrion, and the soil environment. The understanding of insect succession on buried carrion could lead to the development of a post-burial interval estimation. This estimation would be similar to the postmortem interval (PMI) used on surface carrion to determine time of colonization. With this development, it would be possible to date a buried body based on the insect evidence present.

Burial is a popular technique chosen by assailants when looking to dispose of a body. Rarely are bodies buried very deep since digging requires a great amount of time and effort. The longer the assailant is in contact with the body, the more likely they are to be found with the body or leave evidence linking them to the crime. Therefore, assailants usually dig shallow graves to dispose of their victims with depths ranging between zero and three feet, the most common depth being two and one-half feet. A difference in the insect fauna has correspondingly been found when comparing exposed and buried carcasses, yet very little is known about when exactly these insects arrive. The time it takes insects to travel to carcasses at different depths has not been readily studied. It is also unclear how far insects are able to travel through the soil to colonize a carcass because most experiments only looked at a depth of one foot.

By increasing the frequency of sampling and placing out a large number of replicate pig carcasses (42), it was hoped to narrow down the time range

in which certain insects arrive at carcasses buried at one foot and two feet, with the bottom of the hole measuring the depth. A predetermined number of pig carcasses were exhumed after three, five, seven, fourteen, twenty-one, thirty, sixty, ninety, and one hundred twenty days. Insects were collected off of the carcass itself, as well as from the soil above the carcasses via excavating and sieving, and then placed in ethanol for later identification. It was postulated that insects would be able to colonize a carcass at two feet, that it would take one week for insects to reach a depth of one foot, that it would take two weeks for insects to reach a depth of two feet, and that insect succession would progress similarly to exposed remains with fly larvae from the family *Calliphoridae* being the first to colonize.

The results indicate that insects are capable of colonizing a carcass at both one and two feet and arrive after five days and seven days, respectively. The insects present do not correspond with those normally found on exposed remains. Instead, fly larvae from the families *Sarcophagidae* and *Muscidae* are the first to colonize buried remains. From these results we hope to aid in the possibility of dating buried bodies based on the insect evidence present.

#### **Forensic Entomology, Insect Succession, Buried Carrion**

### **G15 Stab Wound in the Neck: An Unusual Case of Suicide**

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The goal of this presentation is to report an uncommon case of suicide by stab wounds in the neck and to underline the need of a complete forensic approach through autopsy with special techniques for the dissection and examinations of the neck structures, histological, and toxicological ancillary examinations, as the only way for a better understanding of the mechanism of death.

This presentation will impact the forensic science community by showing how useful a formaldehyde fixation of the neck organs could be allowing an easier dissection of this area and better identification of the injuries.

In forensic contexts, stab wounds usually have a homicide etiology. The severity of the wounds is based mainly on the location, depth of penetration, the type of weapon (knife length, shape, straight or serrated), and manner of assault.

Stab wounds to the chest are usually lethal. They can result in rupture of the lung, marked bleeding within the chest cavity, or penetration of the heart. Stab wounds to the abdomen could result in trespassing internal organs or vessels with fast or slow bleeding inside the abdomen. In the neck, even a single stab wound, because of the multitude of organ systems in this part of the body, is capable of producing considerable harm.

Suicide caused by a stab wound in the neck is unusual in the forensic literature. A case of suicide is presented, in which the victim, resident in a retirement home and with amputation of lower limbs, uses a small penknife with a 6.5cm blade to commit suicide. He was found in his room, still conscious, bleeding from the neck and expressing his suicide intentions – “*I want to die, I want to cut the carotids.*”

When admitted at the emergency room he has hemodynamically stable but with respiratory distress and hemoptysis. He developed hypoventilation and bradycardia and died after two hours. Resuscitation procedures were carried out.

During the autopsy the external examination found a single fusiform wound in the anterior view of the neck and two superficial wounds, affecting only the epidermis. However, the internal examination shows multiple injuries at the cervical fascia and at the muscles of the anterior cervical region. Injuries at the thyroid cartilage were also found but none at important vessels. The lungs had a mottled appearance. The neck organs were removed *en bloc* and subjected to formaldehyde fixation. A posterior and careful dissection

allowed to better identified the paths of the blade and the injuries, some of them located at the oesophagus and pharyngo-laryngeal mucosa. Histological exams were performed as well as toxicological. The last ones revealed the presence of drugs (midazolam, lidocaine, amitriptyline, carbamazepine, and paracetamol) at therapeutic levels.

This study underlines how in cases of stab wounds of the neck, a careful autopsy involving special techniques of neck dissection must be adopted and combined with the examination of the neck organs after fixation. This previous fixation could be very useful, particularly when no vessel or spinal cord trauma was found, to explain the mechanisms of death.

#### **Stab Wound, Neck, Formaldehyde Fixation**

### **G16 Atypical Hanging Occurring in Two Sisters Within a Short Period of Time: Incidental, Sympathetic, or Something Else?**

*Khalid Jaber, MD\*, Firebrigade Training Centre, Malahide Road, Marino, Dublin 3, IRELAND*

After attending this presentation, attendees learn two cases of atypical hanging occurring in two sisters within a relatively short interval. Both sisters belonged to the Travellers' community of Ireland. Both were married and had sporadically difficult relationship with their husbands. When the paramedical staff first encountered these females separately, ligatures were not found around their necks.

This presentation will impact the forensic science community by presenting the unusual death of two sisters through atypical hangings. Autopsy and scene photographs will be presented showing the atypical features of both deaths.

**Case 1:** The first sister was living with her husband (partner). She had experienced some fertility problems over the past two years prior to her premature death. Her husband somehow was never similarly and or concurrently investigated. She was brought to the near-by hospital, by her husband, clinically in a near hanging situation with unusual burn marks on her left thigh. She was unconscious, with Glasgow coma score of three, with no ligature *in situ*. She remained comatose for less than 24-hours before she expired. The husband's story was that he returned to the house after being away for 20 minutes and found his wife hanging by a rope from the pipe of a boiler unit in a small storage/utility room in an incomplete suspension. Her feet were on the floor. He rushed to remove the noose and loosen the alleged simple slip knot with no apparent difficulty, with one hand while supporting his wife with the other. Upon hearing gurgling noises he realized she was not dead so he called for help from neighbors and began administering CPR. He and his neighbor drove the wife to the hospital. She was admitted and an initial CT scan showed diffuse brain edema and features of hypoxic ischemic encephalopathy. She remained in hospital for less than 24-hours and expired the following day. Upon admission to the hospital, nursing staff noted an unusual burn marks on the side of her left hip and thigh area. The features of the hanging marks and the burn marks are presented and discussed.

**Case 2:** Her sister, a mother of one child, was found dead in her Aunt's house where she sought shelter from her allegedly aggressive husband (partner). The latter apparently had physically assaulted her a few days earlier in her mother's home where she lived with her child. She was drinking with her alcoholic aunt the afternoon when she was found slumped on the floor with her head near the radiator in the center of the wall and her left leg leaning on the edge of the bed positioned along another wall. She was found with ligature marks around her neck by the residents of the house, but with no ligature *in situ*. The scene revealed two TV cables running along two corners of the room and coming from the attic space. The nearest cable to the body was found split at a previously cut point which was taped. The other cable was not considered by the scene investigators as a possible ligature. The features of the hanging marks and the burn marks are presented and discussed.

These two sisters were young females from the Travellers' community in Ireland. Both were married and had experienced uneasy and, at least, sporadically difficult marital life. Both were regular consumers of alcohol and both have been possibly clinically depressed.

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#### **Atypical, Hanging, Sisters**

### **G17 Detection of HCV in a Body Exhumed After Four Months Followed by a Phylogenetic-Tree Analysis Allows Identification of the Origin of a Nosocomial HCV Transmission Associated With the Use of a Multi-Dose Vial**

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The goal of this presentation is to focus on an outbreak of nosocomial transmission of hepatitis C virus (HCV) in a hospital in northern Italy. After attending this presentation, attendees will gain knowledge of a case where a forensic approach by means of exhumation, autopsy, microbiological studies, phylogenetic-tree analysis, and circumstantial evidence led to the conclusion that the outbreak was linked to a patient-to-patient transmission occurred when a multidose vial was contaminated with blood from an HCV-infected patient.

This presentation will impact the forensic science community by demonstrating how important a thorough forensic investigation and a multidisciplinary approach are in order to provide an adequate and high-quality service to the judicial authorities. It will also impact the forensic science community by showing how long HCV can remain active even in a dead body in particular environmental conditions.

Since the introduction of routine screening of blood for anti-HCV and the steep decrease in the incidence of post-transfusional hepatitis, patient-to-patient transmission has become the commonest mode of nosocomial HCV infection.

HCV infection is frequent among patients with hematologic malignancies, especially those with lymphoproliferative disorders, and unapparent routes of infection may be important in this setting. Indeed, many patients acquire HCV infection in the environment in which they receive treatment for their disease.

Outbreaks of HCV nosocomial transmission have been linked to breaches in standard precautions for blood-borne infections during nursing procedures or interventions such as surgery, dialysis, and colonoscopy.

An outbreak of patient-to-patient transmission of HCV through the use of a multidose vial during the rinsing of central or peripheral venous catheters is reported. At the beginning of 2006, acute HCV infection was diagnosed in six patients hospitalized in a hematology ward and a look-back study identified two other HCV-positive patients. Analysis of the events pinpointed the period in which the contamination occurred due to a single episode of exposure from a unique source between 7:00 a.m. and 8:30 a.m. on December 14, 2006. Circumstantial evidence suggested that the only patient having a known HCV chronic infection in this period was the index case; however, since she died on December 29, 2006, there were no samples available to document the source of the outbreak.

It is well known that HCV, like all viruses, is gradually inactivated in the body of a dead host and the presence of heat can have a drastic impact on the

lifespan of the virus. Particularly, the virus is referred to be infectious for about sixteen days at 25°C and two days at 37°C, while it can remain active for more than six weeks at temperatures less than or equal to 4°C.

Regardless of this lability of HCV and the lack of similar cases described in the literature, the cadaver was exhumed four months after burial.

The HCV RNA genome was surprisingly amplified, identified, and genotyped in liver and spleen samples. Genotyping of HCV strains, amplified in various clinical samples from the eight patients, was performed by sequence analysis. The comparative phylogenetic analysis of the strains identified in the patients studied with those from other HCV patients allowed identification of the source of contamination, which was the same for five patients. Moreover, a definite route of transmission has been identified as well as the specific cause-effect relationship for the multidose vial procedure.

Particularly, nursing was found to be inconsistent with recommended safety standards.

In conclusion, the exhumation and appropriate microbiological and phylogenetic investigations allowed for the identification of the outbreak origin and a correct analysis of the professional liability profiles.

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#### **Outbreak of HCV Nosocomial Transmission, Active HCV in an Exhumed Body, Phylogenetic-Tree Analysis**

### **G18 The Virtual Hydrostatic Test**

*Dori M. Franco, DO\*, Edward L. Mazuchowski, MD, PhD, Philip J. Berran, JD, MD, and Howard T. Harcke, MD, Armed Forces Medical Examiner System, 116 Purple Heart Drive, Dover AFB, DE 19902*

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After attending this presentation, attendees will understand the techniques and procedures available to distinguish a stillborn fetus from a liveborn neonate.

This presentation will impact the forensic science community by demonstrating another tool in the determination of live birth.

The hydrostatic test has been used for hundreds of years to help determine if there has been a live birth. Criteria for live births include respiration, heartbeat, food in the stomach, or a vital reaction of the umbilical stump or any injury. The hydrostatic test involves placing the lungs in water to see if they float. If the lungs float, it could be due to aeration or putrefactive gas from decomposition. To exclude decomposition, a portion of liver is also placed in water. If the liver also floats, the test is inconclusive. If the liver sinks, the floating lungs are considered to be aerated. Aeration can also be caused by artificial respiration due to resuscitation efforts and such information should be sought. Histology of the lungs has also been used to assess respiration. Expanded alveoli are consistent with respiration and collapsed alveoli with stillbirth; however, microscopic examination should not be the sole criterion used. Reportedly, there have been stillborns with expanded alveoli and live births with collapsed alveoli.

Computed tomography (CT) is being increasingly used in the practice of forensic pathology. CT is helpful in determining fractures, especially of bones not normally examined during autopsy (e.g., spine), detecting foreign bodies and their trajectories, and detecting air (e.g., pneumothorax). CT can also aid in estimation of gestational age by measuring the femur length.

Presented are two cases of fetal deaths of estimated gestational ages 21-23 and 23-25 weeks in which both the hydrostatic test and CT were performed. In one case, the lungs sank in water and there was no air seen in the lungs or gas in the liver on CT. Histology of the lungs showed collapsed alveoli. In the other case, the lungs floated in water, the liver sank, and air was seen in the trachea, bronchi, and both lungs on CT; and no gas was present in the abdomen. Histology of the lungs showed areas of expanded alveoli and collapsed alveoli. In both cases, the CT was used to corroborate the estimated gestational age.

Because assessment of live birth is a critical and difficult decision, as many factors as possible should be taken into consideration. Postmortem CT offers another assessment to consider in this determination. Further study and correlation with existing methods seems warranted.

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#### **Hydrostatic Test, Postmortem CT, Virtual Autopsy**

## **G19 Methylenedioxypropylvalerone “Bath Salts” Related Death: Case Report and Review of the Literature**

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After attending this presentation, attendees will be familiar with methylenedioxypropylvalerone (MDPV), a new designer drug more commonly known as “bath salts,” and its significance as a current public health trend.

This presentation will impact the forensic science community by increasing awareness of “bath salt” use, the clinical presentation of a MDPV-related death, and the importance of monitoring its emergence as a possible new epidemic in substance abuse.

“Bath salts” are white, tan, gray, yellow, or brown odorless powders sold in “head shops”, gas stations, and convenience stores and on the Internet where they are marketed under multiple names. They also retail as plant food, pond water cleaner, odorizers, research chemicals, potpourri, and incense. “Bath salts” are recreational designer drugs of abuse that are injected, ingested, or inhaled, which unlike traditional cosmetic bath salts have no legitimate use for bathing. These products contain stimulant compounds such as 3,4-methylenedioxypropylvalerone (MDPV) or 4-methylmethcathinone (mephedrone) whose effects are similar to methamphetamine, amphetamine, and cocaine.

MDPV is part of the phenethylamine class of drugs and is structurally similar to cathinone (khat), found in both schedule I hallucinogenic substances and schedule I stimulants. MDPV is currently not scheduled by the DEA; however, many states have banned the sale of “bath salts.” Clinical effects of MDPV include tachycardia, chest pain, hypertension, increased body temperature, diaphoresis, seizures, agitation, paranoia, hallucinations, delusions, aggression, excited delirium, and suicidal thoughts. The psychogenic effects can be prolonged. Symptoms may also progress to rhabdomyolysis and renal or liver failure. Currently MDPV is not found by routine immunoassay toxicology screens.

In April 2011, a 39-year-old white male with a history of depression, back pain, drug, and alcohol abuse was found outside his residence delusional and wandering around in clothing inappropriate for the weather. He was taken to the emergency department (ED) by law enforcement officers under emergency petition for a drug overdose and psychiatric disorder. In the ED, the staff noted whitish powder around his mouth and he admitted he had used “bath salts.” He subsequently became agitated, tachycardic and hyperthermic (106.8°F), and eventually went into asystole. Resuscitation was unsuccessful and he died approximately 12 hours after presenting to the ED. Hospital urine drug screen was positive for benzodiazepines and phencyclidine (PCP). Upon inquiry with the hospital, it was determined that the PCP was most likely a false positive and diazepam and diphenhydramine were given at the hospital.

Autopsy was basically unremarkable except for toxicology. Comprehensive alcohol and drug testing were performed in this case. Therapeutic and abused drug testing was performed on the bile. This included: (1) acid/neutral drug screen by gas chromatography(GC)-nitrogen-phosphorous detection (NPD); (2) alkaline drug screen by GC-NPD; (3) acetaminophen and salicylate by color test; and, (4) morphine and benzodiazepines by enzyme-linked immunosorbent assay (ELISA). The salicylate color test was positive and the alkaline drug screen was positive for diphenhydramine, MDPV, promethazine, diazepam, and nordiazepam.

Salicylate was confirmed by ELISA. The alkaline extractable drugs were confirmed by full scan electron ionization gas chromatography/mass spectrometry. MDPV was quantitated using the alkaline drug screen routinely employed by this laboratory. The heart blood contained 0.1mg/L diphenhydramine, 0.2mg/L promethazine, 0.1mg/L nordiazepam and 0.7mg/L MDPV and the peripheral blood MDPV concentration was 1.0mg/L. Based on the investigative, autopsy, and toxicology findings in this case, the cause of death was methylenedioxypropylvalerone intoxication and the manner of death was accident.

As of July 2011, it is believed that this is the first reported MDPV related death in the United States. Louisiana and states in the Midwest have reported that almost all of their MDPV deaths are traumatic in nature and not due to the drug alone. MDPV is an emerging drug trend in the United States and medical examiner and coroner’s offices need to be aware of the limitations of routine toxicology testing in detecting MDPV. One should consider testing for MDPV in cases with a history of polysubstance abuse with stimulant type and psychogenic symptoms of acute onset and those individuals in a hyperthermic excited delirium type state. Further research on MDPV related deaths is necessary to assist in the determination of emerging drug trends of public health significance.

**Methylenedioxypropylvalerone, Bath Salts, Designer Drugs**

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## **G20 Retinal Hemorrhages Associated With Non-Abusive Sudden Unexplained Deaths in Infants**

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After attending this presentation, attendees will learn how ocular hemorrhages can occur in infants who are found unresponsive and have received cardiopulmonary resuscitation but have no evidence of abuse.

This presentation will impact the forensic science community by increasing the attendees’ awareness of non-traumatic retinal hemorrhages in infants and emphasize that retinal hemorrhages can occur after cardiopulmonary resuscitation and are not specific for abusive head trauma.

Retinal hemorrhages (RHs), considered by many physicians as a key finding in abusive head trauma (AHT), have been reported in about 85% of fatal AHT cases. Published reports have stated that the number, location, and distribution of RHs are significant for differentiating RHs observed in AHT as compared to RHs occurring in accidental head injuries or natural disease processes. A number of studies have indicated that bilateral multiple RHs extending to the ora serrata have particular diagnostic specificity for AHT.

Ten cases are presented of sudden, unexplained infant deaths that had RHs following cardiopulmonary resuscitation (CPR) but no evidence of abusive or accidental head trauma. Postmortem monocular indirect ophthalmoscopy (PMIO) detected the RHs in all cases. Only one infant, who had bilateral RHs detected by PMIO, had a documental clinical fundal examination by a non-ophthalmologist 41 hours after CPR had been initiated. Investigative and autopsy findings revealed no natural diseases, craniocerebral trauma, or other injuries that caused or contributed to the deaths. The cause of death was certified as Sudden Infant Death Syndrome or unexplained infant death.

The infant ages ranged from seven weeks to ten months of age, with a median age of 18 weeks and a mean age of 16.7 weeks. Four infants were born premature at 32 to 36 weeks estimated gestational age. All but one infant was found unresponsive after being placed down to sleep; one was taken to the emergency department after a witnessed episode of loss of consciousness. Four infants who were placed to sleep in a bed or crib were found prone. CPR efforts lasted less than 15 minutes to one hour. Four infants experienced return of spontaneous circulation and survived for 6-56 hours after CPR efforts were started. Resuscitation-related rib fractures

occurred in three infants. Ophthalmologic findings ranged from a single superficial retinal hemorrhage in one eye to diffuse retinal hemorrhages with extension to the ora serrata and optic nerve sheath hemorrhage. The four infants with multiple retinal hemorrhages with extension to the ora serrata all had spontaneous return of circulation and survived between 6-56 hours following the initiation of CPR. The infant with the optic nerve sheath hemorrhage had the longest survival time. Diffuse systemic ischemic and reperfusion injuries affecting the kidneys, adrenal glands, liver, myocardium, intestines, lungs and brain were observed in four cases; focal hypoxic-ischemic organ damage was present in three cases. Neuropathological findings included cerebral edema, intra-falcine and intra-dural extravasated blood, and focal subarachnoid hemorrhage.

Infants found unresponsive invariably undergo resuscitative efforts, often for prolonged periods of time. Forensic pathologists must be aware that RHs can be seen in infants who die suddenly and unexpectedly, following cardiopulmonary resuscitation, and are not specific for abusive head trauma. Infants found unresponsive without evidence of head trauma or natural disease processes that have had CPR, especially those with restoration of circulation, can have RHs that may be few in number or numerous with extension to the ora serrata. It is important for forensic pathologists to perform postmortem ocular examinations on all infants dying suddenly and unexpectedly to identify conditions associated with RHs and not equate RHs solely with AHT.

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### **Retinal Hemorrhages, Sudden Unexplained Infant Deaths, Cardiopulmonary Resuscitation**

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## **G21 Postmortem Artifactual Perimacular Retinal Fold**

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After attending this presentation, attendees will learn that perimacular retinal folds can develop as a postmortem artifact and should not be viewed as pathognomonic or highly specific for abusive head trauma.

This presentation will impact the forensic science community by emphasizing how ocular abnormalities found at the autopsy must be interpreted with caution and never as an isolated finding without appropriate clinical correlation and thorough death investigation.

The classical triad associated with abusive head trauma (AHT) includes subdural hemorrhage, retinal hemorrhages (RHs), and encephalopathy. Several other ocular findings have been described as virtually pathognomonic or highly specific for AHT. These findings include perimacular retinal folds (PRFs), traumatic retinoschisis, optic nerve sheath hemorrhages, and peripheral RHs abutting the ora serrata. There have been numerous cases reports in the last decade of accidental head injuries that have produced these ocular abnormalities. In particular, perimacular retinal folds and traumatic retinoschisis have been increasingly identified in case reports of accidental head injury. However, these cases are considered by many to be rare outliers and the ocular findings continue to be treated as highly specific for AHT. We present a case of accidental head injury with the development of an artifactual PRF during the postmortem interval that could potentially be interpreted as an antemortem PRF.

**Case Description:** The decedent was an 11-year-old girl who was crossing the road to get the mail when she was struck by an oncoming automobile. Emergency Medical Services responded, secured an airway, and transported her to the nearest trauma center. Upon arrival in the emergency department the patient's Glasgow Coma Scale was 3 and CT scan showed subdural hemorrhage, intraventricular hemorrhage, and diffuse cerebral edema with herniation. She was ultimately declared brain dead and life support was withdrawn.

At autopsy, multiple blunt force injuries of the head included subscalpular hemorrhage, subdural hemorrhage, subarachnoid hemorrhage,

intraventricular hemorrhage, Duret hemorrhages, bilateral multilayered RHs, and bilateral optic nerve sheath hemorrhages. Other injuries included a fracture between C7 and T1, a sternal fracture, a splenic laceration, and multiple scattered abrasions and contusions. The left posterior leg had the greatest concentration of subcutaneous hemorrhage 13-17 inches above the left heel, consistent with a site of impact.

Indirect ophthalmoscopy was initially performed four hours after death. The autopsy was done the next day, at which time indirect ophthalmoscopy was repeated and the globes were removed and placed in 10% neutral buffered formalin. The first exam found numerous bilateral multilayered retinal hemorrhages over the posterior pole in all four quadrants extending to and past the equator but no retinal folds. The second exam 22 hours after death revealed artifactual retinal folds. The right eye had an artifactual perimacular fold extending to the fovea and then continuing temporally from the fovea as a linear papillomacular fold, and the left globe had an artifactual papillomacular fold.

These folds are not Lange folds, which are well-known artifacts of fixation in the eyes of infants and children. Lange's folds are described as an inward fold of the neural retina present at the very periphery of the retina and extending to the ora serrata. The folds in this case were perimacular and papillomacular. The folds in the right eye surround the macula in a circumferential pattern that is not consistent with the description of Lange's folds. The folds in both eyes were located primarily between the optic disc and the fovea and did not extend to the periphery.

If the first retinal exam had not been performed and the perimacular retinal fold was identified following ocular enucleation, it may have been interpreted as sequelae of her head trauma. The more concerning aspect would be if the PRF was identified in an infant or young child, in which case, it might be viewed as highly specific for AHT instead of a postmortem artifact. This case further illustrates that ocular findings identified at autopsy cannot be viewed as pathognomonic or even highly specific of AHT without appropriate clinical correlation, a thorough death investigation, and routine postmortem ocular examinations in all infants and young children.

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### **Forensic Science, Perimacular Retinal Fold, Postmortem Artifact**

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## **G22 Sudden Cardiac Death in a Young Male With History of Tako-Tsubo (Stress) Cardiomyopathy: A Case Report**

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After attending this presentation, attendees will learn of a case showing unusual cardiac cause of sudden death in a young male due to stress cardiomyopathy and its clinicopathologic features.

This presentation will impact the forensic science community by educating the attendees on an uncommon disease entity which is usually associated with postmenopausal females, but can also affect younger males.

This case involved a 20-year-old Black male who had a history of a stab wound of the left chest in May 2010. In the hospital a chest tube yielded 750ml of blood. Chest x-rays and a CT scan showed a stab wound perforating left pectoral muscle with hydro- and hemothorax. There were no injuries to the mediastinum, including the heart, great vessels, trachea, and esophagus. The patient subsequently had chest pain. Cardiac enzymes and EKG showed ST elevation and elevated troponin. A cardiologist made a diagnosis of Tako-Tsubo cardiomyopathy secondary to the stress from his stab wound of the chest. He was discharged six days after the stab wound. Four months later he collapsed at home and was transported to a hospital, where he expired.

The autopsy showed that the decedent was 185cm tall, weighed 77.5kg, and had a linear one-inch scar in the left chest area consistent with the previous stab wound. Mild cardiomegaly (460g) with focal pericardial

adhesions was noted. The heart had extensive apical transmural circumferential left ventricular and septal scarring (8 x 5 cm area) with thinning of the myocardial wall (1.0 – 0.5cm) and attached mural thrombus (5 x 5cm). The scarring far exceeded the territory of any single coronary artery. Dissection of the coronary arteries showed no significant atherosclerosis and no narrowing or stenosis. Toxicology analysis revealed the presence of carboxy-tetrahydrocannabinol in the blood. A cardiac pathologist was consulted. He reviewed autopsy findings, previous medical records, and microscopic slides, and he concurred with the diagnosis of Tako-Tsubo cardiomyopathy as a cause of death.

Tako-Tsubo (stress) cardiomyopathy is a non-ischemic cardiomyopathy in which there is a sudden temporary weakening of the heart muscle. Tako-Tsubo cardiomyopathy is a well-recognized cause of acute heart failure, lethal ventricular arrhythmias, and ventricular rupture. It most commonly occurs in postmenopausal women and is frequently precipitated by an emotionally mediated or physically triggered stressful event. Chest pain and dyspnea are typical presenting symptoms. Transient ST-segment elevation on ECG and a rise in cardiac biomarkers are common. Systolic dysfunction with marked left ventricle contraction abnormality, extending beyond the geographic territory of a single coronary artery, and absence of obstructive coronary artery stenosis are characteristic. The hallmark of this disorder is bulging of the apex of the heart with preserved function of the base. The echocardiographic appearance of this syndrome earned the name “tako tsubo,” or octopus trap, in Japan, where it was first described.

In conclusion, this case brings to light an unusual cause of sudden cardiac death in a young male. After literature review it is reported the youngest male (20-years-old) who was diagnosed with and died due to Tako-Tsubo cardiomyopathy.

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**Sudden Death, Takotsubo Cardiomyopathy, Stress Cardiomyopathy**

## **G23 Sudden Cardiac Deaths Related to Ischemic Heart Disease in Postmortem MDCT and MDCT-Angiography**

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After attending this presentation, attendees will learn about the utility of postmortem radiological imaging of coronary arteries in cases of ischemic heart disease, along with their possibilities and limitations.

This presentation will impact the forensic science community by showing that the interpretation of postmortem radiology, especially in the cardiovascular field, is a new field for both forensic pathologists and radiologists. Information obtained from both parties can help to further the understanding of CT and CT-angiography in postmortem examinations and enable its integration into the classical autopsy protocol.

Although postmortem radiology is very useful in demonstrating traumatic lesions, its application is still limited for deaths resulting from cardiovascular pathologies. The goal of this study was to evaluate the diagnostic potential of postmortem angiography for deaths related to ischemic heart disease by comparing findings of native postmortem cardiac multi-detector computed tomography (MDCT), multiphase postmortem CT-angiography (MPMCTA), and conventional autopsy.

Twenty three cases were selected based on clinical history, including 21 men and two women. The age of the victims ranged from 35 to 89 years (mean age 52.3 +/-12.2). All cases underwent a postmortem MDCT, MPMCTA, and complete autopsy which included histological examination of the coronary arteries and myocardium.

MDCT-angiography was carried out using the standardized protocol of MPMCTA, in which a native CT-scan was performed prior to any manipulation of the corps. Liquid samples for toxicological and biochemical analyses were collected under CT-guidance before cannulation of the femoral vessels. Radiological interpretation of all images was performed by both a board certified radiologist specialized in vascular radiology and a board certified forensic pathologist trained in forensic radiology.

Postmortem MDCT and MDCT-angiography showed calcifications of coronary arteries in 18 cases (78%). The findings could be more easily detected and documented with postmortem MDCT or MPMCTA than with classical autopsy, where the calcifications were not systematically described by the pathologist. MPMCTA allowed much better visualization of coronary arteries than MDCT and permitted the evaluation of stenoses and occlusions. In 13 cases, MDCT-angiography revealed at least one coronary artery that was not perfused or had a stenosis greater than 75%. Conventional autopsy of 11 of those 13 cases detected an acute or subacute coronary thrombosis, in one case, a postmortem clot led to a perfusion stop. From 14 cases of coronary thrombosis detected at autopsy, 11 were identified as stop of perfusion during CT-angiography, two acute thromboses related to eroded plaques and an old recanalised thrombus were visualized as partial occlusions. Some artefacts were identified using multiphase postmortem MDCT-angiography: in one case a postmortem clot was suspected in a non-perfused coronary artery and in three other cases a postmortem clot imitated pulmonary embolism. These artifacts were confirmed upon autopsy. A hemopericardium was easily identified by all techniques in one case, but the exact localization of the rupture was better visualized in MPMCTA than in MDCT.

This study shows that native postmortem MDCT has limited value for the diagnosis of ischemic heart disease as only calcifications of coronary arteries or evident cardiac pathological findings, such as cardiac tamponade, can be identified. This technique can not estimate the degree of stenosis or occlusion. Postmortem angiography enables identification of coronary occlusion and significant stenosis, and seems also to be promising to detect signs of myocardial ischemia by evaluation of myocardial enhancement.

This study finds that, coronary postmortem MDCT-angiography, if correctly interpreted, is a useful tool to view the morphology of coronary arteries, rule out coronary artery stenoses and to indicate vascular occlusions suspicious of thrombosis, thereby directing the sampling for histological examination. It is, however, too early to postulate if this technique can provide sufficient evidence to determine if the cause of death was related to an acute or chronic ischemic event.

The goal of this presentation is to present the diagnostic potential of postmortem multi-detector computed tomography and multiphase postmortem CT-angiography in cases of sudden cardiac deaths related to atherosclerotic coronary artery disease.

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**Sudden Cardiac Death, Ischemic Heart Disease, Postmortem Imaging**

## **G24 Sudden Cardiac Testosterone-Related Death in a Young Bodybuilder**

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The goal of this presentation is to examine the cardiovascular effects of AAS chronic abuse. In all cases of sudden death in apparently healthy bodybuilder an accurate circumstantial investigation is necessary to confirm the AAS abuse and then the optoc, histological, and toxicological investigation can ascertain the cardiac pathological features correlated to the abuse of these various substances.

This presentation will impact the forensic science community by communicating the pathological relationship between androgenic-anabolic steroids and sudden cardiac death, underlying the necessity of an accurate anamnestic, circumstantial, histological, and toxicological investigation in cases of unjustified death involving apparently healthy young man to ascertain AAS abuse.

Androgenic anabolic steroids (AAS) are synthetic derivatives of testosterone used in therapeutic dosages in medical practice. In addition, AAS are used worldwide to help athletes gain muscle mass and strength, although the prohormones of testosterone and nandrolone are on the list of forbidden substances of the International Olympic Committee (IOC). To minimize the risk of developing tolerance to any particular agent, AAS are taken as a cocktail of different agents taken at one time. There are several reports in the literature regarding the adverse effects of AAS on various organ systems including endocrine, cardiovascular, and hepatic pathologies. The heart is one of the most frequently affected organs by the chronic and acute administration of AAS. Although the topic is still being debated, and most of the evidence is anecdotal, a consensus is beginning to emerge that chronic AAS abuse may be associated with an increased risk of sudden cardiac death (SCD), myocardial infarction, altered serum lipoproteins, and cardiac hypertrophy.

A case of sudden cardiac death is reported in an apparently healthy bodybuilder who was chronic androgenic-anabolic steroids abuser.

A 28-year-old male was found collapsed in his bedroom early in the morning and he was pronounced dead by an emergency physician called by his father few minutes later. He was an amateur but he had weight lifting workouts at the gymnasium for few hours each day. According to father of the deceased he had been taking anabolic steroids parenterally for several years mainly in an effort to improve his appearance. His room contained a veritable arsenal of drugs, in particular several glass vials, most of which fell into the AAS category, and seven used 2.5-ml syringes.

The body was that of a well-built man (weight 87kg, length 176cm). External examination revealed needle marks on the upper external part of the right buttock and on the anterior part of the left forearm. The autopsy revealed abnormal muscle development and hepatomegaly. The heart had a normal shape and was normal in size (13cm x 11.5cm x 3.5cm) but it weighed 470g. The left and right coronaries showed 75-80% lumen reduction. At the section the left ventricular wall was moderately increased.

Histologically, the myocardial samples showed wide fields of myocardial necrosis characterized by hypercontraction of the myocyte with a breakdown of the whole contractile apparatus with markedly thickened Z-lines and extremely short sarcomeres. Foci of disarray with star-like disposition of adjacent myocytes, aligned obliquely or perpendicular to one another, and joined together by short, generally hypertrophic myobridges, with interconnecting myofibrils were observed. Small groups of disappearance of myofibrils with intramyocardial oedema resulting in empty sarcolemmal tube and with any type of reaction (colliquative myocytolysis grade 1) were also observed.

Histological examination of coronary arteries confirmed 80% lumen reduction in anterior descending branch, left circumflex, and right coronary artery sections, characterized by nodular hyperplasia of smooth muscle cells and elastic tissue with progressive fibrous replacement, associated with calcium salts accumulation. Both gross and histological examinations of other organs did not reveal any pathology, except for pulmonary oedema and polyvisceral stasis.

Complete toxicological examination was negative for drugs of abuse, including ethanol, but positive results for testosterone and metabolites in blood, liver, and kidney were confirmed. The examination of content in six syringes confirmed the presence of testosterone.

In conclusion, in this case the combined effects of vigorous training and the i.m. administration of testosterone led to a stimulation of the sympathetic nervous system and predisposed the young man to myocardial injury and subsequent sudden cardiac death.

#### **Anabolic Steroids, Sudden Death, Toxicological Findings**

## **G25 Traumatic Pericardial Rupture**

*Sara H. Zydwicz, DO\*, and Vernard I. Adams, MD, Hillsborough County Medical Examiner's Office, 11025 North 46th Street, Tampa, FL 33617*

After attending this presentation, attendees will understand the frequency, spectrum, and associated injuries occurring with traumatic rupture of the pericardial sac caused by blunt impact.

This presentation will impact the forensic science community by expanding knowledge base of blunt impact thoracic injuries and help autopsy pathologists recognize traumatic rupture of the pericardial sac as a marker for energy loading of the heart by presenting data from a series of autopsies in which pericardial laceration was associated with high velocity impact trauma.

A retrospective review was conducted of 145 consecutive blunt injury autopsies performed by one pathologist from 2007 through the first quarter of 2011, comprising all traffic fatalities and suicides who leaped from high structures. One-hundred-fourty were traffic accidents and five were suicides by leaping from a height. The age range of the study cases was 17-80 years with a median age of 29 years and a mean of 37.4 years. Thirty-eight cases were selected for review because they had lacerations or contusion of the heart or laceration of the pericardial sac; 17 were motor vehicle operators, six were motor vehicle passengers, six were pedestrians, five were motorcycle operators, and four were suicides who leaped from a height. Of the 38 study cases, 28 had laceration of the pericardial sac, of which 24 had associated cardiac contusion or laceration. A single pericardial laceration was present in 22 of the cases. In five of 13 pericardial lacerations involving the left side of the sac, the heart was statically herniated through the defect. In three of eight pericardial lacerations on the right side of the sac, the heart was statically herniated through the defect. One case has a single pericardial laceration on the anterior sac without herniation. Multiple pericardial lacerations were present in six cases, one with static herniation of the heart. Fractures of the ribs, sternum or ribs and sternum were associated with pericardial laceration in six cases.

The most likely mechanism causing lacerations of the pericardial sac involves the heart being forced through the sac when the space between the sternum and the vertebral column is reduced by impact. Pericardial lacerations are frequently associated with wounds of the heart, great vessels, lungs and skeletal structures; lacerations of the pericardium infrequently occur in isolation and most subjects with pericardial lacerations died rapidly; only five of the 22 cases with pericardial lacerations were transported to the hospital. Isolated surgical case reports have described this type of injury; however, no case series were found in a search of the relevant literature.

#### **Pericardial Sac, Traumatic Herniation, Laceration**

## **G26 Histologic Variation in Cardiac Rupture Complicating Myocardial Infarction: A Forensic Experience**

*Brandy Shattuck, MD\*, Mary L. Anzalone, MD, Merrill O. Hines, MD, and Dwayne A. Wolf, MD, PhD, Harris County Medical Examiner, JAJ Forensic Center, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the spectrum of histologic changes associated with cardiac rupture, particularly in those who do not have preceding medical intervention.

This presentation will impact the forensic science community by providing a range of histologic findings associated with cardiac rupture that presents as sudden and/or unexpected death.

Cardiac rupture as a complication of myocardial infarction is a rare but well known cause of mortality. Traditional thought has placed the danger period of this complication at five to seven days post infarct with up to 25 percent of the events occurring in the first 24 hours. The accompanying histologic findings of granulation tissue that one would expect at five to seven days after the initial event have been used to describe the most common

background of rupture. This natural time course; however, has changed as medical intervention has altered the timing of rupture and did not fully describe the 25 percent who ruptured in the first day after infarct.

Along with improved cardiac survival, there has been a revision of infarct complications due to medical intervention. Research has shown that thrombolytic therapy decreases the overall rate of rupture from four percent to less than one percent; however, it causes many of those who do experience a rupture to do so within the first 24 hours after the initial infarct. Similar research on direct percutaneous coronary intervention placed the danger period for rupture in the first two days. This research, while clarifying the clinical course of the treated infarct and incidence of associated complications, still does not address the spectrum of histologic findings associated with survival less than five days.

The acceleration of the natural time line will change the cellular milieu that can be seen upon histologic examination, making it similar to those patients who do not have medical intervention. Those patients who present with death due to cardiac rupture without intervening medical intervention are most frequently seen in the forensic pathology setting. An examination of the histology seen in this setting can help explain the lack of the classical presentation of fibroblasts, blood vessels, lymphocytes, and macrophages that histology teaching has classically associated with ruptures.

A review of all forensic autopsies performed in Harris County, Texas from January 2006 to July 2011 revealed 37 patients who were found to have cardiac rupture as a complication of myocardial infarction listed as cause of death. Of these 37 patients, nine had evidence of classical histology, i.e., granulation tissue with fibroblasts, macrophages, and blood vessel formation. The remainder did not have the same infiltration of fibroblasts and early blood vessel formation in the area of myocyte death. Signs of early myocyte death, including minimal to extensive neutrophilic infiltrate, dissecting hemorrhage, and varying amounts of lymphocytes and macrophages were present in those who had witnessed collapse, were found unresponsive, or had complaints of chest pain less than 48 hours before death. None of these patients had active medical intervention such as thrombolytic therapy or percutaneous intervention according to available medical records. Many patients, both with and without classic histology, did not have a reported history of chest pain.

These findings provide a histologic correlate to both the originally described 25 percent of patients who present with cardiac rupture in the first 24 hours after infarct and to those who may have an accelerated time to rupture due to medical intervention. These results, even with a small sample size, can provide an explanation for the spectrum of histologic findings associated with cardiac rupture in patients who have no medical intervention and those who have an accelerated time course due to therapy.

#### **Histology, Cardiac Rupture, Myocardial Infarct**

## **G27 A Comprehensive and Systematic Evaluation of Sudden Cardiac Death in a Diverse Urban Population**

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After attending this presentation, attendees will understand the definition of sudden death and its relationship to atherosclerotic coronary artery disease. Attendees will also gain new insight into sudden death and its causes.

This presentation will impact the forensic science community by showing how the background incidence of sudden death due to coronary artery disease is decreasing and is lower than had been reported in the literature previously.

Despite decreases in overall mortality of death from cardiac causes due to better treatment and interventions over the past decade, sudden cardiac death (SCD) remains a significant public health problem in the United States. The true burden of SCD; however, remains unknown; variable definitions and inconsistent ascertainment methods in previous studies have resulted in widely divergent estimates of its incidence. The common paradigm is that sudden death is most likely cardiac (particularly due to coronary artery disease) in origin. However, commonly cited epidemiologic data on SCD are now a generation old, predate modern advancements in cardiac care, and were drawn from homogenous populations. Many relied on retrospective records review and/or death certificate review, which did not require autopsy-proven heart disease in the vast majority of cases, and may overestimate the prevalence of coronary artery disease in the community. Prior gold standard autopsy studies defining the underlying causes of SCD indicated that coronary artery disease (CAD) is the underlying cause of death in 80% of cases; however, these data are similarly outdated and hindered by referral bias of only a small subset of SCD cases. The contemporary epidemiology and pathology underlying SCD in the rapidly diversifying U.S. population is therefore unknown.

In a unique collaboration, the Cardiac Electrophysiology Section at the University of California at San Francisco and the San Francisco Office of the Chief Medical Examiner have worked to establish a robust surveillance method and comprehensive evaluation of all consecutive incident SCDs in San Francisco, a prototypic diverse U.S. community that presages near-term national demographic shifts. The population of San Francisco numbers 700,000 residents and increases to 1.5 million persons during working hours. The annual mortality rate in San Francisco is approximately 6,000 with half of these reported to the Office of the Chief Medical Examiner. Of these cases, approximately 1,400 decedents come under the jurisdiction of the Medical Examiner, including the vast majority of sudden deaths which include those dying outside of a physician's recent (within three weeks) care. The cases accepted by the office are completely investigated with review medical records, statements from witnesses, and evaluation by a American Board of Pathology certified forensic pathologist.

In this study funded by the National Institutes of Health for five years, which officially began in February 2011, all deaths reported to the office are

screened by two physicians (a forensic pathologist and a cardiac electrophysiologist) for circumstances fitting World Health Organization (WHO) sudden death criteria (witnessed death within one hour or unwitnessed without symptoms 24 hours before). These cases then undergo complete investigation, including comprehensive autopsy incorporating a specially designed cardiac protocol, toxicology, and histology. The cardiac protocol includes measurements of the left ventricular compact and full thickness myocardium, and the short axis of the left ventricle at the level of the origin of the papillary muscles. Coronary artery stenoses are assessed systematically at 5mm sections, and microscopically if evidence of plaque rupture is seen. Histology is reviewed by two pathologists and assessed for degree and nature of cardiac fibrosis, as well as other pathology (i.e. myocarditis, cardiomyocyte disarray, etc.). Frozen tissue (heart, blood in EDTA tubes, and skin) is obtained and with next of kin consent, will form a genetic and molecular tissue bank for future study.

To estimate the prevalence of cardiac pathology in the general population, comprehensive autopsy evaluation of a frequency-matched sample of geographically and demographically similar accidental trauma death controls are also performed over the same period. Accidental trauma deaths are those who die with an accidental manner of death (such as falls or motor vehicle collisions) who die at the scene or shortly after transportation to the hospital. Findings from autopsy of these trauma death controls will be compared to SCDs to allow for the evaluation of CAD, other pathology, and cardiac indices (all thickness, mass) as risk factors for SCD.

As of July 2011, approximately 110 SCDs and 14 trauma controls have been evaluated in the study, representing over 85% of all SCDs occurring in San Francisco over the six months since study inception. Of these cases, approximately 40 cases have been reviewed by two pathologists and two cardiac electrophysiologists. Approximately 40 percent of the reviewed "SCDs" so far have had non-cardiac causes of death. At the time of presentation, the study will have been performed for one year (out of a total of three years of autopsy study) and many more cases will have been reviewed with resultant information.

This novel study fulfills a critical need for the precise characterization of the contemporary epidemiology and underlying causes of sudden cardiac death in population subgroups heretofore underrepresented. These data may allow for the elucidation of new independent risk factors for sudden cardiac death and direct new insights into the clinical therapy of this lethal disease.

**Sudden Death, Coronary Artery Disease, Public Health**

## **G28 Osseous DNA Sampling Procedures and Success Rates at the Pima County Office of the Medical Examiner**

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After attending this presentation, attendees will learn the results of a study examining the success rates of an osseous DNA sampling procedure.

This presentation will impact the forensic science community by providing procedural guidelines for a successful, scientifically rigorous, and economical sampling procedure for obtaining DNA profiles from osseous elements recovered from an arid desert environment. Furthermore, it presents the results of a study on the success of these sampling procedures in providing DNA profiles from skeletal elements in varying stages of decomposition.

Over the years, DNA analysis has become elemental in the positive identification of individuals in mass fatality situations and for decedents without appropriate antemortem records for comparison. Increasingly,

forensic anthropologists are responsible for obtaining osseous tissue samples from decomposed, burned, mummified, and skeletonized remains. There are over one hundred cases each year of unidentified decedents without sufficient antemortem records. Due to the extreme climate and desert environment, many of these individuals are in varying stages of decomposition and require an osseous sample for DNA testing. With the large number of cases and the high cost of sampling and testing samples, efforts have been made to apply a procedure that is scientifically rigorous, while simultaneously prompt and economical.

The first step in laboratory preparation at PCOME is the adornment of basic personal protective equipment, such as gloves and a mask. All osseous DNA samples are taken at the analytical table using a non-disposable Stryker saw blade that has been soaked in bleach and cleaned with a brush. Osseous elements with the thickest portion of cortical area are typically selected for sampling, such as the posterior femur, anterior tibia, and parietal. When these are unavailable, samples are taken from other osseous elements, including the humerus, occipital, and mandible. Some skeletal elements may have been processed in simmering water with Alconox and Sodium Carbonate prior to sampling; however research demonstrates that this technique has no effect on DNA extraction rates (Rennick *et al.* 2005; Lee *et al.* 2010). A rectangle of bone weighing approximately 15-25 grams is resected, wrapped in filter paper, placed into a manila envelope, and sealed into a plastic heat-and-seal bag. All samples are then cooled for varying periods of time to await shipment in chilled transport containers. After shipment to Bode, the osseous samples are decontaminated by using a dremel tool to sand the top layers and then put through bleach and ethanol washes. The osseous samples are then re-sampled and powdered using a blender cup or by drilling a hole, depending on the size and quality of the bone. The bone powder (input amount ranging from 0.2g - 2.0g) then undergoes a decalcification step prior to DNA being extracted using a proprietary extraction method, which has been optimized and validated for use on skeletal samples.

For this study data was collected on osseous samples submitted from the past several years (n = 420). Information regarding the estimated postmortem interval (PMI), freshness of the bone sample, quality of the cortical surface of the bone, and skeletal element sampled was collected for analysis. These variables were then compared with the number of STR loci reported and the mtDNA region developed for each sample.

Results demonstrate a high success rate for both STR and mtDNA extraction. In total, 73.5% of all samples developed at least 8 STR loci, the minimum necessary for entry into the Missing Person's DNA Database, and 58.3% of the total sample developed a full STR profile. Of those tested for mtDNA, 93.7% developed an adequate profile. Predictably, there is a statistical relationship between the success rates for developing a full STR profile and the freshness of the bone, the estimated postmortem interval, and the quality of cortical surface of the osseous sample. Regardless, 59% of skeletal remains with a PMI estimate of one to two years, and 37% of remains with a PMI estimate of more than two years developed an STR profile of at least 8 loci. Furthermore, 41% of the samples with sunbleached and weathered cortical surfaces developed at least 8 STR loci indicating the success in obtaining STR profiles from even highly degraded osseous samples.

**Forensic Anthropology, Osseous Sampling, DNA**

## **G29 What Can a Cartridge Case Tell us When There is no Gun? Interpreting Extractor and Ejector Marks on Ammunition That has Been Inserted in a Gun but not Fired**

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After attending this presentation, attendees will understand that comparing spent cartridges with unfired cartridges found at the crime scene in cases of suspected murder is a nightmare even for an expert firearms consultant. To obtain a good, positive match in such cases, it is important not to confine the identification to a simple comparison but extending the analysis to other parts of the bullet.

This presentation will impact the forensic science community by showing that in many cases after shooting, a criminal may throw away or destroy the gun but often forgets unfired bullets at home that have at one time been inserted in the gun but not fired perhaps, on a whim or to check the correct function of mechanical devices, especially the loading and ejection mechanisms. This generally leaves marks on the cartridge (often very evident) caused by the extractor and ejector, but not by the firing pin, of course, that at most may leave slight signs that are useless in such a comparison.

This case reports a 34-year-old man involved with the “camorra” (a variety of mafia), was killed in a narrow but very crowded street in Naples. Several shots were fired by different guns. None of the people present were willing to testify but the presence of a well known “boss” at the crime scene at the time of the murder was confirmed. No gun was found at this criminal’s home but 23 unfired cartridges were found in a drawer, of the same caliber and brand as the spent cartridges found at the crime scene.

The man was convicted, and for this study, by surveying the evidence, obtained surprising results that demonstrated that the cartridges found at the suspect’s home were undoubtedly inserted in the weapon, never recovered, that had been used for the mafia killing two months before.

Beginning by examining the characteristics of the class of spent cartridges found near the victim; this examination was done with a comparison microscope. Even at first sight, the marks of the extractor appeared very distinct, and to vary very little from one cartridge case to another, whereas the ejector marks seemed less useful for comparison. Naturally, firing pin marks were not useful. The class and individual characteristics of all the cartridges, spent or not, were perfectly superimposable, as were the breech marks.

The results of this analysis are illustrated and discussed, specifying the potential and limitations of this type of investigation that has never previously been described in literature. It is believed this case may shed light on new possibilities in forensic investigations, which should be kept in mind as further elements to be used by trained experts, especially in cases with a shortage of evidence that otherwise often remain unsolved. In particular, strategies are proposed for resolving disagreements between experts and thus avoiding contention during the trial that may lead to an unsatisfactory conclusion. It should be kept in mind that considerable experience is required to recognize the true concordance of marks on spent and unfired cartridges, and differentiate them from accidental and not individual marks visible on extraneous cartridges.

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**Firearms Investigation, Unfired Cartridges, Mafia**

## **G30 Foreign Emboli to Multiple Organs Following Catheter Ablation**

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After this presentation, attendees will be aware of an unusual complication to the common procedure of cardiac ablation involving trans-atrial catheterization. Attendees will understand the possible clinical presentation and the likely histopathologic findings seen in patients whose intravascular procedures are complicated by embolization of foreign material from the devices used in these procedures. Attendees will also understand how to investigate and report a possible medical device safety problem.

This presentation will impact the forensic science community by alerting the autopsy pathologist to the possibility that an intravascular procedure may directly contribute to death. This presentation will inform the pathologist as to the likely gross and microscopic findings in cases of foreign material emboli from catheter devices. Finally, this presentation will highlight the role of the forensic pathologist in product safety surveillance.

Catheter ablation is indicated as therapy for patients with symptomatic supraventricular tachycardia, symptomatic ventricular tachycardia, and atrial fibrillation. In patients with atrial fibrillation, catheter ablation is indicated for those who do not tolerate anti-arrhythmic drugs, in whom pharmacologic therapy is ineffective or who have lifestyle-impairing atrial fibrillation. The simplest ablation therapy for atrial fibrillation is radiofrequency ablation of the atrioventricular nodal junction only. However, it is common to ablate more extensive areas of cardiac tissue, including the muscular connections between the pulmonary veins and the left atrium. These procedures may employ a technique in which the cardiac catheter crosses the atrial septum. Embolization of foreign material as a result of catheterization procedures is an uncommon complication.

This case study is a 63-year-old male who developed *Streptococcus anginosus* sepsis and suffered a progressive frontoparietal stroke complicated by extensive pneumocephalus within three months of his catheter ablation procedure for atrial fibrillation. Clinically, he was suspected to have septic emboli from heart valve vegetations. However, at autopsy, his heart valves appeared normal. His brain had bilateral infarctions with subfalcine herniation. On microscopic examination, there were foreign material emboli in his lungs, kidneys, heart, liver, brain, and esophagus. The foreign material was basophilic, non-polarizable, PAS positive, trichrome negative, and had no defined shape. The foreign material in his cerebral vasculature was associated with areas of infarction as well as foreign body giant cells with a granulomatous reaction. In other organs, the foreign material was associated with chronic inflammation and foreign body giant cells, but not necrosis. The histologic appearance of this foreign material is similar to that described in other literature reports of foreign material emboli from intravascular procedures. This case appears to be unique among reported cases of foreign material emboli from intravascular procedures in that this previously healthy patient had a single intravascular procedure that produced foreign material emboli to every organ examined at autopsy and contributed directly to his demise.

The autopsy findings and their implications were discussed with the cardiologist who performed the procedure and with the manufacturer of the device. The cardiologist was unaware of this complication in other cases. The manufacturer of the catheter used in this patient’s procedure was unaware of reports of similar complications from this device. This case was also reported to the Food and Drug Administration as a medical device problem.

This case report details an unusual complication of intravascular procedures and highlights the importance of the forensic pathologist in surveillance of potential product danger to the community.

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**Foreign Material Emboli, Catheter Ablation, Cardiac**

## G31 Extrapulmonary Tuberculosis — Rare Autopsy Findings

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The goal of this presentation is to show how the involvement of heart and pancreas although rare, can be seen at autopsy in multi-organ tuberculosis in developing countries. Tubercular myocarditis can remain clinically asymptomatic, or can present with sudden death, ventricular arrhythmia, heart block, or congestive heart failure. Tubercular necrotizing pancreatitis is an atypical form of the disease, and can result in shock.

This presentation will impact the forensic science community by showing how cardiac and pancreatic tuberculosis is associated with increased fatality rate in systemic tuberculosis affecting both immunocompromised and immunocompetent individuals.

Autopsy study of the heart and pancreas is important in suspected cases of multiorgan tuberculosis although the disease is generally believed to spare these organs as well as skeletal muscle and thyroid. Many times, diagnosis of tuberculosis is made only at autopsy, especially in developing countries. Multi-organ tuberculosis, historically a disease of infants and young children, currently predominates among the elderly and immunocompromised individuals especially those infected with both HIV and Mycobacterium tuberculosis. The involvement of myocardium and pancreas could be considered as potentially lethal especially in multiorgan tuberculosis.

Tuberculous pericarditis with tamponade, myocarditis, and necrotizing tuberculous pancreatitis can be fatal. Systemic tuberculosis with multi-organ failure should be considered as a possible cause of septic shock especially in patients with typical high risk factors such as advanced age, diabetes, alcoholism, and immunosuppression. Extrapulmonary tuberculosis was seen in three males who were not hospitalized and aged twenty to fifty years in the present study.

Cardiac tuberculosis most commonly affects the pericardium, while endocardial, myocardial, valvular, or coronary artery involvement is exceedingly rare. It is estimated that 1% of all cases of tuberculosis have cardiac involvement. Before the introduction of chemotherapy, the overall incidence of cardiac tuberculosis, as detected by autopsy was less than 3%. Tuberculous caseating myocarditis usually results from direct hematogenous seeding from pericardium or mediastinal lymph nodes via lymphatics. The three distinct forms of myocardial tuberculosis are nodular tuberculomas, miliary tubercles, and an uncommon diffuse infiltrative type. In the present study, multifocal areas of caseous myocardial necrosis were seen in three cases. The pericardium showed tuberculoid granulomas in all the three cases.

Pancreatic tuberculosis was seen in 4.7 to 14% of deaths from miliary tuberculosis. Higher incidence of this entity is related to increased frequency of abdominal tuberculosis in immunocompromised patients. Pancreas is biologically protected from infection by Mycobacterium tuberculosis because of the presence of pancreatic enzymes. Hematogenous dissemination from the pulmonary focus or direct spread from adjacent peri-pancreatic lymph nodes causes tuberculous pancreatic abscess, though large lesions are not so common. In the present study, tuberculous pancreatic abscess with necrosis of the large vessels was seen in one case. In all the three cases, there was concurrent infection in the lungs, kidneys, liver, and spleen, with the presence of caseating granulomas. Ziehl Neelsen stain showed tubercle bacilli in these organs.

Mortality from systemic tuberculosis is high and death due to multi-organ failure and septic shock has been reported in both immunocompetent as well as immunocompromised individuals. In conclusion, although myocardial and pancreatic involvement by tuberculosis is rare, it should be suspected as a cause of congestive heart failure and shock, respectively.

**Tuberculosis, Heart, Pancreas**

## G32 Patterns of Injury in Fatal Coyote Attack: A Case Study From Nova Scotia, Canada

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After attending this presentation, attendees will: (1) gain an understanding of the rarity of lethal coyote attacks on human populations; (2) gain an understanding of patterns of injury in coyote attacks; and, (3) be educated about distinguishing animal attacks from postmortem predation and inflicted injuries.

This presentation will impact the forensic science community by presenting a unique and very rare case report on coyote attack fatalities. There have only been two recorded fatalities in North America from Coyote attacks. The presentation will also provide a unique examination of coyote attack patterns during autopsy.

The typical activity pattern of coyotes in the absence of human harassment seems to be largely crepuscular and diurnal, but when predator control activities are under-taken, coyotes shift their activity mainly to nighttime to avoid humans. Conversely, a lack of human harassment coupled with a resource-rich environment that encourages coyotes to associate food with humans can result in coyotes losing their "normal" wariness of humans.

Several factors may have led to the recent increases in predator attacks on humans in North America. Among them are human population growth, suburban sprawl, and protection of predator species that were once harassed. Male coyotes in the wild generally have home ranges from 8.1 to 16.1 square miles (21 to 41.6km<sup>2</sup>) and females 3.1 to 3.9 square miles (8 to 10km<sup>2</sup>).

The motive for attacks by coyotes is not always hunger or protection of dens. Movement, particularly escape behavior, is a key stimulus for eliciting orientation and attack; children's play and running behavior, particularly when running away from a coyote may provide a strong stimulus for attack.

There are only two recorded fatalities in North America from coyote attacks: in 1981, a female toddler in Glendale, California, and, in 2009, an adult female in Cape Breton, Nova Scotia, Canada. This is the case of an adult female who was attacked by at least three coyotes while hiking in the Cape Breton Highlands National Park in Nova Scotia, Canada.

The offending animals included a 32-pound female, 34-pound male (likely a brother of the female), and one 50-pound male (genetically unrelated to the other two animals). During the attack, a group of four hikers scared away the coyotes and called "911."

When emergency crews arrived, she was transported to a regional tertiary care center where she died overnight. An autopsy was performed by the Nova Scotia Medical Examiner Service.

The offending coyotes were captured, euthanized, and necropsied by a wildlife pathologist at the Atlantic Veterinary College, University of Prince Edward Island. All animals were healthy, free of apparent disease and infection, and well fed on postmortem examination. The female coyote contained human remains in her stomach.

This case report will describe the patterns of injury seen at autopsy and consider them in the context of usual coyote attack behavior. It will include considerations of distinguishing animal attacks from postmortem predation and inflicted injuries.

**Coyote Attack, Injury Patterns, Forensic Pathology**

### G33 Takotsubo Cardiomyopathy Following Jeweler's Hold Up: Forensic Considerations

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After attending this presentation, attendees will understand the causal link between a physical assault and a stress-induced cardiomyopathy.

This presentation will impact the forensic science community by providing an example of a stress-induced pathology rarely described in the forensic literature.

Takotsubo cardiomyopathy (TTC) is also known as transient apical ballooning, apical ballooning cardiomyopathy, stress-induced cardiomyopathy, and simply stress cardiomyopathy. The name refers to the morphological features of the left ventricle which look like the Japanese Takotsubo ("fishing pot" used to trap octopuses). It was initially described in Japan by Dote et al. and subsequently recognized in the United States and Europe. The prevalence of stress cardiomyopathy among patients with symptoms suggestive of myocardial infarction is 0.7–2.5%.

The mechanisms of disease remain unclear and the cause has not been established. Hormonal disturbances, diffuse microvascular spasm, multivessel epicardial coronary artery spasm, hidden coronary disease or aborted myocardial ischemia, focal myocarditis, structural changes and oxidative stress theory, hypercontraction of the basal segments, have been suggested as possible causes of this disorder.

The typical presentation of a patient with TTC is a sudden onset of congestive heart failure or chest pain. On the ECG the most common changes are ST segment elevation in two or more leads and T-wave inversion. Markers of myocardial damage and heart failure are moderately elevated.

Left ventricular function was assessed by echocardiography, left ventriculography or cardiac Magnetic Resonance Imaging (MRI). Typically, dyskinesia of the left ventricular apical or midventricular segments with a hyperkinetic basal region was demonstrated. In 2009, a systematic review showed that cardiac MRI is very useful, first for diagnosis, but also for visualization of apical thrombus, left ventricular abnormalities and analysis of the right ventricular function.

The prognosis of stress cardiomyopathy is good, although fatal complications, such as cardiogenic shock, malignant arrhythmias, and free wall rupture of the left ventricle, have been reported.

**Case presentation:** This case is about an 80-year-old man who was victim of confinement during the hold up of his jewelry store. His arms and legs were tied with adhesive tape. He was maintained by three unknown male individuals, first in a vehicle with his wife, and then in a room where he was released. The hold up lasted eight hours.

Following the hold-up and confinement, he vomited and felt a gripping pain with a feeling of tightness that led him to consult a physician. On arrival he presented a badly tolerated tachycardia with low blood pressure at 80/60. The physical examination found subcrepitant rales but no lower limb oedema. No skin lesions were found.

The cardiac troponin presented a small and rapid increase. The ECG showed a sinus rhythm, left bundle branch block and a ST segment elevation of 1mm in lead D1 to VL. On the echocardiography, the left ventricular ejection fraction was 35%, there was an apical akinesis with apical ballooning pattern. On the coronarography, no significant epicardial coronary artery disease was found. He was hospitalized in cardiology department for a TTC.

Two weeks after the admission, a cardiac MRI was performed, showing normalization of the apical wall motion and of the ejection fraction.

**Discussion:** One of the goals of the forensic expert was to establish the link between the assault and the cardiomyopathy.

It is well known that TTC arise after an acute emotional stress and examples of incidents are: after an electroconvulsive therapy, after an earthquake, following dobutamine stress echocardiography, after caesarean delivery.

In this case, all of the four criteria were present:

- Transient hypokinesia, akinesia or dyskinesia of the left ventricular mild segments with or without apical involvement; the regional wall motion abnormalities extend beyond a single epicardial vascular distribution; a stressful trigger is often, but not always present.
- T Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture.
- T New electrocardiographic abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin.
- T Absence of pheochromocytoma, myocarditis.

Furthermore, a physical assault is unquestionably an intense emotional and physical stress. Only three cases reported of a TTC in a judiciary context has been found: in an elder abuse case, following a physical assault and after use of electric weapons.

Considering the alleged facts and the unquestionably link between the stress situation and the TTC, the expert reasonably accepted the direct, certain and exclusive causal link.

Although no studies were found in reference to the maximum time for onset of the appearance of symptoms, they classically appeared quickly after the initial complaint.

In case of death and considering the classic complications, except the free wall rupture of the left ventricle, it is possible to find no macroscopic lesion during the autopsy.

**Conclusion:** Forensic pathologists could be led to discuss the imputability of stress induced cardiomyopathy to a physical assault.

**Takotsubo Cardiomyopathy, Physical Assault, Imputability**

### G34 Two Unusual Cases of Homicide Involving Heterosexual Erotic Hypoxic Games

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After attending this presentation, attendees will gain understanding about deaths, occurring after hypoxic heterosexual erotic games. The important points of this presentation are that hypoxic erotic games are rarely seen in forensic pathology and that the hypoxic games prior to death are not necessarily the cause of death.

This presentation will impact the forensic science community by improving the knowledge about accidental erotic asphyxia and homicide, involving hypoxic erotic games. Knowledge about injuries associated with eroticism is important for forensic pathologists. The incidence of erotic asphyxiation, occurring after or as a result of hypoxic heterosexual erotic games is low and almost every case is highly individual. It is believed that the actual presentation can contribute to improving the diagnosis and differentiation between self-inflicted, accidental sexual activities, and homicide in cases where hypoxic heterosexual erotic games are involved.

Fatal autoerotic asphyxiation is not uncommon, but deaths due to homicidal attended behavior or after accidental erotic asphyxias involving heterosexual partners are rare. Cases, where the hypoxic games were practiced before or as a part of the homicide are difficult to diagnose due to difficulties in separating features of accidental and inflicted injuries. It is even more difficult to differentiate if the diagnosis of death is due to mechanical asphyxia or non-deadly asphyxia, taking place shortly before the death.

This study addresses two cases of homicidal deaths occurring after or due to asphyxiation during heterosexual erotic games. Both cases were routinely autopsied at the Department of Forensic Medicine University of

Aarhus, Denmark. The drug testing was performed at the Department of Forensic Toxicology of University of Aarhus, Denmark.

The main role of forensic pathologists is to give a reasonable hypothesis regarding the cause of death. In the vast majority of cases the asphyxia can easily be diagnosed. The diagnosis of asphyxia is traditionally based on morphologic findings and nonspecific signs, such as ligature marks, fingernail marks, bruises on the neck, petechial hemorrhages, facial congestion, edema, cyanosis, bruises in soft tissue, fracture of hyoid bone, thyroid- and cricoid cartilage. These findings are all characteristic for death caused by asphyxia, but almost in non-deadly asphyxia, taking place shortly before the death. Additionally fractures of hyoid bone, thyroid- and cricoid cartilage can occur after death.

Two cases are presented in order to provide new knowledge and to improve the forensic diagnosis of causes of deaths, occurring after hypoxic erotic games involving heterosexual partners, which are relatively rare events in forensic pathology. The presented cases illustrate that the presence of morphologic signs of asphyxia at autopsy provide evidence of asphyxia shortly before death, but it cannot provide evidence for asphyxia causing the death.

It is realized that even though such complicated cases are highly individual there is some similarity of findings and signs. Since such cases are extremely rare, the collection of knowledge regarding the case history, forensic examination, police investigations, and court records is an important tool, providing an evidence based interpretations of the findings.

**Sexual Asphyxia, Death, Homicide**

### **G35 Antipsychotic Polypharmacy: Lessons to be Learned From Forensic Toxicological Screening**

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After attending this presentation, attendees will understand the challenge of medical treatment of psychiatric patients with several drugs and the importance of the forensic autopsy in psychiatric patients dying suddenly

This presentation will impact the forensic science community by showing an example of what can be revealed from routine forensic toxicological screening and toxicological interpretation and how it can help understanding drug intoxication in psychiatric patients.

The prevalence of antipsychotic polypharmacy among schizophrenic patients is high and increasing internationally although a considerable difference in prescription practices exists. To estimate the overall risk of polypharmacy for the patient, pharmacodynamics, pharmacokinetics, as well as pharmacogenetics (how genetic differences influence patient's response to drugs) also have to be taken into consideration.

A 62-year-old schizophrenic man treated with several antipsychotic drugs died suddenly and unexpectedly. Beside his psychiatric diagnose he was once hospitalized with atrial flutter and ventricular extrasystoles (VES). No cause of death was found. The autopsy was performed and blood samples were taken from the femoral vein in accordance with standard procedures. Toxicological screening revealed the following: a lethal concentration of sulphiride (4,6mg/kg blood), concentrations of amitriptyline (0,43mg/kg blood) and the active metabolite nortriptyline (1,1mg/kg blood) were in levels where symptoms of lethal intoxication are seen, clozapine (1,3mg/kg blood), zuclopenthixole (0,16mg/kg blood) and procyclidine (1,2mg/kg blood) were found in concentrations where symptoms of intoxication are seen, levomepromazine (0,13mg/kg blood) and metoclopramide (0,13mg/kg blood) were both found in therapeutic concentrations. Alcohol was not detected in blood or urine.

Histology showed a simple pneumonia and slightly myocardial fibrosis. The cause of death was stated to be intoxication due to polypharmacy with several drugs. The manner of death was accident. All drugs were found to have been prescribed in recommended doses, and findings at the crime scene did not disclose intended intake or overdose.

The high drug concentration could according to toxicological interpretation not be explained by hepatic metabolic interactions, whereas pharmacodynamics may have played a role; the influence of potential pharmacogenetics was not examined.

Sulpiride is poorly absorbed from the gastrointestinal tract leading to low bioavailability of 25-35%. The patients constipation may have increased the absorption of sulphiride and thereby his serum concentration.

Metoclopramide was prescribed to the patient due to complaints of nausea, a well known symptom of intoxication of the drugs he was treated with. Metoclopramide which share side effects with antipsychotics is; however, not recommended in combination with other antipsychotic drugs. A majority of the drugs this patient was treated with share prolonged QT-interval and cardiac arrhythmia as side effects among others. This, in combination with the history of atrial flutter and ventricular extrasystoles, gives a high risk of cardiac arrhythmia. Furthermore, sulphiride is not recommended in combination with other drugs that may cause prolonged QT interval

This study presents this case illustrating that the potential fatality of polypharmacy in psychiatric patients should be considered and explored. Furthermore, it will reveal hypotheses of how this patient, treated with medication in recommended doses, could reach these high serum concentrations.

**Antipsychotic Polypharmacy, Drug Intoxication, Psychotropic Drugs**

### **G36 Nucleic Acid Degradation and the Postmortem Interval**

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After attending this presentation, attendees will be updated on how to capitalize on the utilization of modern molecular biology techniques, providing new and powerful statistically based tools uniting forensic biology and forensic pathology together with the potential to reveal crucial information regarding the circumstances surrounding a potential crime.

This presentation will impact the forensic science community by providing understanding of how the analysis of DNA and RNA from aged and/or degraded samples may reveal vital information about processes involved in decomposition, as these also affect nucleic acids. This has the potential to be of benefit in the analysis of samples that may traditionally be compromised and difficult to analyze.

While DNA has been used for over twenty years in forensic biology the potential applications for RNA have only recently received attention after the revelation that RNA is more stable than previously thought. By studying DNA and RNA from aged and/or degraded samples we expect to learn more about the processes involved in decomposition, as these also affect nucleic acids. This has the potential to be of benefit in the analysis of samples that may traditionally be compromised and difficult to analyze.

This study measured the systematic rate of decay of nucleic acids (DNA and RNA) in hard tissues such as nails, teeth, and bone. These tissues are more stable against environmental conditions due to the inherent biochemical structure of the tissue. The rate of degradation of nucleic acids in these tissues will be less influenced by external environmental factors and utilization of the rate of nucleic acid degradation found is likely to lead to the development of

postmortem interval indicators for longer time periods. A unique aspect of this research is the ability to take multiple samples from various human cadaver tissues at different time intervals without interfering with the decompositional process. This methodology, incorporating various time intervals, has not been studied before in relation to the degradation of nucleic acids in a systematic manner using human cadavers.

A critical aspect of assessing nucleic acid degradation was the recovery of RNA and DNA without any further fragmentation. Previously studies have shown that the DNA isolation and quantitation extraction system can be successfully adapted to allow the co-extraction of RNA and DNA without compromising the quality of either nucleic acid. An important practical aspect of this work is the further adaptation of this method to co-extract RNA and DNA from bone and nail samples. This will ease implementation into forensic laboratories as many already have the necessary skills and capacity to perform this adapted extraction strategy.

This initial research has identified the most useful genetic markers and established the experimental system required for analyses to assess whether ribs, nails or teeth are preferred as tissues for PMI estimation using this technique.

It was found that nails contain relatively high levels of DNA and RNA. Using reverse transcriptase PCR (RT-PCR), we have amplified four keratin mRNA transcripts and 18S rRNA from nail samples. After placement of nail samples in different environments, including submerged in water, soil, and at room temperature we have found that all four keratin mRNA transcripts are stable under different environmental conditions for significantly long time periods. Using these results, a statistical model has been developed to correlate the rate of degradation of the different keratin mRNA transcripts and 18S rRNA with the time since the material was sampled (PMI). Using nails collected from cadavers, we have applied the statistical model to determine the PMI in real-life situations. This work has shown that DNA and RNA in nails are stable and suitable for use in estimating the PMI.

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#### DNA, RNA, Postmortem interval

### G37 Typical Contrecoup Injury of Fixed Head by a Blow: A Case Report

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After attending this presentation, attendees will be able to learn an unusual case demonstrating a prominent contrecoup injury of fixed head which is caused by a blow and get a lesson about how to identify a cause of head injury through examining a cerebral contusion.

This presentation will impact the forensic science community by presenting an exceptional practical point regarding on how to interpret the occurrence of coup and contrecoup injury, and contributing to a study about the mechanism of the coup and contrecoup injuries by suggesting that several factors including anatomical position of the deceased, a surface area of a blunt weapon, damaged area and etc should be considered together to distinguish between two injuries.

It is critical to distinguish between coup and contrecoup injuries because it provides important information about the cause of the head injury. It is well known that coup injury is more dominant by a blow and contrecoup by a fall. However, several exceptions have been reported, and one of them is that contrecoup would be more prominent when a fixed head to a ground or a wall is struck by a heavy blow

A 45-year-old man was found dead in a prone position on a cabin of his village with a severe head injury. The police investigation proved his son's crime associated with a gambling debt.

On the postmortem examination, there were multiple bruises with semi-circular lacerations on occipital area and posterior portion of left temporal area of the scalp, and small bruises on right forehead and zygomatic region. Widespread subcutaneous hemorrhage and communicated and depressed skull fracture with several linear fractures on occipital bone of calvaria were identified. On the skull base, front to back fracture, which run occipital bone, right side of foramen magnum, right temporal bone, sellar turcica and right frontal bone, and small linear fractures on both orbital roof of frontal bone were noted. The brain showed multiple and large contusions on poles and undersurface of both frontal lobes and poles of both temporal lobes, and relatively small and weak contusions on both occipital lobe and right lobe of cerebellum. The remaining body was unremarkable except a contusion with intramuscular hemorrhage of left shoulder and small abrasions of right elbow.

The deadly weapon used in the crime was a hammer with an octagon-shaped surface, which is 35.0cm in an overall length, 10.0cm in length of the head, 10.2cm<sup>2</sup> in surface area and 978g in weight, and it turned out that he was hit by a hammer on the occipital area in a sitting posture, and then more than 10 times in a prone position.

There were several factors which were associated with injury pattern such as anatomical position of the deceased, a type and a surface area of a blunt weapon, damaged area, material quality of the floor, and the number of impacts. This deceased placed a face on wooden floors and received repetitive blows on the occipital area by a heavy hammer with relatively large surface.

In conclusion, this case showed that a blow can cause contrecoup more significantly than coup injury and this was more likely to be interpreted when several factors, particularly that head is fixed to a ground or a wall, were considered together.

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#### Cerebral Contusion, Coup, Contrecoup

### G38 Man With a Blue Brain: A Case of Hydrogen Sulfide Poisoning

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The goal of this presentation is to illustrate an unusual case of suicide within a closed vehicle by mixing household chemicals to form hydrogen sulfide gas.

This presentation will impact the forensic science community by illustrating an unusual case of suicide that results in a hazardous materials (Hazmat) situation for first responders and medical examiner personnel.

**Introduction:** Suicide is one of the most important public health issues and represents the eleventh leading cause of death in the United States. Suicides comprise approximately 12% of the caseload of the Allegheny County Medical Examiner's Office in Pittsburgh, Pennsylvania. Suicide rates for this country have been relatively stable over the past decade averaging approximately 10 per 100,000 populations. The most common method of suicide in the United States is the use of a firearm.

Chemical suicides have plagued the United States since 2008, and continue to be on the rise. This method of suicide originated in Japan in 2007, where they have seen over 2,000 such cases. Chemical suicide, or detergent suicide, involves mixing common household chemicals to create deadly hydrogen sulfide (H<sub>2</sub>S) gas, which is lethal in contained areas. Hydrogen sulfide, H<sub>2</sub>S, is a colorless gas that has a strong odor of rotten eggs or sulfur. H<sub>2</sub>S inhibits enzymes in mitochondria by binding with Fe<sup>3+</sup> of cytochrome oxidase. This reaction blocks cellular respiration, and interferes with oxygen utilization at the cellular level.

**Materials and Methods:** The case involved a 32-year-old Caucasian male with a history of a recent suicide attempt via sleeping pills. The decedent was recently depressed over losing his job and the threat of his car being repossessed. He was last seen alive the previous night.

The decedent was located by his father the next evening in his car in the rear of a large parking lot. The father recognized that his son was unresponsive and smashed the car window to gain access to his son. He then drug his son partially out of the car before being overcome by noxious fumes. First responders arrived at the scene and pronounced the son dead. His father was evaluated by medics and taken to a local hospital for observation.

There was a note taped to the driver's side window of the car stating "Danger! Hydrogen sulfide gas! Call police! Stay away!" There was a large size plastic container in the back seat of the car containing a blue liquid.

**Results:** The body was decontaminated at the scene by local fire department and Hazmat crews by dousing with water.

The external examination revealed a Caucasian male that had a blue-gray hue to his skin color and wet clothing. There were areas of skin slippage to his trunk and extremities. There were no detectable noxious smells or gases detected with a Hazmat CAD device. The autopsy revealed bilateral congested lungs and white froth in the airways. Upon removal of the skull cap, a rotten egg smell was observed and the brain was blue.

The household chemicals used to produce the hydrogen sulfide gas were lime sulfur spray (containing the active ingredient calcium polysulfide) and a strong chlorine acid.

**Conclusions:** Responders must do a thorough scene safety check before attending to a vehicle with unresponsive patients. Responders should take extra time to peer into the vehicle and look for buckets or other mixing containers in the front or rear seats, containers of acids and pesticides, a yellow or green residue in the vehicle, and vents that may be taped off. Responders need to be extremely cautious when investigating suspicious odor calls inside a structure. Once recovered from the vehicle, the body should be thoroughly washed with water and transported in a body bag to the morgue. During the autopsy, decedents may off-gas from their lungs and in this case their brain.

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**Hydrogen Sulfide Gas, Hazmat, Suicide**

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### **G39 Application of Single Nucleotide Polymorphisms (SNPs) to Forensic Casework in Malaysia**

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After attending this presentation, attendees will learn another reliable SNPs panel developed from the SNPforID markers that can be applied for population study or forensic human identification. Data obtained showed the SNP markers and platform chosen are informative and suitable to be used in forensic casework, where samples with minute amount of DNA input (as low as 30 picogram) can be detected.

This presentation will impact the forensic science community by introducing reliable and sensitive SNaPshot assays in order to genotype the population or to be used in human identification. Data obtained from the study will provide knowledge about the allele frequencies distribution in three major ethnic groups in Malaysia (Malay, Chinese, and Indian).

The analysis of degraded DNA can be problematic. Recent advances in the identification and analysis of single nucleotide polymorphisms (SNPs) have demonstrated the advantage of these markers over short tandem repeats (STRs) in that they only require small amplicons. However, before applying to casework, it is important to develop allele frequency databases from relevant populations. The purpose of this phase of the study is to characterize three Malaysian major ethnic groups: Malay, Chinese, and Indian, using 52

autosomal SNP markers that have been identified in the SNPforID project.

*Sanchez et al.*, 2006 reported a multiplex of 52 SNP markers in one PCR reaction with two single base reaction (SBE) in the detection of SNPs using capillary electrophoresis (CE). The amplicons for PCR ranged from 59 bp to 115 bp. Whilst for SBE reactions ranged from 16 nt to 92 nt. In their study, full complete profile was obtained from 500 pg DNA input. The study was carried out on three major populations: African, Asian, and European.

As in this study, a total of 150 Malaysian samples (50 samples from each ethnic group) were genotyped. In order to genotype the population samples reliably and robustly, four sets of 13-plex SNPs were developed. Sensitivity and reproducibility studies demonstrated that the assays were highly sensitive, requiring as little as 30 pg of DNA. Full, complete, and clear profiles were generated. Data were collected and evaluated statistically for forensic usefulness.

Across the three ethnic groups, few significant departures from HWE were observed in Malay, Chinese, and Indian ethnic groups. At marker rs2107612, no heterozygosity was observed at all in Malay group ( $H_o=0$ ) but the Indian group showed higher heterozygosities (above 80%). Whereas, for marker rs1413212, Malay group showed higher heterozygosities (above 80%) compared to Chinese or Indian groups. Major departure from HWE also was observed in both Chinese and Indian ethnic groups at rs1528460 marker.

The combined mean match probabilities for the 52 SNPs of Malay, Chinese, and Indian are  $2.1974 \times 10^{-18}$ ,  $6.0042 \times 10^{-18}$  and  $1.1756 \times 10^{-18}$ , corresponding to a combined power of discrimination of  $>99.99999999\%$ , respectively. Paired  $F_{st}$  values obtained in the study showed, as expected, that Malay group is closely related to the Chinese population, with the Indian population being more distant.

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**SNPs Marker, Malaysian Population, SNP for ID**

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### **G40 Firearm Suicide Demographics in Harris County, Texas**

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After attending this presentation, attendees will have a better understanding of the rate of firearm suicides among the different racial groups in Harris County, Texas.

This presentation will impact the forensic science community by providing insight into the rate of firearm suicides among different racial groups, particularly Hispanics, in an urban setting with high firearm ownership rate.

Suicide continues to be a common cause of death among all ethnic groups in the United States and using firearms to complete suicides is the most frequent method. As has been previously documented, White and African American males are more likely to complete suicide by firearm than males of other ethnic groups. It has also been found that nationally, Hispanics have a lower overall suicide rate than either Whites or African Americans. Studies have shown that regions that have a high prevalence of firearm ownership have a higher rate of suicide by firearm over other methods.

The literature has had fewer assessments of Hispanic suicide statistics; however, given the growing population of Hispanics, confirming these results or finding regional trends is useful to better understand this segment of society. To that end, a review of the suicide cases in Harris County, Texas was performed to see if these national statistics were applicable, given both the large Hispanic population and the high prevalence of firearms.

All deaths classified as suicides by the Harris County Institute of Forensic Sciences from January 2006 until July 15, 2011 were reviewed for age, sex, race, and method of suicide. Statistical analysis using student chi-squared testing with contingency tables was performed. In line with previous

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\* Presenting Author

research, a significance value of  $p < 0.05$  was used. All statistical analysis was performed using spreadsheet software. The total number of suicides classified during that time period was 2,411, with males comprising 1,827 or 75.6 percent and females comprising 584, or 24.4 percent. The ethnic composition was 1,610 White (66.8 percent), 434 Hispanic (18 percent), 261 African American (10.8 percent), and 106 Asian (4.4 percent). These numbers vary from the ethnic distribution of Harris County according to the 2010 census. The percentage population is as follows: White (33.0 percent), Hispanic (40.8 percent), 261 African American (18.9 percent), and 106 Asian (6.2 percent).

Statistical analysis shows that Hispanics in Harris County are less likely than both Whites and African Americans to commit suicide by firearm ( $p$  value  $< 0.04$ ). Results also show that females of all races are less likely to commit suicide by firearm than their male counterparts, confirming previous research. There was no statistical significance to women of any ethnicity using firearms. It was found to be statistically significant ( $p < 0.004$ ) that Hispanic men are less likely than men of all other ethnicities to use firearms to commit suicide. Interestingly, Hispanic women were just as likely as women of other ethnicities to use a firearm to commit suicide ( $p = 0.41$ ). It had been hypothesized that the given the prevalence of firearms in this geographic area that both genders of all ethnic groups would be more likely to use firearms but the analysis did not show this to be the case.

These results were surprisingly in line with previously documented research. While this analysis does not provide any additional information on the trends in Hispanic suicide, it does confirm the national data from a region with both a prominent Hispanic population and a large population that owns firearms.

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#### **Suicide, Firearms, Demographics**

### **G41 Reconstruction of Homicide by Stabbing Through Clothing Position**

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After attending this presentation, attendees will understand basic principles of recording clothing position in homicide cases involving stabbing. A case example will be discussed

This presentation will impact the forensic science community by providing information on the importance of analysis of clothing position in homicide cases involving stabbing.

Stabbing is the most common method of homicide and it represents several investigative challenges. Its reconstruction is a complex process where factors such as stabbing site, body position and dynamics, type of clothes, knife type, blood distribution, crime scene condition, pathology, and psychology are important information parameters to be considered. Major questions required to be answered in stabbing cases are differentiating between homicide and suicide, the victim and perpetrator positions, and the sequence of stabbing.

There is little attention directed towards clothing position analyses. This factor can be lost or affected during or after moving the body in the crime scene or the morgue which could produce changes in the clothing position profile.

Clothing position can be divided into three stages which are before stabbing, during stabbing, and after stabbing positions. This research will attempt to reveal clothing position during the stabbing process. It will be achieved mainly through matching stabbing sites in clothes and body tissues. The stab sites will be assessed individually and in relation to each other. This will help to determine the movement and the interaction of the victim with the perpetrator during the stabbing process and help to determine the

sequence of wounding. In addition, other evidence such as blood distribution on the victim and the perpetrator's clothing can help to indicate clothing position during stabbing.

A case of a son with a history of psychological disorder, who killed his father by stabbing is presented.

Examining the victims t-shirt showed seven stab sites. In correspondence there are six stabs sites in the left chest and left back areas of the body. The clothing position analysis demonstrated differences in the t-shirt alignment after matching stab sites in the clothing and the body. This can be explained by different pulling forces applied vertically and horizontally on the t-shirt during the stabbing process. The son confessed that he held his father after stabbing to prevent his movement which could produce the pulling force that is supported by the blood distribution evidence.

Understanding clothing position and blood distribution on the victim and the perpetrator helped to elucidate some of the events during the stabbing. Clothing position and blood distribution should be recorded and analyzed in the crime scene first then at the mortuary and in the laboratory. There is a need of more research in this vital area of stabbing reconstruction.

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#### **Reconstruction, Stabbing, Clothing Position**

### **G42 The Prevalence of Hepatitis Among the Forensic Autopsy Population of the South Alabama Region**

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The goal of this presentation is for attendees to become familiar with the prevalence of hepatitis among the forensic autopsy adult population of the South Alabama region in a cross section study.

This presentation will impact the forensic science community by determining the estimated prevalence of hepatitis among the forensic autopsy adult population in the South Alabama region.

From 887 cases investigated by the Alabama Department of Forensic Sciences (ADFS) during the calendar year 2003, demographic information was obtained and all adult cases that had had a postmortem examination were included in the study. Of the 887 cases, 775 had an autopsy and of these 711 were over 18 years of age. From the 711, 580 liver hematoxylin and eosin slides were available and retrieved from the ADFS files.

A total of 550 slides have been reviewed by at least one reviewer, 127 by two reviewers and 75 by three reviewers. The reviewers include one pathology resident, one surgical pathologist, and one medical examiner/forensic pathologist. The Batts-Ludwig modification of the Scheuer grading system designed for chronic hepatitis cases was used by the reviewed to standardize the information, acknowledging that most of these cases may not represent chronic hepatitis in the first place or that the information needed to make such diagnosis is not available. The information obtained includes the degree of inflammation (grade) and the degree of fibrosis (stage). The presence or absence of steatosis was also recorded and graded if present and any other significant pertinent findings recorded.

Paraffin embedded blocks were retrieved from 75 of the cases that had shown morphologically some degree of inflammation. Four micron sections were obtained on coated slides and immunohistochemistry for the hepatitis B core antibody was performed.

From the 550 reviewed, 229 (41.6 %) had no significant inflammation or fibrosis scored by one or two pathologist, indicating no morphologic evidence of hepatitis. Another 48 (8.7 %) cases had no significant inflammation or fibrosis by one reviewer and variable degrees of inflammation or fibrosis by additional reviewer/s. An additional 122 (22.1 %) cases by one, two or three reviewers had minimal inflammation and mild fibrous portal expansion; this pattern with no clinical information may only represent chronic triaditis, a common finding in liver tissue from medical examiner adult autopsy with no clinical significance. The remaining 145

(26.3 %) cases had mild to severe portal and lobular inflammation and fibrosis that ranged from fibrous portal expansion to cirrhosis. Six cases had marked autolysis and were not graded for inflammation or staged for fibrosis.

Cases with minimal to marked changes were included in the group that was selected for immunohistochemistry. All except one case were negative for Hepatitis B core antigen. The positive case had cirrhosis.

This study shows that approximately one fourth of the cases had morphologically features that would indicate chronic hepatitis in the appropriate clinical setting. The prevalence of hepatitis B appears to be low in this cohort. The prevalence of hepatitis C remains to be investigated in this group.

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**Prevalence, Hepatitis, South Alabama**

## **G43 Fatal Latrogenic Pseudomonas Aureginosa Meningitis After Epidural Anesthesia: A Case Report**

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After attending this presentation, attendees will become familiar with the risk factors associated with iatrogenic meningitis, as well as the proper approach to follow by the forensic pathologist.

This presentation will impact the forensic science community by emphasizing the importance of: (1) a thorough investigation in potential infection-associated fatalities: and, (2) surveillance for infections in medico-legal cases.

**Case report:** A 22-year-old pregnant woman at her 35<sup>th</sup> gestation week was admitted to the hospital after spontaneous rupture of membranes. She had a past medical history necrotizing faciitis during childhood and a previous episode of meningitis. An emergency cesarean section was performed due to failed labor induction. Epidural anesthesia was placed at this time. The baby was born with low Apgar scores and was taken to the intensive care unit (ICU). The patient was discharged three days later and soon developed headaches, agitation, and altered mental status. A lumbar puncture performed at the ED showed a very elevated white blood cell count on her cerebrospinal fluid (CSF). She was admitted to the ICU, where she had rapid decrease in consciousness, and erratic breathing, and needed to be intubated shortly thereafter. A magnetic resonance imaging (MRI) showed severe cerebral edema, suggestive of meningitis. CSF cultures grew *Pseudomonas aureginosa*. A ventriculostomy was performed; her condition continued to deteriorate to the point where there was no sign of brain activity. The family withdrew care and she was pronounced dead.

Autopsy revealed bacterial meningitis with fibrinoid necrosis and multiple intraparenchymal microabscesses. Postmortem CSF cultures were negative for *Pseudomonas aureginosa*

Latrogenic meningitis, along with epidural abscess and vertebral osteomyelitis, are rare but clinically important known complications of spinal anesthesia. Some of the potential patient-associated risk factors to develop meningitis after spinal procedures include: bacteremia; diabetes; immunodeficiency; alcoholism; chronic renal failure; malignancy; and, spinal trauma. Other described causes include: poor antiseptic technique; starch powder from gloves; and, aerosolized oropharyngeal secretions from medical personnel.

Almost half the cases of postdural puncture meningitis are caused by *Streptococcal* species. *Pseudomonas* species are an infrequent cause of meningitis and occur almost exclusively as a nosocomial infection.

Clinicians should maintain a high index of suspicion in a patient with altered mental status that has undergone spinal anesthesia, since early

diagnosis is key to a successful treatment regimen. Equally important is to follow strict aseptic techniques during all spinal procedures.

Being a preventable entity, each case of potential iatrogenic meningitis should be investigated thoroughly by the forensic pathologist with specific inquiry regarding the circumstances surrounding the death, and postmortem CSF cultures as to try to elucidate a specific cause.

Only a minority of deaths due to infections fall under the jurisdiction of medical investigators. This makes communication between forensic pathologists and clinicians essential because the sentinel case of an outbreak can often be detected at a medical examiners office.

Surveillance for infections in medico-legal cases is of utmost importance for medical examiners; it can frequently help achieve an organism-specific diagnosis, and may also identify potential high risk populations.

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**Meningitis, Pseudomonas, Epidural Anesthesia**

## **G44 An Unusual Case of Whipple's Disease With Fatal Outcome in a Young Woman: A Rare Disease and Diagnosis Failure**

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After attending this presentation, attendees will gain knowledge on macro and microscopic aspects of the rare disease, Whipple's disease, in order to make the difficult diagnostic process of this illness easier.

This presentation will impact the forensic science community by showing how a "diagnosis failure" which does not always imply a professional negligence; it stresses the importance of not relying only on the epidemiological evidence for the diagnostic prediction of the disease.

**Introduction:** Whipple's disease is a rare, systemic infectious disease caused by the bacterium *Tropheryma whipplei*. First described by G.H. Whipple in 1907 and commonly considered a gastrointestinal disorder, Whipple's disease primarily causes malabsorption but may affect any part of the body including the heart, lungs, brain, joints, skin, and eyes. Weight loss, diarrhea, joint pain, and arthritis are commonly presented symptoms, but their presentation can be highly variable and approximately 15% of patients do not have these classic signs and symptoms. Whipple's disease is significantly more common in men (87% male) and the incidence has been estimated in around 1/1,000,000, even if no valid estimate of the incidence is available. The disorder has been described most frequently in white people and in Western Europe. For these reasons, in Italy the disease is naturally placed on the National Register of Rare Diseases (RNMR) of ISS.

When recognized and treated, Whipple's disease can usually be cured with long-term antibiotic therapy; untreated the disease is ultimately fatal.

**The case:** The case of a 27-year-old woman who died six days after admission in a Sicilian hospital for persistent fever, anaemia, and weight loss is presented. For the symptoms presented, doctors evaluated a broad spectrum of diagnoses, not identifying this rare disease. For this reason, the court ordered an autopsy, to clarify the causes of death and any professional liability. The autopsy, together with the histological and microbiological investigation, allowed the diagnosis of Whipple's disease, with systemic manifestation and predominant intestinal involvement (lipodystrophy), heart (fibroadhesive pericardial disease), brain (lipid thesaurismosis), pulmonary and renal fat embolism and erythrophagocytosis.

**Discussion and Conclusions:** After explaining all aspects of forensic pathology, accompanied by macro and microscopic investigations, the exclusion of other causes of death, attention was focused on the concept of "diagnosis failure" of a rare condition such as Whipple's disease, which does not always imply a professional negligence, as in this case.

What is stated above, doesn't want to mean that a "rare disease" is the same as a "failed diagnosis", rather it wants to point out that a rare illness must be carefully investigated and therefore needs a manifold elaboration "differential diagnostic path" (with other more common pathological conditions) which require more time and more specific examination.

Moreover, it stresses the importance of not relying only on the epidemiological evidence for the diagnostic prediction of the disease.

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#### **Whipple's Disease, Diagnosis Failure, Autopsy**

### **G45 A Freak Human-Human Collision: Application of MSCT 3D Modeling to Incident Reconstruction**

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After attending this presentation, the attendee will be able to appreciate the usefulness of MSCT as an adjunct tool to the routine forensic autopsy in providing valuable objective and more detailed documentation, and the value of 3D reconstruction of MSCT data in understanding the mechanism of injury in cases of trauma, and its application in assisting the investigation into circumstances and accident reconstruction.

This presentation will impact the forensic science community by showing how 3D reconstruction of MSCT information provides better spatial and orientation information in the total understanding of pattern of injuries, aiding in accident reconstruction.

3D reconstruction of CT scan damage is a useful method in assisting in medicolegal death investigation and scene reconstruction, particularly in traumatic deaths.

The use of MSCT has become more common place. Multiplanar reconstruction (MPR) is the usual mode of reviewing of CT images in the identification and 2D documentation of skeletal injuries. This case study of two simultaneous deaths arising from a human-human collision demonstrates the application of 3D reconstruction in understanding the pattern of injuries, which might have otherwise required painstaking and lengthy forensic anthropological preparations.

While 2D CT MPRs provide a basic documentation of injuries, application of 3D reconstruction provides additional spatial information in a speedy and non-destructive manner. Its use should be considered in more routine forensic work.

In traumatic deaths with severe skeletal injuries, while identification of first level documentation of presence of fractures may be easily done through the routine autopsy method, it is not often possible, without significant effort to examine deep seated structures, such as the pelvis, scapula, and transverse processes of vertebrae. Much painstaking effort is also required if one desires to examine the pattern of fractures so as to understand the mechanism of injury. Additional steps involving further mutilation of the body, recovery, and retention of body parts, and forensic anthropological processing in a laboratory are required. This takes time and additional resource such as a proper anthropological facility. This is made more complicated where retention of human tissue becoming more sensitive medicolegal and ethical issues in the community at large.

Further, removal of the material for processing involves the loss of original spatial orientation and related information. For example, recovery of a severely comminuted fractured skull will disturb the original orientation of the pieces, run the risks of loss of minute fragments, and incur the loss of information on the original fracture pattern. Severe comminution also makes reconstruction difficult due to generation of numerous similar looking pieces and loss of 'fit' characteristics.

In recent years, CT scanning has been used in more and more jurisdictions. Multiplanar reconstruction (MPR) tends to be the most common manner in which CT information is viewed and findings gleaned. The Forensic Medicine Division, Health Sciences Authority Singapore, acquired a multi-slice helical CT scanner and operationalised it in late

2010/early 2011, using it to scan cases of traumatic deaths routinely. Besides reading the MPR images, 3D reconstruction of the skeletal system is routinely carried out. The bulk of traumatic deaths in Singapore are accounted for by self-inflicted fall from a height (tall buildings), road traffic accidents, and hangings.

3D reconstruction has been found to be very helpful in understanding the pattern of skeletal injuries, in cases where severe trauma has been afflicted. To illustrate its application, a case study will be presented to demonstrate the value of 3D reconstruction in a freak accident involving two adult Chinese females who were found side by side dead at the foot of a block of apartment flats, both sustaining serious injuries. There was a lack of eye-witness accounts and investigators were not certain how both women could have come to rest in that situation. Initial investigations appear to suggest that one or both deceased could have fallen from a substantial height. A suicide pact scenario was considered. There has been no previous documented incident where a human falling from a height had impacted another individual.

Prior to the autopsies, both deceased persons were scanned and forensic autopsies were conducted. Following the scanning, 3D reconstruction of CT data was carried out. Analysis of the 3D reconstructions enabled the medical examiner to postulate the mechanism of injuries in both individuals, by looking at the overall patterns and direction of injuries. It was then possible to subsequently make a determination on the relative postures and positions of both deceased persons at the time of impact and form a conclusion that one of the deceased persons had fallen from a height and contacted the other deceased in a back to back manner, resulting in severe injuries to both individuals and instantaneous deaths.

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#### **MSCT, Fall From Heights, Human-Human Collision**

### **G46 Heart Study in Magnetic Resonance: Birth of a Protocol**

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After attending this presentation, attendees will understand the specific approach to create a protocol for ex-situ cardiac MRI (Magnetic Resonance Images) based on the clinical experience

This presentation will impact the forensic science community by showing which elements (physics, materials) must be considered to create an easy applicable and reproducible protocol for examining a heart ex-situ by MRI.

In recent years, MDCT (Multi-detector Computed Tomography) and MRI (Magnetic Resonance Imaging) are becoming increasingly important tools for investigations in forensic medicine. Both modalities can be used on the same case. In the Institute of Legal Medicine, Lausanne-Geneva, multi-phase postmortem CT-angiography (MPMCTA) using the oily contrast agent mixed with paraffin oil is executed regularly. Because of this technique, the complete vascular system can be examined in detail, which is especially important in order to investigate the coronary arteries in cases of ischemic heart disease. However, MDCT is not appropriated to investigate cardiac tissue. Cardiac MRI could solve this problem. Additionally, no adequate scanning protocol and no guidelines for interpreting postmortem cardiac MRI are available at this time. The purpose of this study is: (1) to create a protocol for an ex-situ study permitting the systematic examination of cardiac tissue of patient suspected of heart attack; and, (2) to investigate the performance of the oily contrast agent used for MPMCTA for its application on MRI.

This study was performed on a MRI unit using a 32 channels-head coil. Different scanning protocols were tested using six porcine hearts and one human heart. The protocols included: a) the preparation of the organ including contrast-agent injection and the positioning of the heart inside the coil, b) the scanning parameters used for the MRI acquisition. The following detailed the protocols:

- a) In order to define a reproducible position of the heart inside the coil, different samples were tested made of plastic and glass containing paper, sand, or flour to avoid vibration artifacts due to the low weight of the examined heart. To unfold the left ventricular wall, different materials were inserted through the aortic valve such as a balloon catheter, polystyrene balls, or modeling clay. Different positions of the heart have been tested and a material for marking anatomical landmarks had been searched. During contrast agent injection into the coronary arteries, different types of catheters were inserted, the influence of vascular ligature, and the concentration of the oily contrast agent were tested.
- b) As we performed the MRI sequences with a 32 channels-head coil, we had opportunities to use a small slice thickness (0,6 mm to 3 mm), a relative small field of view (180-250 mm) with a good signal-to-noise ratio. The choice of the different T1-or T2-weighting sequences depended on the original software equipment of the MRI unit and of the suspected pathology.

This study shows that the oily contrast agent had a good signal in T1-weighted sequences, which makes this contrast agent adequate for the MRI examination. This can be explained by the fact, that it is an oily liquid. The filling of the coronary arteries with the oily contrast agent depended on the morphology of the vessels and on their size. In some cases, the result was disappointing; however, this problem could be solved by the use of an angiographic catheter and the dilution of the contrast agent with a solvent in order to decrease its viscosity.

To find consequential edema of cardiac infarct, T2-weighted sequences were performed in the small axe of the left ventricle, in analogy to clinical examinations and the cardiac dissection technique used in our center. In some sequences we observed a bad image quality due to the movement of the heart produced by radiofrequency waves and the gradients. This problem could be overcome by the filling of the recipient with heavy materials. A glass recipient filled with flour turned out to be optimal because it didn't interact with the magnetic field and therefore no artifacts were observed.

For an easy orientation on the resulting images of the heart, a tablet of vitamin E was used (oily contrast in MRI) as landmark that was introduced in a cork stopper which was inserted into the aorta.

This study demonstrates: (1) that an optimal scanning protocol for cardiac ex-situ MRI contains the performance of T1-weighted sequences to evaluate the coronary arteries and of T2-weighted sequences for the imaging of the cardiac tissue, and that the preparation of the organ is essential for the quality of the exam; and, (2) the application of an oily contrast agent is providing an excellent contrast for coronary arteries on MRI.

#### **Postmortem MRI, Cardiac MRI, Forensic Imaging**

### **G47 Logistic Challenges in the Initiation and Development of a Forensic Computed Tomography Scanning Service at a United States Medical Examiner's Office**

*Jamie Elifritz, MD\*; Mike Spinoe RT, Barry Daly, MD, Zabiullah Ali MD, and David Fowler, MD, State of Maryland Office of the Chief Medical Examiner, 22 South Greene Street, Baltimore, MD 21201*

After attending this presentation attendees will become familiar with the process that the medical examiner's office (OCME) went through to develop

protocols for specific forensic indications, in order to train autopsy technicians and forensic investigators and to initiate scanning of decedents.

This presentation will impact the forensic science community by demonstrating how the early experience gained from the development of a PMCT service in a medical examiner's office will be valuable for other forensic institutions planning to introduce a similar service.

The use of postmortem CT (PMCT) examination as part of the investigation of death is gaining interest in the forensic community.<sup>1-6</sup> PMCT may be utilized to complement and in some selected instances, to replace conventional autopsy. PMCT has potential in cases of blunt or penetrating trauma, elder or child abuse, drowning, burns, suicide, unknown cause of death, unidentified remains, decomposed remains, as a triage tool in mass fatalities, and in cases of cultural or religious opposition to autopsy. In suspected accidental or natural death, PMCT can be used as a triage tool to determine if cases may or may not need conventional autopsy. It can also be used as a tool for 3D documentation of complex findings, especially in blunt trauma. To date, several medical examiner offices in the United States have implemented PMCT scanning as part of their practice.

There are many variables to consider when incorporating CT scanning into forensic practice. One must consider economic factors including the purchase or lease cost of the scanner, maintenance contracts, and the operational expenses of scanning. The need for availability in a 24/7 operational environment prompted the initiation of a training program to teach autopsy technicians and forensic investigators how to operate the CT scanner. This training program was supervised by a part time CT technologist with forensic experience. Input was also provided by forensic radiologists from the University of Maryland.

A Lightspeed RT16 Widebore CT scanner with a wide aperture gantry was installed in January 2011. Three basic scan protocols were created, (whole body, head only, dental), adaptable for a wide variation in body sizes. All protocols were tailored to be completed in a 15 minute timeframe. Most 3D reconstructions were created as needed on a separate dedicated 3D server by forensic pathologists or radiologists. All protocols were easily accessible in electronic format on the scanner desktop for step-by-step reference by the autopsy technicians and forensic investigators both during and after training completion. PMCT training was provided in real time by the CT technologist and backed up by a visual presentation on the scanner desktop.

After installation of the CT scanner, nine staff members (six autopsy technicians and three forensic investigators) successfully completed the training program in PMCT applications in an 8-10 hour period each. In order to assure that all trainees produced high-quality diagnostic images upon training program completion, a grading system for technical quality of trainee-performed examinations was used, coupled with ongoing quality assurance. Establishing a radiation safety program and guidelines was an important additional component of the training program. Interpretation of PMCT studies has been performed by on-site forensic imaging fellows and state medical examiners with oversight by off-site forensic radiologists with internet access to the imaging studies. As experience accrues, more complex techniques such as postmortem angiography may be introduced.

In summary, this experience describes the development of forensic indications and basic protocol development for PMCT in a U.S. medical examiner's office. It also demonstrates that autopsy technicians and forensic investigators can be appropriately trained in the technical aspects of PMCT in a short timeframe, thus addressing the economic challenge of providing this service over a 24/7 timeframe. Close co-operation between the OCME staff and both an experienced CT technologist and radiologists interested in forensic imaging has been an important part of this process.

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#### Computed Tomography, Autopsy, Postmortem Investigation

### G48 Replica Remains: The Current State of Virtual and Physical Conservation Methods in Forensic Medicine

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After attending this presentation, attendees will have an understanding of the variety of cutting edge technologies available to forensic medicine for the reproduction and conservation of fragile human remains.

This presentation will impact the forensic science community by serving to increase scientific knowledge of new technologies and methods available to the forensic community for the protection of human biological specimens. It will also attempt to advance the state of conservation techniques by proposing alternative methods to the usage of actual human specimens in forensic research.

Many collections of human skeletal remains are exhibiting evidence of wear and tear from years of handling by researchers. Damage to often fragile specimens by repeated analyses from calipers has increased the likelihood of biased data and conclusions. Additionally, evidentiary concerns by law enforcement agencies regarding the documentation and preservation critical physical evidence call for conservation techniques that protect the specimen while providing an anatomically accurate alternative to the actual remains, particularly for use in the courtroom.<sup>1</sup> The goal of this study was to document the process of developing an anatomically accurate virtual and physical reproduction of a human skull that could be used for legal, educational, and research purposes.

In this study, a complete Caucasian male skull with intact dentition was utilized for computed tomography (CT) scanning, three dimensional (3D) computer modeling, 3D laser scanning, rapid prototyping, and casting. Using protocols developed and presented previously by the researchers,<sup>2</sup> the skull was 3D modeled and specific regions of interest were highlighted (ex: individual teeth and selected cranial bones) in order to maintain fine anatomical detail. Visualization and texturing of the final 3D model was completed in a 3D rendering software package. After the completion of the virtual 3D skull, stereolithographic models were exported for rapid prototyping using two different commercially available printers.<sup>3</sup> The actual specimen was 3D surface laser scanned and then shipped to a bone-casting expert for a proprietary reproduction process. After a museum quality replica cast was produced, the virtual and physical models (rapid prototypes and France cast) were compared quantitatively and visually to the actual specimen.

Using a binocular surgical microscope with a micrometer precision measurement reticle, the physical replicas were inspected at all regions of interests for the quantification of anatomical features. The replicas were also examined in the areas in which a high level of anatomical detail is required for biological profile analysis, such as suture lines and dental features. The results of this study suggest that accurate anatomical replicas can be made both virtually and physically to substitute for handling of actual osteological specimens. It was discovered that all of the imaging techniques were all useful tools, and that they were useful in somewhat different ways for different applications.

The benefit of this study is that there is an open discussion of the strengths and weaknesses at each stage of the invasive and noninvasive analyses, which will in turn increase the understanding and scope of forensic conservation. With both virtual and physical replicas, these resources can be used in court as visual aids for juries and also for teaching purposes in osteological labs.<sup>1</sup> The detailed replicas also address the need of existing osteological collections to preserve their materials for long-term display and study. Validated replicas such as those used in this study are providing a reliable alternative to the everyday use of actual osteological specimens.

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#### Virtual Remains, Rapid Prototyping, Physical Evidence

### G49 Investigation of Sharp Trauma by Postmortem Multi-Phase CT-Angiography

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After attending this presentation, attendees will understand the advantages and limitations of investigating cases of sharp trauma by postmortem multiphase CT-angiography.

This presentation will impact the forensic science community by showing how the performance of a pre-autopsy postmortem CT-angiography can increase the quality of the medico-legal exam. It also shows the importance to complete the radiological exam by a conventional autopsy and the necessity to further develop this new approach.

While the performance of postmortem Multi-detector Computed Tomography (MDCT) has already become a routine investigation in some institutes of legal medicine, postmortem CT-angiography is still a field of research. At the University Center of Legal Medicine in Lausanne, a

standardized technique to perform this kind of exam has recently been developed and is already getting its way into daily routine. The so called multi-phase postmortem CT-angiography (MPMCTA) consists in the performance of a native (without contrast-agent injection) MDCT scan and at least three angiographic phases (arterial, venous, and dynamic phase). As a result of this technique, the vascular system of the head, thorax, and abdomen can be investigated in detail and in a minimally invasive way. Also the examination of soft tissue (musculature, subcutaneous tissue) is significantly increased.

The goal of the presented study was to investigate the performance of MPMCTA on cases of sharp trauma.

More than 170 medico-legal cases have been investigated by postmortem MDCT angiography. From this collective, cases with sharp trauma on which the standardized protocol of MPMCTA has been performed were selected. The findings obtained by native MDCT, postmortem CT-angiography and conventional autopsy have been compared. Additionally, the impact of the radiological findings on the final interpretation of these cases was explored.

Ten cases (six Suicides, four Homicides) were selected for this study. Subjects were primarily men (seven male, three female) and age varied from 17 to 64 years (mean age=37.7). Causes of death were mostly due to exsanguination and hemorrhagic choc (n= 6). Three persons died due to a cardiac tamponade and one victim, on whom the sharp trauma was combined with manual strangulation, died due to asphyxia. More than 75 lesions due to sharp trauma were described in the final autopsy reports (including radiological and autopsy findings). They consisted of 49 stab wounds, 26 cuts, and multiple scratches.

While native MDCT could only identify major injuries due to the presence of air in the soft tissue or due to bone lesions visible on the trajectory, the sensitivity of the radiological examination could be significantly increased by performing postmortem multi-phase computed tomography angiography (PMPCTA). Due to the enhancement of the injured soft tissues, trajectories of stab wounds and even superficial cuts could be rendered visible. The 3D-software of the CT-workstation permitted also different reconstructions that could be used to explain the lesions to medical laypersons and to plan the conventional autopsy. While in most of the cases, the depth of the injury could be measured easily, it was underestimated in some cases because smallest lesions were not visible in the radiological images (e.g., small impact on a vertebra, lesion of the pericardium). On the other hand, the radiological exam revealed vascular lesions that have been missed during the autopsy and could detect the exact source of bleeding. Such findings can be important and can even explain the cause of death (e.g., cardiac tamponade due to a small lesion of branches from coronary arteries).

The sensitivity of PMPCTA to detect mayor lesions (stab wounds, cuts) in the head, thorax, and abdomen was extremely high (100 %). The protocol of PMPCTA allows a complete opacification of the vascular system in these regions; however, the examination of the limbs is not included in the scanning protocol. Therefore, the opacification of the vessels in these regions is not regular and, until now, no standardized protocol exists for the limbs. In cases where their vessels were perfused, the lesions could be identified. Without an optimal perfusion, the radiological exam failed to visualize the morphology of the lesion.

In conclusion, postmortem CT-angiography using the protocol of MPMCTA is a powerful tool to investigate lesions due to sharp trauma localized in the head, thorax and abdomen. The detection of the exact source of bleeding represents an advantage over conventional autopsy. However, this exam should be followed by a conventional autopsy, as smallest lesions could be overseen and the depth of stab wounds can be underestimated. In order to investigate also the limbs, standardized protocols have to be developed that permit the filling of their vascular system.

#### **Postmortem CT, Sharp Trauma, Forensic Imaging**

## **G50 Postmortem Multi-Phase Computed Tomography Angiography: Investigation of “Hemodynamic” Perfusion Parameters and Their Utility in Forensic Cases**

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After attending this presentation, attendees will understand the interest of monitoring the “hemodynamic” pressures while performing a postmortem multi-phase computed tomography angiography in order to optimize the vascular filling of the corpse.

This presentation will impact the forensic science community by showing the significance of monitoring the “hemodynamic” perfusion pressures in order to understand their relation to parameters of the examined body and for developing an optimized injection protocol for the perfusion.

Since November 2008, the University Center of Legal Medicine performs postmortem Multi-detector Computed Tomography (MDCT) angiography on autopsy cases in order to complete the autopsy report by a detailed investigation of the vascular system. The recently developed method of Postmortem multi-phase computed tomography angiography (PMPCTA) permits to use a standardized protocol and includes the perfusion of the body with an oily contrast-agent mixed with paraffin oil using a specialized perfusion device. This method reveals its advantages in cases showing vascular lesions or abnormalities. Until now, the attention was focused on the obtained images for the forensic radiological interpretation. The quality of the radiological interpretation depends, among others, on the filling of the vascular system that remains in some cases incomplete, especially concerning the venous system of the head.

The present study focuses on the examination of the perfusion parameters. Employing the PMPCTA protocol, standardized injections parameters for each of the three phases (arterial, venous, and dynamic) are used. During each phase, the hemodynamic pressures can be measured. This study shows interest of investigating these pressure data, in order to determine: (1) if they are related to the filling of the vascular system; (2) if they could be useful to optimize the angio-CT perfusion protocol; and, (3) if they are related to the cause of death.

In order to answer these questions, the data of 64 PMPCTAs, realized between February and November 2010, were analyzed. They included especially the measured perfusion pressures (visual observation of pressure indicator on the perfusion device) during the three different phases, the filling of the venous system, the radiological interpretation of the images and the cause of the death determined by the forensic pathologist. In order to compare the filling of the vascular system, a grading system was used, assessing the opacification of the venous system of the head. The radiological diagnosis was extracted from the radiological report, performed jointly between a forensic pathologist trained in forensic imaging and at least one radiologist, specialized in vascular imaging. Cause of death was determined by the forensic pathologists in charge of the cases after conventional autopsy and additional analyses (histology, toxicology, neuropathology, etc.).

The different data was reported on a computer spread sheet for statistical analysis.

The comparison of the hemodynamic data revealed a relation between the pressure and the filling of the vascular system. Therefore, the necessity of exploring the possibility of performing the acquisitions with specific “intelligent” injection protocols seems evident. It was hypothesized that better filling could be reached by developing a new software for the perfusion device that adjust in real time the injection parameters in conjunction with the measured pressures instead of using fixed injection parameters. Concerning

the relation between perfusion pressures and the cause of death, we noticed an indirect influence that could be observed in a better filling of the vascular system in persons who died without long periods of agony (cases of polytrauma, instant death, etc.).

The measurements of the pressure during the injection for the different phases lead to a software upgrade of the perfusion device which will include the automatic measure of pressure values and their backup. Additionally, new injection protocols could be calculated by the perfusion device depending on the perfusion pressure measured in the body.

In conclusion, our study showed that the measured perfusion data are related to the filling of the vascular system and indirectly to the cause of death. The development and implementation of intelligent perfusion software, based on real-time pressure monitoring, will be the next step to optimize the vascular filling during postmortem multi-phase computed tomography angiography.

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**Postmortem CT-Angiography, Vascular Perfusion, Pressures Monitoring**

## **G51 Postmortem Vertebral Arteriography in Forensic Investigations**

*Philip J. Berran, JD, MD\*, Howard T. Harcke, MD, and Edward L. Mazuchowski, MD, PhD, Armed Forces Medical Examiner System, 116 Purple Heart Drive, Dover AFB, DE 19902*

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After attending this presentation, attendees will understand the techniques and applications of postmortem vertebral arteriography using computed tomography (CT) as a supplement to the forensic autopsy.

This presentation will impact the forensic science community by detailing methods to evaluate vertebral artery injury and pathology using CT that may obviate the need for tedious dissection and/or removal of the neck block.

Pathology and injury to the vertebral arteries are often difficult to detect and document at autopsy. Traditionally, the vertebral arteries are injected with a radio-opaque dye and a radiograph taken. Extravasation of contrast indicates a defect in the artery wall and the area is further explored with either *in situ* or en bloc dissection. A limitation of this technique is the inability to view the artery and surrounding structures in three dimensions. Using CT in place of radiography overcomes this limitation.

A retrospective review of a series of 14 postmortem vertebral arteriograms in 10 individuals was conducted to clarify the optimal technique for antegrade and retrograde vertebral arteriography using CT. Location and method of vascular access, injection volume, and timing were varied. Arteriography was incorporated into the autopsy protocol and findings correlated with the procedure.

In six of 14 studies the vertebral artery injections were done retrograde following removal of the calvarium and brain. Cannulation of each intracranial portion of the vertebral arteries was done with a 5F angiocatheter. Injection volumes of 15-30 cc were used. All six injections were diagnostic. There were five intact arteries and one lacerated artery. In 8 of 14 studies injections were done antegrade following cannulation of the vertebral artery at its take-off in the neck. Both 5F catheters and embalming cannulas were used with some injections made before organ block removal and some made after block removal. Both techniques were satisfactory. All antegrade injections were performed before opening the cranium. A single injection of one vertebral artery with 60cc filled the contralateral vertebral artery and segments of both internal carotid arteries. Bilateral vertebral injections of 30cc achieved better generalized filling. There were four lacerations and five intact vertebral arteries. In one case retrograde filling of the left vertebral following right vertebral injection showed a left vertebral laceration.

Successful postmortem vertebral arteriography can be done during autopsy using an antegrade or retrograde approach. The antegrade technique done before opening the cranium is preferred since it offers the advantage of perfusing the Circle of Willis. Incorporating vertebral arteriography with CT

into the autopsy protocol aids assessment and may negate the need for an intricate dissection of the vessels.

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**Postmortem CT, Vertebral Artery, Arteriography**

## **G52 Postmortem Computed Tomography (CT): A Tool for Assessment of Emergency Medical Intervention**

*Howard T. Harcke, MD\*, Philip J. Berran, JD, MD, and Edward L. Mazuchowski, MD, PhD, Armed Forces Medical Examiner System, 116 Purple Heart Drive, Dover AFB, DE 19902*

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After attending this presentation, attendees will be able to explain the value of postmortem CT in assessing medical devices used by first responders to emergency events.

This presentation will impact the forensic science community by demonstrating that postmortem CT affords the opportunity to evaluate some aspects of the use of emergency medical devices. These observations can improve design and application of devices and the training of first responders.

Medical examiners are increasingly using postmortem CT as a preliminary to autopsy. The Armed Forces Medical Examiner System (AFMES) has done this since 2004 and learned that CT can yield helpful information about the use of medical equipment by first responders in the attempt to save a life. However, this requires that resuscitative equipment not be removed from the body until after imaging.

Intraosseous intravenous devices (e.g., tibial, sternal, humeral), cricothyroidotomy devices, thoracentesis needles, and supraglottic airways have been studied by the AFMES in conjunction with autopsy. The CT images non-invasively show precise location of devices in areas not routinely incorporated into the internal examination. Placement of tibial devices in 44 cases has been 95% successful, sternal device placement in 98 cases has been 80% successful and humeral device placement in 24 cases was 83% successful. Recommended length of thoracentesis needles was changed from 5cm to 8cm. It should be noted that military first responders are typically in environments that differ appreciably from civilian emergency situations, results of intervention attempts may therefore differ. Feedback communication of autopsy observations to the military medical community has been used to improve design of devices and upgrade training in device use.

It is important to point out that the postmortem observations do not assess function of a device with regard to its effectiveness in resuscitation or stabilization because the conditions and circumstances surrounding placement of the devices is not always known. Also for any data gathered from postmortem CT consideration must be given to the possibility a device position may have changed during transport and handling of the body.

Attention to details of emergency medical interventions seen on postmortem CT can result in observations that aid first responders in assessing equipment and procedures used in pre-hospital emergency care.

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**Postmortem CT, Emergency Medical Treatment, First Responders**

## G53 Development and Validity of a Postmortem Radiological Alteration Index: The RA-Index

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After attending this presentation, attendees will understand that it is possible to quantify the state of alteration of bodies using postmortem multidetector computed tomography (MDCT) imaging and will learn how to use the new Radiological Alteration-Index.

This presentation will impact the forensic science community by showing that presence of gas from only seven sites is a valid mean to measure the distribution of gas in the entire body. The RA-index is a rapid and easy to use instrument to indicate radiologically the alteration state of the body, which remains reliable for non-experienced users and valid for non-traumatically and traumatically deceased.

Postmortem imaging examinations, performed before autopsy, are more and more used in forensic medicine. Multidetector Computed Tomography (MDCT) is the most often used technique, and preferred to MRI for a number of reasons, including the rapid achievement of the examination, its relatively easy handling and its lower cost. Furthermore, the spatial resolution and high sensitivity of MDCT allows the detection of small collections of gas in bodies which is relevant because such small quantity of gas cannot be detected during standard autopsy techniques.

When investigating cause of death, it is fundamental to be able to distinguish gas formed during postmortem cadaveric alteration and gas due to a vital air embolism. It has previously been shown that cadaveric alteration and gas formation can thus be quantified. This procedure is nevertheless time consuming and requires qualified personnel. There is a need to simplify procedures to detect gas and make them accessible to non-trained physicians.

The objective of the present study was therefore to develop an easy to use radiological alteration index (the RA-index) adapted for cases seen in forensic medicine and verify whether this index can reliably be measured by non-experienced forensic pathologists.

The RA-index, ranging from 0 to 100, quantifies the state of cadaveric alteration analysing only seven sites. It was derived from postmortem MDCT data from 119 non-traumatically deceased people. One hundred additional scanned bodies (including 50% traumatically deceased) were retrospectively examined by two independent observers. Presence of gas on 82 sites was assessed by a radiologist, whereas a forensic pathologist only investigated the seven sites used for the RA-index.

The RA-index was derived using seven sites that were shown to be highly predictive of the overall presence of gas in all 82 sites ( $R^2=0.979$  in the derivation set, and  $R^2=0.843$  in the validation set). From the 119 cases analyzed in the derivation set, 25 had a RA-index of 0, 64 had a RA-index of 15 or less (no or slight alteration), 18 had a RA-index of over 30 (heart cavity full of gas), and six over 80 (invasion of gas to all tissues). Even if assessment semi-quantitative evaluation of gas presence in each site showed moderate reliability (Cohen's kappa ranged from 0.406 to 0.781), the overall RA-index was very reliable ( $ICC_{2,1} = 0.945$ ; CI95% 0.919 to 0.962).

This study derives and validates an index quantifying the state of cadaveric alteration of bodies exclusively based on postmortem MDCT imaging, and ascertains the reliability of the results obtained by a radiologist and a forensic pathologist with no experience in postmortem imaging.

Furthermore, the results were extended to traumatic deaths. Indeed, the seven selected sites for calculating the RA-index are sufficiently distributed all over the body to overcome the bias due to gas expansion from open wounds. Therefore, the RA-index is also applicable in cases of trauma such as gunshot or sharp trauma.

The RA-index, presented in this study, is the first approach that allows quantifying the alteration in an objective way. It can be used to indicate the state of the examined body for radiological reports and cases included in different research studies. This means that the selection of cases for different studies could depend on the RA-index and some diagnosis such as the presence of gas embolism should only be made in cases showing a low RA-index. The RA-index can also be important to decide if a body should undergo further examinations such as postmortem CT-angiography.

**Thanatology, Postmortem MDCT, Putrefaction Gas Index**

## G54 Biochemical Characteristics of Diffuse Axonal Injury by Fourier Transform Infrared Micro-Spectroscopy

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After attending this presentation, attendees will learn a new method of Fourier transform infrared (FTIR) Micro-spectroscopy to study the diffuse axonal injury.

This presentation will impact the forensic science community by providing participants with a better understanding of the biochemical and histopathological characteristics of diffuse axonal injury.

Fourier transform infrared (FTIR) imaging and microspectroscopy have been extensively applied in the identification and investigation of both healthy and diseased tissues. FTIR imaging can be used to determine the biodistribution of several molecules of interest (carbohydrates, lipids, proteins) for tissue analysis, without the need for prior staining of these tissues. Molecular structure data, such as protein secondary structure and collagen triple helix exhibits, can also be obtained from the same analysis. Thus, several histopathological lesions, for example diffuse axonal injury, can be identified from FTIR analyzed tissue images, the latter which can allow for more accurate discrimination between healthy tissues and pathological lesions.

The goal of the study was to assess whether Fourier transform infrared spectrometry (FTIR) micro-spectroscopy could produce distinct spectral information on biochemical characteristics of diffuse axonal injury (DAI) and to set them as molecular markers to diagnose atypical DAI.

**Method:** DAI was produced by rotational acceleration in rats and rabbits. Paraffin-embedded brain tissues from rats with head trauma were studied by hematoxylin and eosin staining, immunohistochemistry (please specify the staining), silver staining, and FTIR micro-spectroscopy. The characteristics of DAI were analyzed morphologically and molecularly. Biochemical parameters of DAI in rabbits' brain tissues were also conducted by using a chemical FTIR mapping.

**Results:** The most relevant bands identified were the amide A, B, I, and, II showing crucial spectral differences between apparent normal region and DAI region, including the peak position blue shift and the increased intensity of DAI. Comparing to single spectral band, the I1650/I1550 ratio was increased and rationally used as a molecular marker for diagnosing DAI. These novel preliminary findings supported further exploration of FTIR molecular profiling in clinical or forensic study, and were in accordance with histopathology.

In rabbits study, the N-H stretch ( $3290\text{ cm}^{-1}$ ) of amide A, the  $\text{CH}_3$  symmetric stretch of mainly proteins ( $2873\text{ cm}^{-1}$ ), fibrocollagenous tissues ( $1638\text{ cm}^{-1}$ ), nucleic acids and phospholipids C-O stretch represented by  $\text{PO}_2^-$  symmetric stretch ( $1081\text{ cm}^{-1}$ ) were tested. All of the above represent changes of proteins, collagens, nucleic acids and phosphonolipid. Red, green, and their gradient color of the Infrared spectra (FTIR-mapping) represented the IR absorptive intensity of the tested microstructure. Red indicated strong

absorption and thus means a greater quantity of material is present at this peak; Green means a weak absorption, indicating of little quantity of material at the peak. The infrared spectra of the above four vibration peaks were different even if the infrared spectrum was consistent with the result of HE staining. Hence, each vibration peak represented a different chemical structure. With the help of different peaks of the infrared spectra, the extent of a certain chemical substance that is involved in the pathological change can be explored, to reveal the pathophysiological process. The results of this study showed that N-H stretching ( $3290\text{ cm}^{-1}$ ) of amide A,  $\text{CH}_3$  symmetric stretch of mainly proteins ( $2873\text{ cm}^{-1}$ ), and nucleic acids and phospholipids C-O stretch represented by  $\text{PO}_2^-$  symmetric stretch ( $1081\text{ cm}^{-1}$ ), all widely participated in the diffuse axonal injury. The red region is mostly consistent with diffuse axonal injury lesion. The approaches described in this manuscript significantly enhance the rate and quality of spectroscopic analyses of tissue specimens, allowing realization of the statistical sampling and further numerical analysis to explore associations between molecular chemical changes and pathologic information. This imaging method also provides accurate information about the exact distribution of each component in the composite material, which is crucial for understanding its performance when in contact with pathologic processes.

In summary, it is proposed that FTIR be used as a new tool integrating both molecular and histopathological assessments to investigate the degree of biochemical and pathological characteristics of DAI diffuse axonal injury.

**Fourier Transform Infrared (FTIR) Micro-Spectroscopy, Histopathology, Diffuse Axonal Injury**

## G55 Multislice Computed Tomography in Two Fatal Stab Wound Cases With Knife *In Situ*

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The goal of this presentation is to present two fatal cases with multiple sharp injuries in which MSCT was performed before autopsy. The relevance of imaging contribute in forensic analysis in the assessment of penetrating injuries and sharp trauma casualties facilitating autopsy planning and increasing the overall detection frequency of traumatic findings is underlined and surprising images collected with knife *in situ* makes the case peculiar

This presentation will impact the forensic science community by demonstrating the relevance of MSCT as a valuable tool in detecting lethal stab and incised wounds and their course through the body and the hereby injured structures. Postmortem imaging does not replace autopsy, but rather aims at providing additional information for a more carefully planning of autopsy (virtuous autopsy). The autopsy can be improved upon but not replaced. Description of macroscopic features like margins, edges remain as indispensable for a correct framing of the death.

Fatal injuries due to sharp trauma are common in everyday forensic practice, be it in a homicidal, a suicidal, or in an accidental setting. The main common denominator of the injury-inflicting objects in sharp trauma is that they can pierce and slice the human body and thus cause internal damage. Death due to this harming of the body's integrity is manifold; exsanguination by injury of blood vessels is the most frequently encountered form. In the assessment of sharp trauma, issues such as the wound morphology, which may help to determine the type of weapon involved, the number, and location of the injuries, the wound channel, the injuries inflicted to the soft tissues and the skeleton and viscera in order to undertake a reasonable incident reconstruction. Traditional forensic autopsic approach provided by dissecting layer by layer has been generally integrated involving X-ray to detect gross injuries of bones or to detect foreign bodies but it reduces a three-dimensional corpse to a two-dimensional image, thus complicating reconstructive

attempts. With the invention of spiral computer tomographs, two-dimensional reconstructions of radiological images in every possible plane or even three-dimensional reconstructions are possible. These multislice computed tomographs (MSCT), which have become everyday clinical standard, have been implemented in forensic pathology with promising results also in the assessment of sharp injuries. Two fatal cases with multiple sharp injuries are presented; MSCT was performed before autopsy in both.

**Case 1:** A 50-year-old man went to the local military police reporting to have killed his wife after a quarrel and that the cadaver was outside in the car. The cadaver of a 52-year-old woman was found sitting normally in the front passenger side of the car, wearing clothes. A knife handle came out from the left thorax in the precordial area. The prosecutor was immediately alerted and a crime scene investigation was performed by forensic pathology crew. Bloody stains were collected from the car. The knife had a plastic handle with a pointed 16.5 cm long single edge blade 74 gr in weight. MSCT scan was performed before autopsy. A complete postmortem examination was performed two days after death. Multiple stab wounds were recorded in the neck (four), in the precordial area (one) where the knife was found, in the left thorax (one), and in the abdomen (five). Defense wounds of palm of hands were also recorded. Gross examination of the head was unremarkable except for a mild cerebral edema. Neck wounds went deep into the muscle layers except for two in which cartilage of trachea and body of the 7<sup>th</sup> cervical vertebra were interested. The wound in the precordial area went deep into the pericardium and the anterior wall of the right ventricle stopping in correspondence of the left atria where a small laceration of the wall was observed. Abdominal injuries included hepatic lacerations and wounds to the anterior and posterior wall of the stomach. Histological examination with H&E stain revealed mild cerebral edema and acute emphysema was also recorded at microscopic examination of lungs. Samples of heart wounds were collected revealing typical aspect of cutting edge lesions. Intraparenchymal hepatic hemorrhages were also observed. Vessels were generally poor of blood.

**Case 2:** A 44-year-old man affected from psychiatric disorder attempted suicide by means of multiple stab wounds inflicted to the thorax and abdomen and incised wounds to the flexor surface of wrists, bilaterally. He was found unconscious by rescuers and immediately taken to the local hospital; resuscitation maneuvers were unsuccessful. At external examination multiple stab wounds to thorax (seven) and abdomen (seven) were recorded. In the precordial area the handle of the knife was sticking out and left *in situ*. Multiple superficial cuts observed in the flexor surface of the wrists, bilaterally were interpreted as hesitation marks. An incised wound through the muscular layer of the left arm was also detected lacerating radial artery. MSCT was performed before autopsy in order to study the channel of injuries in the thoracic and abdominal cavities. The corpse was placed in the supine position on the CT-table for the non-contrast CT. Non-contrast CT was performed with the knife *in situ*. Two- and three-dimensional (2D and 3D) reconstructions were calculated and assessed by both, a radiologist and a forensic pathologist experienced in postmortem imaging. The non-contrast postmortem CT of the thorax displayed position of the knife through the sternum and surprising images were collected. There was a small gas collection in the subcutaneous fat, at the level of the entry wound. The blade of the knife passed through the sternum. The knife was plastic handle with a pointed 9.8cm long single edge blade 65gr in weight. Autopsy was performed the day after death. Mild hemorrhagic infiltration of subcutaneous fat was observed in correspondence of the thoracic and abdominal wounds; thoracic wall examination was unremarkable except for sternum. No blood was collected in the thoracic and abdominal cavities. Pericardium was also unremarkable. Heart was normal in size and volume, with conical shape, as well as lungs except for a mild edema, with white foam on the main bronchi. Abdominal viscera examination was unremarkable. A complete laceration of left radial artery was recorded. A complete histopathological study with H&E stain was performed. Toxicological examination was negative for substances of abuse. Hemorrhagic shock from laceration of the left radial artery was indicated as the cause of death.

Experience with sharp injuries demonstrate the relevance of MSCT as a valuable tool in detecting lethal stab and incised wounds and their course

through the body and the hereby injured structures. Postmortem imaging does not replace autopsy, but rather aims at providing additional information for a more carefully planning of autopsy (virtuous autopsy). The autopsy can be improved upon but not replaced. Description of macroscopic features like margins, edges still remain as indispensable for a correct framing of the death.

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**Sharp Injuries, MSCT, Autopsy**

## **G56 A New Tool for Coding and Interpreting Injuries in Fatal Airplane Crashes**

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After attending this presentation, attendees will understand the importance for forensic pathologists, investigators, and safety experts to have at their disposal the tools to collect and interpret injuries in case of an aircraft accident. The presented tool will be used in a real case to illustrate its possibilities.

This presentation will impact the forensic science community by proposing a new coding system and software to record, code, and analyze injuries sustained in aircraft crashes.

Unfortunately, every year in the world, more than 500 passengers die in air disasters involving commercial airplanes. There are numerous questions to be answered following an aircraft accident: What caused the accident? Who was involved? What were the causes of deaths or mechanisms of injuries? What can be done to prevent it?

In such cases, forensic pathologists are facing pivotal challenges, first to identify the victims but also to perform injury analysis in order to reconstruct the sequence of events and to estimate human-machine interactions. Crash reconstruction is generally obtained using data from the flight recorder but these are often supplemented by physical evidence coming from the site of impact especially the damage of the different parts of the airplane but also from documented injuries pattern linked to position of the victims in the airplane. Detailed records of injuries when many victims are involved are; however, sometimes overlooked with the priority being placed on identification procedures. This proved to be unfortunate, in particular when flight recorders could not be recovered, but also to issue recommendation in the area of injury prevention.

The objectives of this presentation are to describe a new tool to facilitate this work. A coding system has been derived from the AIS (Abbreviated Injury Score), which was developed from a nomenclature dedicated to research and statistics for trauma epidemiology. Numerous modifications and additions were necessary as the AIS system was developed to predict survival from injury severity while the goal is to code lesions that can be observed on corpses. Severity scores were replaced by scores corresponding to the amount of energy that caused the trauma (ECT).

In a second step, a software was developed to compute summary variables that will be further related to the position (assigned seat) of each of the victims in the airplane. Three types of variables are available:

- Variables representing the number, potentially weighted, of injuries coming from a group defined by the user (for instance, number of lesions of the pelvis).
- Variables representing a maximum ECT score among a group of injuries defined by the user (for instance, maximum ECT score for injuries of the upper limbs).
- Variables representing a sum, potentially weighted, of the ECT scores of a group of lesions defined by the user (for instance the sum of the ECT scores for all the coded lesions of the head).

When relevant, each score can take into account the laterality of a lesion.

A real case will illustrate the use of the coding system and software and the interest to collect and to interpret the pattern of injuries as a consequence of the circumstances of the air crash.

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## **Air Crash, Injury, Coding**

## **G57 Jay Dix Memorial Bonus Day**

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After attending this presentation, attendees will learn how and why deaths temporally related to custody; related to sports and recreational activities; related to the environment; and, related to substance abuse occur. Attendees will learn a systematic approach to investigate these types of deaths. Attendees will learn about rare causes of death and how to evaluate difficult cases.

This presentation will impact the forensic science community by providing a comprehensive review of what causes and contributes to deaths involving custody, the environment, substance abuse, sports and recreation, and rare and difficult to evaluate entities. Attendees' knowledge in these areas will be expanded and focused and attendees will better be able to systematically evaluate these types of deaths when encountered in the attendees' daily practices.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of lectures is intended to provide the non-forensic pathologist forensic scientist a comprehensive basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance inter-discipline communication.

This year's lecturers will discuss deaths related to custody, the environment, sports and recreation, less common drugs of abuse and rare and difficult entities.

Although there are myriad diseases and injuries that can cause sudden unexpected deaths, most of these are familiar to experienced death investigators and fall into familiar scenarios. However, there are a number of rare entities that cause death and a number of entities that rarely cause death. If these entities are not properly recognized and assessed, deaths in which they are involved may be erroneously certified. In other deaths, a variety of factors may lead to difficulty in case assessment and/or certification. This lecture will comprehensively and systematically delineate and discuss rare entities leading to death, deaths due to entities that rarely cause death and "hard cases."

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are related to the environment. To understand and appropriately assess these deaths, the normal relationship between a person and the environment must be considered. In addition, physiologic changes that occur in response to changes in the environment must be recognized. Diseases, injuries and/or drugs (therapeutic and recreational) and alcohol can play significant roles in the interaction between a person and the environment. This lecture will comprehensively review human and environmental factors that potentially affect a person's ability to survive under a variety of environmental conditions. What constitutes "hostile" environmental conditions, how humans attempt to adapt to changes in the environment and what prevents successful adaptation to changing environmental conditions will be discussed. There will be discussion of how humans get into hostile environments, how they attempt to adapt to these conditions and what factors prevent adaptation and/or escape from inhospitable environments. Deaths caused or contributed to by cold, heat, altitude, drowning, animals and lightning will be among the topics discussed.

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to participating in and, occasionally, while being a spectator at sporting or other recreational activities. Understanding these deaths requires understanding of the physical requirements to perform particular activities, susceptibility of particular diseases to stresses associated with particular activities, effects of various chemical and/or biological agents that may be taken to enhance performance and physical injuries associated with particular recreational activities. This lecture will provide a comprehensive review of these issues in the context of investigating deaths that occur in relation to sports/recreational events. Understanding factors that are involved in deaths occurring in these circumstances also helps in instituting appropriate safety measures to protect participants and spectators.

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to custody. The custody process can be divided into several stages—pre-custody, pre-incarceration and incarceration. Particular diseases and injuries tend to occur and/or become manifest during each of these stages. This lecture will systematically review what diseases and injuries cause/contribute to death in the custody process, how they affect physiology and anatomy, when they are typically operative and how they are manifest. Recognizing what occurs during the various stages of custody allows a systematic approach to assessing deaths that occur during the custody process. This lecture will review the conceptual and practical aspects of understanding and investigating custody-related deaths.

The last three decades have seen an impressive expansion in the range of drugs available for abuse. The psychedelic revolution of the 60's spilled over into the 70's and 80's with the advent of "designer drugs" like Ecstasy (MDMA), joined in the 90's and 00's by the increased abuse of psychoactive drugs like ketamine and GHB, typically in nightclub and rave settings. A second pattern of expanded drug use involves increase in consumption in rural areas poorly served by the traditional distribution network for cocaine and heroin. The heartland of America has seen explosive growth in the use of methamphetamine, whether prescribed, homegrown or cartel-distributed, and in diversion and abuse of prescription medication. These other substances are commonly used in characteristic scenarios and have somewhat stereotypical death scenarios. Recognition of the patterns of abuse of these agents helps in assessing the role, if any, of these agents in particular deaths. This lecture will provide a comprehensive review of both increasingly and infrequently encountered agents in the context of investigating deaths.

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**Jay Dix, Death Investigation, Forensic Pathology**

## **G58 Explosion Scene in Sri Lanka**

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After attending this presentation attendees will gain knowledge about specific injury patterns pertinent to suicide bomber related explosion scenes. It further studies two different types of suicidal bomb explosions compared to other circumstances and touches on the clinical aspects of victim management.

This presentation will impact the forensic science community by highlighting extend of damage and complexness of investigations when close target explosion attacks are made by using relatively smaller quantity of explosive substance in a form of a "body bomb."

In our days, explosives manufacturing has found rapid advancement and available in mass scales to become a weapon of choice in many parts of the world for military, commercial, criminal, and organized terror activities due the ease in handling, storage, and transportation; need in lesser quantity to cause devastating effects; simplicity of detonating mechanisms, etc. Injuries due to high explosive devices were often seen in Sri Lanka during past two decades, especially during the period from 1995 to 1997 and from 2004 to 2008 due to escalations of civil conflict. The suicide bomber became a hallmark of the Sri Lankan explosion scene. Two categories of suicide

bombers have been observed: "body bomb" which may be described as a person with high explosive device attached to his body in different forms of attire including that of a pregnant woman; and, "Carried bomb" using other movable device, explosive effects of which had distinguishably reflected in the overall injury pattern.

There have been at least 125 attacks on civilian and military targets since 1986. The incidents have predominantly taken place in the capital city and autopsies on most of the cases were performed at the Office of the Judicial Medical Officer in Colombo. The findings of autopsy examinations, scene examination, and information gathered from the police sources and evidence given in the subsequent Court proceedings have been used for this study. The number of victims was excluded from the study due to unavailability of details of autopsy examinations conducted at different places. The present study is mostly emphasized on qualitative comparison of injury patterns with six principal types of explosive injuries described in the literature.

Analysis of injury patterns observed in the suicide bombers and the victims of these incidents revealed characteristic features of medico-legal and scientific importance. The following classification of explosive injuries is found to be more applicable to the local explosive scene, especially in the cases of individual suicidal bomb attacks: (a) a suicide bomber; (b) combined effect injuries including total disruption; (c) blast wave injuries; (d) burns; (e) injuries due to flying missiles; and, (f) circumstantial effects. The absence of shrapnel wounds, severe disruption of the trunk, and extensive burns of the transected body margin and presence of a cyanide capsule made a suicide bomber clearly distinguishable from other victims. The injury pattern of the victims was modified by the effects of the surroundings and the distance from the centre of explosion.

The recognition of the above injury patterns and their bodily effects found to be important in crime-scene investigations, subsequent legal proceedings, and organization of preventive measures. The effects of the modern vehicles with impact resistant designs on the gravity of injuries have also been considered.

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**Explosion Injuries, Suicide Bomber, Injury Pattern**

## **G59 Stab Wounds and Drowning - A Fatal Combination**

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After attending this presentation, attendees will: (1) be able to state the percentage of cases in which more than one modality of fatal injury is used to kill; (2) recall the percentage of cases of homicide by stabbing; (3) recall the number of cases of homicide by drowning; (4) be able to quote the literature on the number of reported cases of death by drowning after infliction of stab wounds; (5) appreciate a case of homicidal drowning after the infliction of stab wounds of the chest; (6) review the pathological features of classical drowning; and, (7) review the utility of comparative diatom analysis as an ancillary investigation in cases of drowning and determine its appropriateness as an ancillary investigation.

This presentation will impact the forensic science community by showing how the use of more than one modality of fatal injury in homicides sometimes occurs. It is rare in forensic practice to have death from drowning following multiple stab wounds of the body, especially with penetrating stab wounds of the chest. An interesting case of homicide is presented in which death by drowning in an environmental watercourse occurs after an attack with a knife in which multiple penetrating stab wounds of the chest are sustained. This case is presented due to its rarity and to revisit the utility of the comparative diatom analysis as an appropriate and available ancillary investigation in cases of suspected drowning.

The use of more than one modality of fatal injury in homicides sometimes occurs. It is rare in forensic practice to have a death from drowning following multiple stab wounds of the body, especially with penetrating stab wounds of the chest.

An interesting case of homicide is presented in which death by drowning in an environmental water course occurred after an attack with a knife in which multiple penetrating stab wounds of the chest are sustained. This case is presented due to its rarity of presentation and to revisit the utility of comparative diatom analysis (Diatom Test) as an appropriate and available ancillary investigation in cases of suspected drowning.

A 17-year-old single mother was missing for two days. Her body was recovered from a canal in a fully clothed state with multiple stab and incised wounds. Investigations revealed that she was lured to the secluded area via text messages and phone calls sent by both her present boyfriend and ex-boyfriend. External examination at postmortem identified multiple stab wounds with four penetrating stab wounds of the chest and multiple defensive stab and incised wounds of the upper limbs. Internal examination revealed pale, hyperinflated lungs that overlapped in the anterior midline with no evidence of collapse from haemo/pneumothorax. There was stab wound injury of the lung and a small volume of residual blood within the right pleural cavity. The cause of death was given as Drowning with Multiple Stab and Incised Wounds. It was evident that the decedent was alive on entry into the water. Comparative Diatom Analysis provided ancillary support for drowning.

Drowning is not necessarily the cause of death when a body is recovered from water. The possible scenarios are:

**Death before immersion in water**

- natural disease
- injury

**Death while in water**

- natural disease
- injury
- drowning
- death from effects of immersion other than drowning

The autopsy diagnosis of drowning is a major problem in forensic medicine as the pathological proof is often difficult or impossible to obtain, especially when there is a delay in recovery of the corpse. The diagnosis of drowning can often be easily established in a fresh corpse that has been recovered early. The task becomes more difficult when putrefaction sets in and the signs become obscured!

Modell (1981) defined drowning as “*suffocation by immersion, especially in water.*” This is the classic and most widely used definition. The World Congress on Drowning (2002) defined drowning as “*the process of experiencing respiratory impairment from submersion or immersion in a liquid.*”

The pathological diagnosis of drowning can be made through identification of the following signs:

- Champignon d’mouse
- Froth in the tracheobronchial tree
- Emphysema aquosum - heavy, waterlogged and hyperinflated lungs – (600-700 g each) that fill the chest cavity, exhibit rib indentations (visible and palpable grooves), overlap/meet in the midline such that they obscure the bare area of heart and obliterate the anterior mediastinum. They are pale and crepitant and do not collapse when placed on the dissection board.

10 - 20% of undoubted drownings are “dry lung” cases with no excess weight (Copeland 1985)\*

- Paltauf’s hemorrhages
- Pleural effusions
- Water in the stomach\*
- Middle ear hemorrhage\*
- Low spleen weight\*

\* Too inconsistent to be of diagnostic use

**Diatoms** – Diatoms are microscopic algae that live in water and range in size from 2µm to 1mm with most being 40-80µm. The smaller species of the order of 2-5µm can easily penetrate tissues. There are about 15 000 species which are almost equally divided between fresh water and brackish/seawater. They are covered by a heat and acid resistant siliceous exoskeleton (frustule) which are physical properties which are taken advantage of in their laboratory extraction. Diatoms can be demonstrated as a microscopic finding in the tissues in drowning.

**Diatom Test** – The basis of the diatom test is premised on the aspiration of water into the lungs on entry of a living person into water during drowning such that such that the contained diatoms also enter the lungs. The aspirated diatoms then penetrate the alveolar walls, enter the pulmonary venous circulation and then the systemic circulation such that they are transported and deposited with distant organs and tissues such as the brain, kidney, liver, and bone marrow. Passive entry of diatoms into the lungs can occur in deceased bodies which inadvertently end up in water, through wave action. As there would not have been a functional circulation at the time of entry of these bodies into water, the diatom species of concern will not be present in the distal organs.

The diatom test was first applied by Revenstorff 1904 as an ancillary investigation in possible drowning deaths. It involved the identification and comparison of recovered diatom species to assist in the confirmation of drowning and possibly identify site of drowning. The validity of the test is strongly debated in the medical literature since diatoms can be demonstrated in non-drowning deaths.

The presence of diatoms in non-drowned bodies has been associated with ingestion of seafood, inhalation from the environment and laboratory contamination of glassware and reagents. Despite these, some believe that the diatom test is still the most reliable “drowning test” to date.

Hendey’s criteria (1973) set out two (2) criteria for accepting a positive result from a diatom test as evidence of drowning in that:

- The species of diatoms recovered from pathological specimens are all present in the sample from the site of drowning.*
- species are present in the same order of dominance for the admissible size range and in approximately in the same proportions*

Samples for Comparative Diatom Analysis/Sample preparation – Diatoms must be extracted from the body of water in which the corpse was recovered and compared with those extracted from the tissues. As such, the following samples are needed:

- Water sample from place of recovery of body (at least 500ml)
- Fresh tissues retrieved from the body in a sterile manner
  - lung (one lobe)
  - liver (150g)
  - whole kidney (100-150g)
  - long bone (femoral) marrow

To prevent cross contamination, it is necessary to take each sample *in-situ* with a change gloves and use of sterile instruments and blades when procuring each tissue sample. No co-mingling of the tissues must occur.

The laboratory preparation of the specimens entail:

**1. Diatom extraction from tissues**

- Acid digestion (HNO<sub>3</sub>) *or*
- Water maceration *or*
- Incineration (most useful with fatty tissue eg. bone marrow)

**2. Microscopic examination of the residue**

**3. Comparison of diatom species** – The diagnosis of drowning can be made with reasonable degree of certainty from correlation of:

- History/Circumstances of the Death
- Gross lung findings
- Microscopic lung findings
- Diatom examination
- Other ancillary investigations

The Diatom Test should be used as an indicative aid and NOT as legal proof of drowning.

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**Drowning, Stab Wounds, Comparative Diatom Analysis**

## **G60 Female Suicides by Firearm From 1990 - 2010 in Montgomery County, Ohio**

*Robert L. Hunkeler III, MFS, and Kent E. Harshbarger, MD, JD\*, Montgomery County Coroner's Office, 361 West 3rd Street, Dayton, OH 45402*

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After attending this presentation, attendees will understand trends relative to firearm suicides by females over a 20-year timeframe in Montgomery County Ohio, an area encompassing 460 square miles with a population over 535,000.

This presentation will impact the forensic science community by examining female suicides by firearm and identifying any trends relative to age, sex, race, firearm type, wound location and alternate methods of suicide. This data can be used to compare to other studies to determine any geographical trends related to firearm suicides by females.

Suicide continues to be a very sensitive topic and significantly impacts families and the community at large. Firearm deaths have become commonplace in today's society and usually are seen in homicides, followed by suicides and accidental deaths. Although firearms are a predominate mode in male suicide, it has had a lower representation in female suicide. Montgomery County Coroner's Office began seeing higher numbers of female suicides by firearms and a study was conducted in order to determine the significance of these numbers and if they translate to an increase in rate for female suicides over time. In addition, Montgomery County Coroner's Office began seeing females in higher age categories selecting firearms as the mode for suicide; therefore, the study addressed age as well, in an effort to determine rate and if there was statistical significance to the findings. A total of 30 of the 87 cases involve females age 55 and over. Another interesting aspect is wound location, with Montgomery County Coroner's Office seeing a higher number of cases involving gunshot wounds to the head, versus historically seen wound locations of the chest and abdomen.

It is imperative for coroners and medical examiners to understand identified trends in order to determine fluctuations within their jurisdictions. It is vital for communities to understand trends associated with suicides. By identifying trends for our serviced communities, various services can be provided, or actions can be taken in order to design suicide prevention programs in an effort to prevent additional deaths. An example of this can be seen in Ohio where a number of pain management clinics have been shut down in the southern part of the state due to the amount of pain medication being distributed, resulting in overdose and death. By taking action, it will be interesting to see if the affected counties see a downward trend in overdose deaths.

This presentation will discuss the analysis of 87 female suicides by firearms over a 20-year period in Montgomery County Ohio. Analysis will include age, sex, race, firearm type, and wound location and determine if these factors are statistically unique for females as compared to male suicides by firearm for the same period of time. In addition, other methods of female suicide will also be compared to determine if there is statistical significance relative to female suicide by gunshot in the 324 female suicides from 1990 - 2010. Rates per 100,000 will be calculated for each year on each method for the population of Montgomery County, which has declined from 573,809 in 1990 to 535,153 in 2010.

**Suicide, Firearm, Gunshot Wound**

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## **G61 Suicide and Profession**

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After attending this presentation, attendees will be aware of why it is of paramount importance to know the profession or skills of the victim before performing the autopsy.

This presentation will impact the forensic science community by serving to understand how the profession, knowledge, and or skills of the victim should influence the decision of what is the best way to commit suicide in a simple, rapid, intelligent, and effective way.

Methods of suicides most common used and examined at the National Center of Forensic Medicine in Israel for the last several years were by hanging, jumping, shooting, stabbing, and cutting. To choose the appropriate way to commit suicide, is probably a very complicated issue, and depends on many factors that certainly, the profession and skills of the victim are an important parameter to take into consideration.

In general, the people know the location of the heart or neck's great vessels, so those are usually the places of choice for cutting or stabbing. In most cases, the victim doesn't have the appropriate anatomy knowledge which tells him where to cut for achieving the most "effective" place to harm himself and usually they doesn't know how much pressure to apply in order to create the most effective lethal damage.

This lack of knowledge guides the typical victims to select the most inappropriate places for cutting which leads to produce several superficial and parallel cuttings (*hesitation wounds*). Such wounds indicate repeated attempts of self-inflicted damage before to build-up of sufficient courage for the final deep gash that injures major blood vessels or expose the trachea or larynx to die. Those lesions not always appear near the deeper fatal injury.

In these cases not being able to achieve their goal by this method, they usually finish their life by hanging or jumping or choosing another way to die. Death may have been due to a cause other than exsanguination.

In self-inflicted incised wounds of the extremities, right-handed individuals usually cut the left wrist or forearm, typically found on the flexor surface and radial aspect of the forearm. Groins are not such a common place.

The story can take different angle when the victim has anatomy knowledge such as painter (artist), hunter or a physician when chosen the cutting method. In these cases, they know where to cut, at which depth, and may be they know how much pressure to apply on the skin. In such cases one can asked if hesitation marks must appear. The answer is: probably not.

This presentation will show an unusual case of a convict physician, who committed suicide on jail by cutting his own groin vessels.

For the first time, as far as the records at the The National Center of Forensic Medicine tells, the body of a person who committed a suicidal act by cutting the main blood vessels in the groin, with only a few hesitation wounds, was brought in for autopsy. A similar case like this has not been previously described in the forensic literature to the best of our knowledge.

Undoubtedly, the victim had a background of anatomical knowledge, and unquestionably this point helped him to select the proper area of his body to do a lethal damage.

In this case there are some interesting questions to ask:

1. It looks that in this case the appearing of hesitation marks are not a result of lack of knowledge of the amount of pressure to apply. Maybe it was the result of not enough courage to execute the act?
2. Even though the victim had knowledge in Anatomy; one of the groins' damaged wasn't effective enough to cause death. Why?
3. Why the victims choose the groin vessels as a target of damage instead of neck vessels?
4. Did the victim weigh another alternative which is more common option to commit suicide in jail like hanging?

Obviously, it is impossible to give answers to these questions today.

**Suicide, Profession, Hesitation Marks**

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## G62 An Unusual Homicide-Suicide Modality in an Elderly Couple

Margherita Neri, MD, PhD\*, Natascha Pascale, MD, Sara Vita, MD, and Irene Riezzo, MD, PhD, Department of Forensic Pathology, University of Foggia, Osp D'Avanzo, Viale degli Aviatori, 1, Foggia, 71100, ITALY

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The goal of this presentation is to present a case of homicide-suicide in an elderly couple, characterized by an unusual complex suicide achieved by three different tools: a knife, a razor blade, and a claw hammer.

This presentation will impact the forensic science community by presenting the case of a homicide-suicide in an old couple, where the murderer garoted his wife with an elastic band, and then he tried to kill himself first hitting his head with a claw hammer and then cutting his neck and arms with a razor blade, and stabbing the chest and neck with a knife. Moreover claw hammer self-inflicted lesions on the scalp are very rare, in particular in association of knife and razor blade and in a case of homicide-suicide.

Complex suicide is very rarely described in literature; in this case it was used three different kind of tools: a knife, a razor blade and in particular a claw hammer.

Homicide-suicide (HS) is defined as that lethal event in which an individual kills another and subsequently commits suicide within one week. The unusual injuring tool and the relevant injuries which are produced the suicide were studied and analyzed to approach the case of dyadic death. The most important question for the forensic pathologist is to distinguish between a real homicide-suicide and a double murder.

On June 2, 2011 at 1:00 p.m., a woman called the police and said that she found her old parents dead. The police and the forensic pathologist went to the crime scene and found the body of a 72-year-old Caucasian woman inside of the bedroom of her own house. The woman lived in the house with her husband. The corpse was lying supine on the bed; she was fully and tidily dressed. She had an elastic band wrapped around her neck; this band was a length of 240cm, width of 11cm. The thanatological data recorded at 4.00 p.m., showed early rigor mortis, hypostasis that was totally blanched with fingers pressure, but congruous with the position, the rectal temperature was 35°C and ambient temperature was 25°C. Inside the bathroom, on the floor, was lying the body of a 78-year-old Caucasian man, his corpse appeared extensively blood-stained with a surrounding blood stain. Close to the left arm of the man, on the floor, was a knife, it was extensively blood-stained and it was length of 31cm, blade length of 19cm, blade maximum width 2.2cm. Close to the left foot, was a razor blade also blood-stained, with a length of 4cm, width 1cm and a hammer with double prong also smeared of blood. The thanatological data recorded, at 4:30 p.m., the body showed early rigor mortis, and hypostasis that was in part blanched with fingers pressure, but congruous with the position, the rectal temperature was 34.5°C and ambient temperature was 25°C. The Prosecutor arranged the autopsy on the bodies because it was necessary to clear up the circumstances of the death and distinguish between homicide-suicide and double homicide. A complete autopsy was performed 24-hours after death on both bodies. At the external examination, the old man showed peculiar lesions of the head: multiple couple of linear lacerations of the scalp including tissue bridges because of the gap between the two claws, although some of the injuries caused marks of the skull. The damage was probably inflicted with a claw hammer. Moreover, it was present multiple slashed wounds on the neck, both forearms and chest; in particular on the neck three stab wounds: two superficial on the left side and one deeper than others on the right side. Section of the neck revealed hemorrhages in the subcutaneous tissues and at the right stub wound sternocleidomastoid muscle and the right jugular vein showed a deep tear. The other organs did not showed specific alterations except for an intense anemia. The toxicological analysis was negative and the cause of death was a hemorrhagic shock.

The woman showed, at the external examination, a remarkable cyanosis of the face, lips and nails; skin petechial hemorrhages in frontal and periorbital region and mucosal petechiae on oral vestibule and both conjunctivas were also detected. The face was covered with various bruises and superficial skin tears. On the neck a horizontal mild blue bruise areas

was present with nails marks. Section of the neck revealed hemorrhages in the subcutaneous tissues and in both sternocleidomastoid muscles and thyroid muscles. Esophagus, larynx, and trachea were unremarkable. Subpericardial and subpleural petechiae were detected. The other organs did not showed specific alterations except for an intense vascular congestion. Skin sections for histological examination were removed at the neck in long strips perpendicular to bruises. Sample of muscle tissue were also taken at the neck. The histological examination showed mild hemorrhages in the cutaneous and subcutaneous tissues, and in the muscles. The stratum corneum of the epidermidis was detached and the dermis was split from the epidermis. An immunohistochemical study was performed to evidence the vitality of the skin injury. The toxicological analysis was negative. According to the examination of neck bruises, autopsy findings and histological data, the mechanisms of death consisted with an asphyxia. Death was attributed to an external neck compression; the tool that caused the death was perfectly compatible with the elastic band found on near the body.

**Homicide-Suicide, Claw Hammer, Self-Inflicted Injury**

## G63 Eventual Suicide by Self-Inflicted Intracardiac Needle on the Eleventh Attempt

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After attending this presentation, attendees will be aware of the fatal complications due to intracardiac self-inserted needles.

This presentation will impact the forensic science community by illustrating a type of injury that is known but rare, showing how prompt surgical removal of the foreign body could be life-saving, preventing the complications related to the sharp nature of needles.

The migration into heart chambers can cause cardiac tamponade, infection, peripheral embolism, valve dysfunction, pneumothorax, and death.

Cardiac injuries produced by sharp penetrating foreign bodies are not uncommon. Bullets, acupuncture needles, fragments of Kirshner wires, and sewing needles represent the object usually inserted. Rarely the heart lesions result from an auto-aggressive behavior in patients with an underlying mental disorder or with opioids abuse. When injury is self-inflicted it's usually an expression of personality disorders, schizophrenia, major depression, mania, and gender identity disorders and can be associated with certain medical diseases or represents a suicide attempt. The major symptoms are chest pain and dyspnea, although patients may be asymptomatic.

Long-term survival with needles and other foreign bodies in the heart has been described in numerous cases. Furthermore there are reports of incidental findings on autopsy of needles embedded in the heart. Though immediate death is uncommon, the overall mortality exceeds 50%.

It is believed that needles should not be removed if the patient is asymptomatic, a conservative approach is also recommended for old wounds: with time most foreign bodies become securely encysted and do no damage. Instead, in the case of early diagnosis needle injury to the heart should be treated surgically, regardless of the presence of symptoms to reduce further myocardial damage and excluding complications including death.

A case is presented in which a young male prisoner who died due to cardiac tamponade after multiple self-inserted intracardiac needles.

The male prisoner was a psychiatric patient admitted to the Emergency Service because of sharp chest pain. The patient didn't disclose any suicidal behavior. However, clinical history was characterized by previous introduction of foreign objects in his chest left *in situ*. The man was oriented, his vital signs were stable, but his heartbeat rate was high. Electrocardiography disclosed no abnormality. Myocardial necrosis enzymes were slightly increased. PA- X-ray of the chest confirmed the presence of multiple intrathoracic and intracardiac metallic string-shaped objects.

The diagnosis was acute coronary syndrome. Nitrates and antiplatelets were administered and the man was immediately moved to the cardiology

unit. Because of a worsening of the cardiorespiratory functions, and the suspicion of a pulmonary embolism, a scintigraphy was requested but not performed because of the urgency of the situation. In the Coronary Intensive Care Unit, the patient had a cardiopulmonary arrest that, despite several resuscitation attempts lead to death after 13 hours after admission.

During forensic examination, acupuncture signs were detected at the precordial region. The autopsy showed chronic constrictive pericarditis, cardiac tamponade, and copious partially coagulated blood in the left hemothorax.

A needle, 11cm in length, was found penetrating the pericardium with about 3cm of its length infixed in the left ventricle of the heart. Another 10 metallic objects were found embedded in the thorax surrounded by a thin fibrous lamina: one at the base of the left costal arch on the midclavicular line and under the subcutaneous fat, one at the peritoneal level inside a fibrous mass near the greater curvature of the stomach, one inside the omentum, five at the precordial level out of the sternal plastron, one in the second intercostal space and one in the third. Accessory tests were non-contributory.

This case is out of the ordinary for the forensic scientist because of the injury pattern found at autopsy, it also involves clinicians of the Emergency Department and cardiac surgeons who have to make an accurate assessment of psychiatric patients with penetrating foreign body for a proper management to reduce myocardial injury and avoid complications.

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#### **Intracardial Needle, Suicide, Autopsy**

### **G64 “One-Punch” Fatalities**

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The goal of this presentation is to show how special forensic and pathological issues associated with brief physical altercations result in fatalities. The cases presented and relevant published material will review typical circumstances, procedures for case analysis hopefully leading to proper forensic interpretations. Those attending should expand their knowledge base for immediate application to the forensic exercise at their own facilities.

This presentation will impact the forensic science community by highlighting that though “One Punch” fatalities are not especially common, they can pose significant forensic challenges and challenges for the justice system. Proper case analysis and interpretations can materially affect proper adjudication of these often sensational and notorious cases.

A relatively uncommon occurrence that often attracts attention of the media involves a physical altercation, usually between intoxicated young men in which sometimes a particularly forceful blow with a fist by one of them will result in the rapid death of another. The blow scenarios may involve a fist impact to the face, head or jaw, or neck, and sometimes to the chest. Other scenarios involve some other form of single impact. Following the blow-impact the stricken individual may immediately fall backward, often striking the head on a hard surface, with immediate unconsciousness. If the victim does not fall backward striking the head, there may be a period of confusion or stupor, then followed by unconsciousness. The victim is usually transported to hospital but may die en route or shortly after admission. Autopsy findings may range from major basilar and/or other skull fractures with or without epidural or subdural hemorrhages, or major subarachnoid hemorrhage with or without skull fracture or epidural or subdural hematoma. Tearing or avulsion of circle of Willis arteries, carotid, or vertebral-basilar arteries may also be found with or without dissections and/or thromboses in the absence of skull fracture. There is a significant literature on traumatic injuries to intracranial arteries by Krauland (*Verletzungen der intrakraniellen Schlagadern*, Springer-Verlag, Berlin, 1982), but being in the German Language may have escaped the attention of those with no knowledge of this language. The 31 cases of Krauland and other contributions will be reviewed and compared with cases of our own. There are significant technical problems associated with the pathological examination in these types of cases

which will be reviewed with recommendations on how to avoid or minimize them.

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#### **Traumatic Subarachnoid Hemorrhage, Fatal Head Injury, Arterial Injury**

### **G65 Homicidal Deaths in the Republic of Ireland Through the Work of the Office of the State Pathologist**

*Khalid Jaber, MD\*, Firebrigade Training Centre, Malahide Road, Marino, Dublin 3, IRELAND*

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After attending this presentation, attendees will gain information on the prevalence and incidence of homicidal deaths in the Republic of Ireland, as seen through the workload of the Office of the State Pathologist.

The presentations will impact the forensic science community by presenting, possibly for the first time, internationally, the figures and characteristics of homicidal deaths reported in the Republic of Ireland for one complete decade (2001 to 2011). The office of the state pathologist is an administrative branch of the Department of Justice in Ireland and is responsible for the forensic pathology investigation of all these deaths. This presentation will attempt to provide an overview of the breakdown of the types of homicidal deaths and their respective demographical and forensically- pertinent features.

Homicide is defined literally the killing of the human being. Ireland, the Island of the saints and scholars is not totally immune to this regrettable human tendency. The archival system of the Office of the State Pathologist in the Republic of Ireland (covering the Island of Ireland except the six counties in the province of Ulster, known as Northern Ireland) has been searched between 2001 and 2011 for all reported and investigated homicidal deaths. The population of the Irish republic is just over 4.0 million inhabitants. This office is part of and works within the guidelines and rules set and approved by the Department of Justice and Law Reform.

This chosen period covers a socioeconomic time when the economic cycle and individual disposable income in the republic literally oscillated between one extreme of a boom position (known as the Celtic tiger era, 2000 to 2007) to literally the juxtaposed position of widespread bust period with significant and recession (the bursting of property market bubble, 2008 to present).

Homicidal deaths account for 30% to 40% of the office’s case workload. The rest of the cases dealt with are, also, regarded by the Garda Siochana (police force) as suspicious deaths. The office handles a wide variety of homicidal deaths. The data collected briefly reveal that fatalities due to firearm - related, sharp force trauma, and/or blunt force trauma each accounts for one third of the total figures.

Increasing number of firearm related - deaths, involving handguns, rifles, and shotguns have been identified during this period. The increase within this period is attributed to a noticeable rise and conspicuousness of the activity of multiple and rival criminal gangs engaged in illegal activities. Following the Good Friday Accord between the republic of Ireland and United Kingdom over the state and future of Northern Ireland, some dissident paramilitary groups have increased their involvement in different criminal activities, bringing them into either direct turf struggle with the established criminal gangs or enhanced co-operation in criminality. Despite the perception and to some extent the evidence pointing to the presence of large varieties of firearms in the country (mostly smuggled) there appears a noticeable absence, during this study period, of military - style assault rifle related injuries.

Blunt force trauma is still a major cause of concern, despite its relative ratio decline, compared to what it used to account for in the past. The data confirms the international findings that major urban population centres are relatively more violent than urban communities.

Stabbing and incisional wounds which are still encountered reasonably regularly are discussed in terms of their relative impact into the homicidal death scene.

The data presented also includes homicide by motor vehicle accidents (hit and run, dangerous driving leading to deaths) but not other types of road traffic accidents fatalities, as these are not handled by this office. Drugs, especially ethyl alcohol, constitute an important part in the Irish homicidal death scene, especially non - firearm related. Alcohol still is a major health concern in the overall mortality figures in Ireland in general.

Despite the recent concerns voiced nationally over the activities of paedophilic priests, and the traditionally high relative birth rate when compared to the rest of the European Union countries, it appears that the incidence of fatal child abuse and fatal non-accidental infant and child traumata are noticeably relatively very low.

Men were more likely to be victims of homicide or serious assault than women in Ireland, like the rest of the European Union. Like many other countries, women were more likely than men to be victims of intimate partner homicidal death and homicide-suicide deaths. The latter are encountered relatively infrequently occasionally.

At midpoint of this study period there were 58 victims of homicide in Ireland in 2005. Of these, 49 were men. Almost half of male victims were aged between 21 and 30 years of age. One third of female victims of homicide were aged between 21 and 30 years of age.

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**Homicidal, Death, Ireland**

## **G66 Elementar Analysis of Gunshot Residue to Differentiate Bullet Type and Firing Distance**

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After attending this presentation, attendees will be familiar with the elemental analysis of gunshot residue (GSR) using inductively coupled plasma mass spectrometry (ICP-MS).

This work will impact the forensic science community by demonstrating how elemental analysis can be used to identify GSR in various stages of decomposition.

Porcine tissue was shot with jacketed and non-jacketed ammunition at two different firing distances. Samples were collected throughout decomposition and analyzed by ICP-MS to determine element composition of the GSR. Element concentrations were statistically assessed to investigate differentiation of bullet type and firing distance based on chemical concentration of the GSR.

In the fresh state, bullet entrance holes and a characteristic GSR pattern may be readily visible, enabling identification of a gunshot wound. However, due to chemical and biological processes occurring during decomposition, as well as larval activity, the entrance hole and GSR patterns can be obliterated. Thus, the natural decomposition of a gunshot victim in an outdoor setting can hinder the identification of a bullet entrance wound. The ability to detect GSR on corpses that have undergone advanced stages of decomposition would aid pathologists and medical examiners in cause of death determination, while the ability to identify the bullet type would aid law enforcement in narrowing down the suspect pool.

Differentiation of bullet type based on elemental composition of GSR has been demonstrated previously in our laboratory. Porcine tissue was shot with jacketed and non-jacketed ammunition and tissue samples were analyzed by ICP-MS for elements characteristic of GSR (antimony, barium, and lead), as well as elements characteristic of bullet type (copper, iron, and zinc). However, in these previous studies, the jacketed and non-jacketed ammunition contained different smokeless powders, all wounds were shot with a firing distance of 5cm, and only moderate decomposition was investigated.

The objectives of this research were to further investigate differentiation of bullet type based on chemical composition of GSR, as well as to investigate differentiation of firing distance based on GSR composition.

Samples were collected throughout full decomposition, to determine if differentiation according to bullet type and firing distance was still possible.

In this study, control (unshot) samples were collected from each of four euthanized pigs. Two pigs were then shot eight times each with jacketed ammunition using a .357 Dan Wesson revolver (blued steel barrel, 1.5 inch in length); one was shot using a firing distance of 5 cm, while the second was shot using a firing distance of 10cm. The remaining two pigs were shot in a similar manner, except using non-jacketed ammunition. All ammunition cartridges were hand loaded with the same smokeless powder to ensure no elemental variation was due to differences in composition of the powder. One tissue sample was collected from each pig immediately after GSR deposition. The pigs were then allowed to decompose naturally and the remaining wounds were collected throughout the decomposition process.

The control samples and tissue samples containing GSR were microwave-digested in nitric acid and then analyzed by ICP-MS. Full mass scans were initially used to identify those elements present at significantly higher concentration in the shot tissue compared to the control tissue, for both bullet types. Once identified, the instrument was calibrated for the elements of interest and the tissue samples were analyzed using selected ion monitoring for those elements. Element concentrations in the fresh tissue were statistically assessed to differentiate tissue shot with jacketed versus non-jacketed bullets, as well as to differentiate tissue shot with a 5 cm firing distance versus a 10cm firing distance. Then, element concentrations were compared for samples collected throughout decomposition, assessing the persistence of GSR and the ability to continue differentiating bullet type and firing distance.

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**Gunshot Residue, Bullet Type, Firing Distance**

## **G67 The Presence of Hyoid Bone Fractures in Self-Inflicted Gunshot Wounds to the Submental Region of the Neck**

*Alice J. Briones, DO\*, Philip J. Berran, JD, MD, Edward L. Mazuchowski, MD, PhD, and Howard T. Harcke, MD, Armed Forces Medical Examiner System, 116 Purple Heart Drive, Dover AFB, DE 19902*

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After attending this presentation, attendees will understand how hyoid bone fractures may occur in association with self-inflicted gunshot wounds to the neck and head and how postmortem Computed Tomography (CT) can facilitate the identification of hyoid bone fractures in gunshot wounds to the submental region of the neck.

This presentation will impact the forensic science community by providing data demonstrating the presence of hyoid bone fractures in self-inflicted gunshot wounds to the submental region of the neck, a scenario not previously considered as a significant cause of hyoid bone fractures.

**Hypothesis:** Although within the forensic science community fractures of the hyoid bone are commonly considered to be seen in cases of manual strangulation, hyoid bone fractures can occur in association with self-inflicted gunshot wounds of the head and neck.

**Methods:** A retrospective review of Armed Forces Medical Examiner System cases 2005-2010 revealed 400 cases of self-inflicted gunshot wounds. The final autopsy reports were reviewed to identify the circumstances of death, the weapon used, entry and exit wounds, and the description of the hyoid bone.

**Results:** Of the 400 self-inflicted gunshot wounds, 55 were to the submental region of the neck. Of these 55 cases, eight had hyoid bone fractures. When the specific type weapon used was evaluated within the total number of gunshot wounds to the submental region (55); hyoid bone fractures were identified in: one of the five shotgun cases, six of the 35 rifle cases, one of the 10 handgun cases, and zero of the five cases with unknown weapons.

In the eight cases of hyoid bone fracture, postmortem CT was available in five cases and showed hyoid bone fractures in all three. Since this series was reviewed, two additional self-inflicted submental gunshot wound cases

were autopsied at our facility. Both cases demonstrated a fracture of the hyoid bone on CT which was confirmed at gross dissection.

**Conclusions:** Hyoid bone fractures occur with self-inflicted gunshot wounds of the neck and head involving a variety of weapons to include shotguns, rifles and handguns. Forensic pathologists should recognize that hyoid bone fractures may exist in this type of case and postmortem CT is capable of identifying these fractures. The presence of hyoid bone fractures in the setting of gunshot wounds of the neck and head does not necessarily indicate manual strangulation.

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**Self-inflicted GSW, Hyoid Bone Fracture, Postmortem CT**

## **G68 Fatal Suicidal Gunshot Wound to the Head With Lack of Immediate Incapacitation**

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After attending this presentation, attendees will learn special wound ballistics of the head, and general mechanisms of incapacitation that make sustained capability to act possible in rare cases of gunshot wounds to the head.

This presentation will impact the forensic science community by showing how questions concerning the possibility of physical activity following a given gunshot wound are often raised in court and can be of major importance in reconstruction of a crime and in differentiation between homicide and suicide.

**Introduction:** A case of physical activity following a suicidal gunshot to the head including perforation of the right frontal lobe from a 9mm is reported. The woman was able to walk a distance of 18m before dying. A review of the literature showed that the potential for physical activity following penetrating gunshot wounds to the head is related to general principles of wound ballistics and mechanisms of incapacitation.

**Case report:** a 45-year-old woman was found dead in a wooded area. The body was lying on the ground near a car and the face was covered by maggots. A suicide note was found inside the car. The decedent was known to suffer from severe depression and was last seen alive the day before by her estranged husband. The investigators first thought that the victim had died from fatal intoxication, as several empty blisters of alprazolam were found nearby. However, secondarily a 9mm carbine was found 18m distant from the body. At autopsy an entrance wound was located at the right temple. A circular depressed fracture of the right temporal bone was noted. There was an acute subdural hematoma of the right cerebral hemisphere. Several pellets were removed from the surface of the brain. Neuropathology showed traumatic injuries of the right frontal lobe. As the frontal brain does not include areas immediately essential to acting or consciousness, it was concluded that physical activity was possible in this case and that this woman had been able to walk a short distance before dying. Toxicology analysis was not requested by investigators. Death was ruled a suicide.

**Discussion:** Penetrating gunshots to the head are presumed to cause immediate incapacitation by subsequent disturbance of cerebral functions. In the literature, incapacitation has been defined as "a physiologically based inability to perform complex and longer lasting movements independent of consciousness or intention." However, not every severe or even fatal gunshot wound causes immediate incapacitation. Physical activity is possible if the trajectory is restricted to the frontal brain or one temporal lobe only and if a projectile of low wounding potential has been used for the gunshot. In our case, neuropathology showed that no central nervous area essential for physical activity was wounded directly, and the woman's capability to walk following the gunshot wound was of major importance in differentiation between homicide and suicide.

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**Forensic Pathology, Gunshot Wound, Incapacitation**

## **G69 Sudden Unexpected Death Caused Probably to Eagle's Syndrome: A Case Report**

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After attending this presentation, attendees will understand the profound implication of vagal reflex in relation to blow inside the neck by ossification of stylohyoid ligament.

This presentation will impact the forensic science community by highlighting the possible existence of higher number of deaths attributed to reflex cardiac arrest.

**Case Report:** An autopsy was performed to a man who was found dead in his car. The initial investigations determined that this young man without health problem went to his job at 5:00 a.m. He was making backing up his car in a parking lot and suddenly died. The policemen remarked that the back wheels crossed the abutments of parking protection. External examination of the body showed that this man was 175cm in height and weighed 73kg. There was no wound at the inspection. Internal inspection revealed bilateral bony hard vertical structures. The length of each ossified stylohyoid ligaments were 5 cm on left and 3cm on right. The carotid vessels were normal without any injury, especially at the fork area. The macroscopic examination of the organs was normal and unremarkable. There were absence of congestion, cyanosis, and petechiae. Toxicological examinations did not reveal any recent or old consumption of toxic substances. Microscopic organ investigations were normal. The elongated stylohyoid ligaments were ossified with the presence of cartilage, bone, and bone marrow. Based on the necropsy findings, the cause of the death was suggested to be a cardiac arrest caused by the vasovagal reflex. This research reviews the embryology of styloid chains and the anatomy of the styloid process - the stylohyoid ligament and the lesser cornu of the hyoid bone form the stylohyoid apparatus. The ossified stylohyoid ligament is known as Eagle's syndrome which is an uncommon sequel of elongation of the stylohyoid process. It is an aggregate of symptoms that includes foreign body sensation, recurrent throat pain, dysphagia, facial pain, and vertiginous. The diagnosis is made by radiography. One part of subtypes is the stylo-carotid artery syndrome. The frequency of elongated stylohyoid process is estimated approximately 4% of the population. The term of calcified is erroneous, ossification is considered more precise because hyperplasia of the styloid process is exhibited by histology. Not as obvious, the ligament could be ossified and form a solid structure. The ossified stylohyoid ligament may press upon the adjacent structures in the neck such as the carotid sinus complex. The reaction could be a vagus nerve mediated reflex cardiac inhibition. The mechanism of cardiac arrest resulting of pressure on the carotid neural complex will be discussed. The referenced causes of reflex cardiac arrest such as manual strangulation, hanging, and blows to the throat or neck will also be reviewed.

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**Forensic, Eagle, Reflex Cardiac Arrest**

## **G70 Harness Hang Syndrome and a Death While Rappelling**

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The goal of this presentation is to review the nature and effects of suspension by a harness and to discuss a death that occurred under such conditions. Attendees should gain a better appreciation of the potential hazards of prolonged harness suspension. The goal is to inspire others to do further research on the topic.

This presentation will impact the forensic science community by introducing the unusual topic of harness hang, also known as suspension stress or suspension trauma. The impact of the presentation will be a better

understanding of the possible mechanisms and nature of deaths occurring under the condition of harness suspension. This experience may be helpful in future investigations of similar cases.

Rock climbing is a popular outdoor activity that may involve suspending oneself within a harness while climbing or descending. Harnesses as a component of a personal fall protection system are used in other recreational and industrial activities. The hazards of falls are obvious, but less well known are the possible hazards of prolonged harness suspension. The harness hang syndrome, also known as suspension stress or suspension trauma, is the result of the physiological response to a motionless body being suspended in a vertical position for a period of time. It is believed that this can lead to cardiovascular alterations, loss of consciousness and even death. Although the medical and forensic literature has little on this subject, other types of suspension and asphyxial deaths are well-known. The most common type of suspension death, of course, is hanging. But other types of asphyxial deaths may involve suspended or trapped body positions such as traumatic or positional asphyxia. The death of a young male who was rappelling down a rock face in close proximity to a waterfall is presented.

This 24-year-old man was found suspended in his rock-climbing harness along the rock wall of Mildred Falls in the Cleveland National Forest on evening of February 11, 2011. A hiker had reported hearing cries for help, but had to hike a long distance to get cellphone reception. The time of the 911 call was 5:08 p.m. By the time rescuers responded to the scene the decedent was motionless and unresponsive. His body was described as arched backward with his chest and face towards the falls. His recovery from the scene was delayed due to darkness and environmental conditions. The next day, he was pulled up from the falls, confirmed dead, and transported to the Medical Examiner's Office. He was known to be an avid rock climber and his car was found parked at the trailhead to the falls. The decedent had left his house in the early afternoon and had told his roommate that he was going to make the descent at the falls. According to family, his medical history was unremarkable.

At autopsy he had multiple scrapes and contusions of the skin, but no evidence of internal trauma. At least some of the abrasions appeared to have occurred during the recovery. Other findings were suggestive of asphyxia including numerous periorbital and conjunctival petechiae. No internal neck, head, chest, or abdominal trauma was found, and no pre-existing natural disease was noted. No alcohol, basic medications, or common drugs of abuse were detected on toxicologic testing. There was no evidence of neck compression and no specific findings suggestive of drowning. It is believed the mechanism of death was a result of mechanical asphyxia and probable hypothermia.

If conscious, someone in the decedent's position would be expected to exert oneself in an effort to escape or regain control until the point of exhaustion, unconscious, or death. The exertion itself along with panic or other emotional factors may also play a role. In this case, the rock wall was wet and covered with vegetation so there was little chance of climbing back up or even getting a foothold in order to transfer body weight from the harness. Of course, one would be unable to escape being suspended if an injury or other preceding event had already caused unconsciousness. Other factors considered in this death case were the possible roles of drowning and hypothermia. As seen and inferred by the video the body was not constantly or consistently in the water and the autopsy did not show evidence of drowning. Therefore this mechanism is unlikely. However, hypothermia may well have been a factor.

The literature theorizes that suspension stress or harness hang syndrome is due to orthostatic hypotension along with other possible cardiovascular mechanisms; however, this has not been verified experimentally. It is believed the mechanism is probably respiratory, not cardiovascular, and that these types of deaths should be considered a form of mechanical asphyxial. This may be the result of direct compression on the chest or abdomen impeding the ability to breath (traumatic asphyxia) or a body position that interferes with the ability to maintain an open airway (positional asphyxia). The body compression may be from the harness or its attached ropes and straps and may be influenced by the body's position. The position may vary depending upon the state of consciousness, the type of harness and

attachments and how they are positioned. It is uncertain how long one must be suspended in order to have adverse effects, and obviously the time frame could vary depending on the circumstances.

Until further research and analysis of similar deaths are undertaken, the nature, physiological effects and dangers of harness suspension are still largely unknown. However, one should be aware of the potential risks of prolonged harness suspension while unconscious or otherwise.

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### **Harness Hang, Suspension, Rappelling**

## **G71 Unusual Patricide: Case Report**

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After attending this presentation, attendees will have a better understanding of the classification of homicide called case of patricide.

This presentation will impact the forensic science community by stimulating discussion on whether or not mental illness should be considered a "trigger point" for offenders.

Patricide is defined as a homicide in which victims are parents and the killers are their children. "Patricide" is the definition given to the murder of father, while "matricide" refers to the murder of the mother. International literature reports that this crime is often associated with psychiatric morbidity and is usually committed by males. Patricide committed by sons is the most frequent form. In Italy, patricide is quite rare and it represents less than 3% of all murders (59% matricides and 41% patricides). The typical profile of the offender is a young adult male who is single and unemployed. The offender typically lives with victim (quite often an old and disabled person), and is suffering from mental illness with co-morbidity of alcohol or drug consumption and/or abuse. Usually he is not able to separate himself from his parents or to assume responsibilities. The homicide usually takes place at home at the end of an argument, and common tools found in the house are often used as weapons. A 73-year-old man was found dead at home, on the kitchen floor, after his son confessed the murder. He killed his father early in the morning, after yet another argument with him. He then called the police, and sat on the floor awaiting the officers, fully aware of his guilt. A pair of scissors and a cross-head screwdriver, both with traces of blood, and a wooden board were located close to the body. At crime scene, the victim was supine and showed multiple injury pattern. Clothes were raised upon the thorax and multiple thoracic-abdominal lesions were found together with bruises and abrasions of the head. The sternal area showed two larger stab wounds, while eleven penetrating cross-shaped puncture wounds affected precordial, epigastric, and mesogastric regions. Four other shallow, cross-shaped punctures of the skin surrounded these lesions. At autopsy, gross examination of organs showed visceral lesions with cardiac, hepatic, and intestinal involvement, widespread hemorrhagic infiltrates of cranial soft tissues, skull fractures (vault), and diffuse subarachnoid hemorrhages (SAH). Multiple and serial rib fractures were also found. The cause of death was related to traumatic shock due to blunt head trauma and multiple stab wounds. All autopsy data allowed drawing the following crime reconstruction: victim's head was initially hit with the wooden board and the man fell down, striking his head on the floor. Then the offender uncovered the anterior thorax and abdominal anatomical regions of his father and stabbed him with the pair of scissors and the screwdriver. Finally, he raised the victim's body, crushing the chest with his weight. The offender's criminal profile revealed a 45-year-old man, with elementary school education. At a young age, he became a construction worker at building sites in Northern Italy. The father, a construction worker too, was described as extremely strict with the son. The mother, a housewife, was characterized as a warm-hearted woman. The offender was very shy, the second of six children, and the only son. He did not have any recent romantic interests and he remembered only

one relationship with a woman in the past. No psychiatric disorders were in his medical records. He had a history of drug-addiction (intravenous heroin) and during the last years, he admitted to occasional use of cannabinoids and cocaine sniffing. However, the drug screen was negative when he was imprisoned. His mother had died a few months before, and due to an accident at work, the offender had lost his job. He was at home with his father every day. The mother's death broke the intra-family balance, and co-habitation caused relationships to deteriorate. The domestic context and the special violence of the crime induced the Court to impose a forensic psychiatric assessment of the offender that excluded factors of mental illness or psychiatric disorders at the time he killed the father. On these bases, the presented case is an unusual patricide that departs from those typically found in the literature.

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**Forensic Pathology, Patricide, Forensic Psychiatry**

## **G72 Accidental Decapitation Due to Tamping Machine: A Case Report**

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The goal of this presentation is to report an uncommon case of accidental decapitation in a worker of a railway line.

This presentation will impact the forensic science community by enhancing worker knowledge of the risks associated to railway activities by showing an unusual incident of work related beheading by heavy machinery.

Decapitation is represented by separating the head from the body with cuts in the soft tissues of the neck and resection of the spine at the level of the last cervical vertebrae. Generally speaking it is referred to the act of intentional or accidental decapitation and it can be the result of an explosion, car or industrial accident, or other violent injury. Suicide by decapitation is unusual. Death by beheading is quickly fatal and it consists in the resection of the neck structures (vessels, nerves, cervical spine).

Presented here is a case of a 45-year-old man who worked on the railway line with other four colleagues, in close proximity to a ballast machine. A ballast tamper or tamping machine is a machine used to pack (or tamp) the track ballast under railway tracks to make the tracks more durable. For each rail there is a tamping unit attached to the main frame by means of vertical guide columns and a lifting/lowering hydraulic cylinder. The operations are controlled from the control cabin by an operator using three pedals, while the lining bogie holds the track in its lifted and slewed position. The Ballast Cleaning Machine (BCM) carries-out deep screening of ballast, which is an important maintenance activity to improve drainage and the resilience of the track. The cutter blades of the BCM dig out ballast from under and around the sleepers, and a conveyor belt transfers it to the on-board cleaning equipment where the ballast is passed over screens which remove fine debris. The clean screened ballast is returned to the track and the fine screened residue is ejected to one side, usually into a hopper wagon on an adjacent track. The machine goes into reverse and the blades rotate counter-clockwise.

The victim was located to the left of BCM, near the cutter blades, checking the correct progress of operations, while another worker was driving the machine the other two were together on the other side. All workers were dressed with safety equipment such as helmet, headset, and reflective vest. The victim was probably bent near the chain when he was slipped due to the instability of the ground, being hit on the helmet or on the jacket by the teeth the chain. The speed of the machine did not allow the victim to move away from the chain and he was transported to the right arm of the BCM, inside of which entered his head, but whose size (45 centimeters height and 40 centimeters wide) did not allow the passage of whole body.

At the autopsy time we found the head and the right forearm detached from the rest of the body. The longitudinal diameter of the head was 28.5 centimeters and the diameter of the laceration at the base of the neck was 15x10 cm. In the right parietal region there was a large lacerated and bruised wound the total length of 15cm and in the left fronto-temporal region there was another lacerated and contused wound with margins diastase that affects the entire skin thickness; also he had fracture of the left lateral eye socket. The laceration line passed through the high left lateral to the low right lateral and posterior part of the upper cervical region. Head and neck were covered with powdered material. The airway was severed at the trachea level. The laceration present in the cervical region had a longitudinal diameter of 21.5 centimeters and transverse diameter of 16 centimeters and through it passed heart and part of lungs, traction by trachea and neck vessels. Excoriated streaks and de-epithelialisation area were observed in whole body even if mainly in the dorsal region; these injuries were due to the action of sliding on the stones present in the binary. The helmet and the headset were broken.

No other similar cases are reported in literature.

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**Decapitation, Railway, Ballast Cleaning Machine**

## **G73 Seasonal and Environment Effects on the PMI Estimation Using the Entomological Approach**

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After attending this presentation, attendees will receive novel information about the advantages of practical application of forensic entomology in different environmental conditions (indoors, outdoors, water) and in different seasons.

This presentation will impact the forensic science community by offering new data about the composition of the carrion breeding fauna particularly during the winter season and the effect of global warming on the entomofauna. This presentation will offer as well a statistical approach for the estimation of the season in which the death occurred. This topic is of particular importance in "old cases."

Forensic entomology is a branch of forensic science in which insects are used as evidence in legal investigations relating to humans or wildlife. The examination, identification, and analysis of insects associated with human remains, combined with the knowledge of insect biology, can provide a further level of detail in addition to medical and anthropological data in the reconstruction of events occurring close to the time of death. In particular, necrophagous insects are useful in studying Postmortem Interval (PMI), postmortem transfer, and presence of drugs or poisons.

Seasons, environment, concealment, and accessibility play an important role in the selection of the carrion breeding insects. It is worth mentioning that during the last 25 years global warming and globalization have modified the species distribution with important effects on the application of the entomological method for the PMI estimation (Turchetto and Vanin, 2010, in Amendt et al., *Current Concepts in Forensic Entomology*, Springer).

Twenty cases have been considered from an entomological point of view. The cases occurred, during the last two years, in Central Italy which is an important area for the understanding of the global warming effect on the entomofauna.

The bodies were discovered between February and November and the estimated minimum PMI ranged from few days to several weeks. Socially isolated people, drug or alcohol addicts, or old people living alone were involved in the majority of the cases considered. The causes of death included natural, homicide, suicide, smothering, and drug overdose. In four

cases the bodies were burned due to fire or electricity. In one case, the body was found in a saltish lagoon.

The insects have been recovered during the autopsy and, where possible, also during the body recovery following the standards and guidelines proposed by the European Association of Forensic Entomology (EAFE, Amend et al., 2006, Int J Legal Med 121: 90-104).

Statistical analysis of the results reveals a strong correlation between fauna composition and decomposition stage of the body, between the fauna composition and the season of the death (winter vs. summer), and between the fauna composition and the environment (outdoors vs. indoors). No statistical differences have been detected comparing the causes of death. In burned remains, insects of the first waves of colonization (Calliphoridae and Sarcophagidae) arrive without any delay compared with other cases, confirming observations and experiments carried out by Vanin and Cattaneo (Vanin et al., in prep).

Species belonging to Diptera (Trichoceridae, Psychodidae, Stratiomyidae, Phoridae, Muscidae, Fanniidae, Calliphoridae, and Sarcophagidae), Coleoptera (Cleridae, Staphylinidae and Silphidae) and Hymenoptera (Formicidae) were collected. The genera *Trichocera*, *Calliphora* and *Hermetia* showed a fall/winter phenology whereas *Lucilia* (particularly the species *L. sericata*), *Sarcophaga* and *Chrysomya* a summer phenology, with the later species present also during the fall.

The recovered data highlight that *Hermetia illucens* (Stratiomyidae) (species introduced in Europe during the last century) and *Chrysomya albiceps* (Calliphoridae) (reported only from the Southern regions till 1985) are now present in the whole Italian peninsula. In addition the data underline the important role of *Megaselia scalaris* (Phoridae) in the colonization of indoors bodies.

Postmortem injuries caused by arthropods have been reported in two cases. An indoors case shows the activity and the possibility to have postmortem injuries by ants also inside an house, whereas the body recovered from the saltish lagoon showed different lesions on the face and on the body performed by aquatic Isopods (Crustacea, Isopoda).

This work summarizes and statically analyzes information coming from different cases. The data, here presented, represent an important point of reference for the definition of the season of death, for the comparison between different environments. In addition our data confirm the spread of alloctonous species, particularly from the southern regions. This information is important in order to avoid mistakes in estimation of body transfer. Moreover, the significant attention to indoors cases is related with the observation that over 70% of the cases occur in this environment (Vanin et al., in prep).

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**Forensic Entomology, Global Warming, Statistical Analysis**

## **G74 Decapitation: Unusual Feature of a Fatal Dog Attack**

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After attending this presentation, attendees will understand the concerns in a case of a fatal Rottweiler dog attack on a 52-year-old man who was found dead on a farm where he worked.

This presentation will impact the forensic science community by showing how the evaluation of fatal dog attacks requires an integrated approach in association with veterinary pathologists and forensic geneticists,

involving review of the circumstance of death, examination of the death scene, CT analysis and autopsy examination of the victim and the histological and immunohistochemical examination (IL-15, CD15 and tryptase).

The cases were all characterized by extensive and mutilative stripping of soft tissues from the upper limbs, face, and scalp, progressing to decapitation in the man. Investigators found the entire skull, completely skeletonized, of about 800 meters away from the decapitated body. A complete methodological forensic approach by means of autopsy, histological and immunohistochemical examinations and an integrated study in association with a veterinary doctor and forensic genetics is an important part of such investigations. It may provide information helping to establish the identity and ownership of the animal, along with trace evidence confirming that the dog was involved in the attack with comparison of the bitemarks on the victim and the dentition of suspected dog.

Deaths due to animal attacks are rare. Deaths caused by dog attacks appear to be growing as the population of both humans and dogs has increased. Many types of dogs have been involved in fatal attacks on humans, with at least 25 different breeds reported in 238 deaths in the United States over a 20-year period. The majority of cases (60%) have, however, involved pit bull-type dog, rottweiler, and german shepherds, most of whom were unrestrained on their owner's property. Diagnosis of a wound as a bitemark is generally not difficult considering the rather pathognomonic wound pattern of "a hole-and-a-tear" together with skin abrasions and claw marks. Any part of the body can be the site of a dog attack. An unusual concentration of severe injuries to the head and neck regions is typically reported in the literature. Bites to the forearms are also common as the limbs are generally raised in an attempt to protect the face. Dog attack deaths usually result from exsanguinations through opened body cavities and/or large vessels and/or asphyxia.

**Case:** A 52-year-old Caucasian man was found dead on the sidewalk in front of his home on the farm where he worked. A multidisciplinary forensic approach, including CT analysis, autopsy findings, histological and immunohistochemical examination, and bitemark analysis was performed. A complete autopsy was performed 48-hours after death. The external examination revealed the presence of multiple and coarse lacerations of the skin in the upper limbs and clusters of superficial, linear, parallel abrasion from a dog's claws were present in the skin adjacent to bitemarks. In the thorax, cluster of ribbon and parallel abrasions with the same trend were observed bilaterally. The most striking finding was represented by decapitation, characterized by a laceration of the circumference of neck exposed second thoracic vertebra, the trachea completely dissected, the vessels of the neck and the paravertebral muscles and shoulders, strong friction and hemorrhagic. The CT analysis of the skull demonstrated small scratches at the neurocranium made by the canine teeth and/or paws. There were no other injuries on the body. The internal examination revealed a hemorrhagic area around the neck vessels that appeared frayed. Histological study of tissue samples confirmed the vitality of the skin lesions. The immunohistochemical examination of the bitemark specimens revealed a positive reaction for antibodies anti CD15, IL-15 and tryptase. The death was attributed to decapitation and hemorrhage due to vascular disruption.

An integrated study with a veterinary doctor was performed by creating the dog's dental cast. Dental casts were superimposed on the victim's wound samples collected at autopsy and analyzed for compatibility with the patterns taken from the jaws of suspected dogs could be clearly adapted on the bitemarks. The results of this investigation allowed the confirmation that the bitemarks found on the corpse matched the cast of the dental arches of the suspected rottweiler. The genetic laboratory was able to match the human DNA profile from the samples (blood and fecal impaction containing hair of the man) the suspected dog. The human DNA profile found was identical to that of the victim. Genetic typing was performed with 19 canine microsatellites markers, co-amplified in a single multiplex PCR reaction with "Canine Genotypes Panel 1.1 (Finzymes Diagnostics, Finland). The search for genetic traces of the dog was performed on samples of skin and muscle of the victim.

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**Dog Attack, Decapitation, Bitemarks**

## **G75 A Case of a Death Related to Underwater Propeller at a Desalination Plant During Illegal Scuba Fishing**

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The goal of this presentation is to show how injuries by propeller, amputation, and death by drowning are rarely reported events in forensic literature. A complete methodological approach with complete autopsy and histological study must be required as forensic approach to these cases.

This presentation will impact the forensic science community by educating them on a complete autopsy and histological study of all organ and surfaces of mutilated skin to determine the phases of death are of impact for attendees.

A self-contained underwater breathing apparatus (SCUBA) is often used in diving; it is practiced both as a sport and for some commercial activities. Most developed countries have legislation ensuring the safety of divers, but despite this, deaths are still recorded, especially among amateurs. In Puglia, many amateurs scuba dive for fishing for shellfish, such as mussels and clams, as well as octopus and bluefish. The most frequent cause of death, associated with this activity is drowning. In Italy, drowning occurs at a lower incidence than in other countries but is a highly lethal phenomenon. In almost half of the 800 events per year, the person involved dies (387 cases in 2007) and in the remaining 55% of the cases, people are hospitalized (near-drowning cases). Of these, it is estimated that in 2010 almost 10% occurred during SCUBA fishing expeditions. In contrast, propeller injuries are rare events and are thus seldom reported in the international forensic literature. According to a previous report, the frequency of propeller accidents is greater than 1 in every 20 boating accidents, and the fatality rate in propeller accidents is 15–23%.

Most propeller injuries occur at water recreational facilities such as those equipped for water skiing, boat racing, and skin and scuba diving. Injuries by propeller are typically multiple, deep, parallel lacerations that can result in permanent scarring, substantial blood loss, traumatic or surgical amputation, or death. Research has not shown any previous reports of injuries or reported deaths related to propellers from a desalination plant. This case study presents a scuba diver's death that occurred during an illegal scuba fishing trip in the vicinity of a desalination plant. During a scuba diving fishing trip at around 6:30 a.m., three scuba divers were fishing about 3–4m deep and about 30m away from the beach. One of the divers decided to fish close to the plant, probably attracted to the enormous quantity of "big mussels" attached onto the surface of the underwater parts of the desalination pump. After a sudden and unexpected start of the pump, a few minutes later, the other fishermen noticed a large, bloody patch in the water and then the lifeless body of their friend. They dragged him by swimming to the beach and then alerted local authorities.

An underwater scene investigation was conducted by an engineering team, studying the mouth of the pump and the dynamic characteristic of rotating propeller blades. An autopsy was performed the day after the death.

The external examination of the body showed multiple amputations of arms and legs; a large bluish/red abrasion/contusion of the right thoracic wall extending to the superior part of the ipsilateral abdominal wall was also observed. Autopsy confirmed the complete sectioning of the right arm, the left forearm, and both ankles, and corresponding great vessels. A contusion of the thoracic soft tissue without any fractures was also noted. Examination of all other organs was unremarkable except for pulmonary and cerebral edema. Vessels were completely free of blood. Lungs were increased in volume and size, with few subpleural hemorrhagic spots. Mild cerebral edema with focal subcortical hemorrhagic spots and focal pulmonary edema associated with extended areas of atelectasia, acute emphysema and "ballonee" cells inside the alveolar spaces were observed at histological examination with standard H&E staining. Histological examination of the heart was unremarkable except for few foci of contraction band necrosis.

Histological examination of the skin of the amputated surfaces revealed the presence of red cells on the derma.

In accordance with the data obtained, the dynamics of the fatal event were reconstructed and the cause of death was a result of drowning and massive hemorrhage due to propeller injuries.

Histological study of all organ and surfaces of mutilated skin to determine the phases of death (drowning and after mutilations of lifeless body?) must be required as forensic approach to similar cases.

The public implications of this event will be discussed especially in regards to criminal charges initiated against the owners of the desalination plant.

**Amputation, Underwater Propeller, Scuba Fishing**

## **G76 The Reconstruction of an Unusual Pedestrian Road Trauma Using Forensic Pathology and Forensic Veterinary Medicine**

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After this presentation, attendees will understand how cooperation between experts in pathology and veterinary medicine can solve a crime involving both humans and animals.

This presentation will impact the forensic science community by highlighting the fact that experts with knowledge of the anatomy of different animal species can contribute to solving a crime.

Traffic accidents have increased in the last decade. Pedestrians are most affected group among the victims of vehicle accidents. At autopsy, it is evident that the most common cause of pedestrian death is central nervous system injury, followed by skull base fractures, internal bleeding, lower limb hemorrhage, skull vault fractures, cervical spinal cord injuries, and airways compromise. The determination of fault can be assigned through the reconstruction of the dynamic of the road accident. The reconstruction of the accident is made through on-site investigation, a survey of the vehicle involved and victim examination.

A case study concerning a car accident where both humans and pets were involved is reported here. During spring 2011 an old man and his dog walking along the road were knocked down by a SUV. The driver stated that the man and his dog were walking in the middle of the road.

The investigation and the reconstruction of the crime scene were conducted by a team composed of a forensic pathologist and a forensic veterinarian.

During the investigation, the pedestrian and his dog were recovered on the brink of the road. It was determined that an autopsy should be conducted on the man and a necropsy on the dog. In addition, a complete inspection of the SUV was conducted.

The results of the autopsy and necropsy were compared following the histological analysis. This information was also used to reconstruct the collision.

Both the man and his dog showed lower limbs fractures with features indicating a collision on the dorsal side of the body. Furthermore, the man and

his dog showed cervical spinal cord injuries because of traumatic neck bend. The comparison between the human and the animal fractures and the autopsy results excluded allegation that the man and his dog were in the middle of the road. In fact, the biomechanics of injuries was suggestive of a collision that the pedestrian and his dog were struck from behind causing the propulsion along the roadside. This finding was supported by the presence dog hairs and fibers of clothing belonging to the man on the right front bumper of the car.

This unusual case was solved through the collaboration between the investigation using forensic pathology and veterinary forensic medicine.

**Forensic Sciences, Forensic Pathology, Forensic Veterinary**

## **G77 Wounds Vitality Evaluation in Decomposed Corpse: The Application of Monoclonal Antibody Against Hemoglobin Alpha Chain**

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After attending this presentation, attendees will become familiar with this immuno-histochemical method for wound vitality evaluation. This presentation will also identify the difficulty of diagnosis of injury vitality in putrescent corpses.

This presentation will impact the forensic science community by demonstrating a method that allows for the assessment of whether wounds are antemortem or postmortem in decomposed bodies. This can benefit the forensic community by adding to the methodology available for the identification of wounds vitality in decomposed bodies, especially in murder.

Forensic pathologists usually evaluate injury vitality through the identification of red blood cells in wounded tissues. Hematoxylin and eosin stain is one of the most commonly used histology stains to do this evaluation. Unfortunately this microscopic technique is not reliable in decomposed bodies, because erythrocytes are often lysed and not recognizable.

Although a lot of researches have attempted to use histological and immuno-histochemical methods to analyze the vitality of lesions of soft tissues, there is still no conclusive tools to determine whether a wound is antemortem or postmortem in decomposed corpse.

The inability to recognize red blood cells in histological samples with hematoxylin and eosin stain does not exclude the possibility to identify red blood cells specific components. In particular hemoglobin chains seem to be one of the most resistant elements to putrescence.

The immuno-histochemical method was used in some cases in which corpses were decomposed and where it was really hard to understand which lesions were caused antemortem and which were postmortem.

In this case, a homicide presented problems regarding injury diagnosis, because there were no existing tests that allowed the confirmation of the diagnosis of vitality. Gross observation and light microscopy examination of the samples (H&E histological samples) seemed to indicate that only some wounds had characteristics of vitality. That was important to determine because all the wounds were potentially dealt either to kill or to try to dismember the corpse. Application of monoclonal antibody direct against Hemoglobin alpha chain allowed the detection of the remaining red blood cells in soft tissues near some of wounds and not in others. Therefore, it could be identified, with some certainty, which wounds were inflicted in the attempt to hide the corpse. These results agreed with the case's reconstruction made by the police.

In another homicide case, when a female corpse was found about three months after the young lady disappeared, the immuno-histochemical method was used to determine which wounds were caused intra-vitam during the struggle and which were made postmortem.

This method was also used in a third case of homicide, when a decomposed body was found in a wooded area, about four months after the disappearance. In this case, it was important to understand which wounds were antemortem and which lesions were due to animal scavenging. The majority of the wounds took place in the neck and it was difficult to determine which were made by a blade and which by animal teeth, because of the decomposition of the skin. This method confirmed the hematoxylin and eosin light microscopy analysis and helped to determine which lesions were really meant to kill and which were not.

Preliminary data presented here suggests that this method will be of great use in determination of wound vitality, even greater number of samples will have to be observed to validate this method for forensic routine use.

**Wounds Vitality, Immuno-Histochemical, Decomposed Corpses**

## **G78 Estimation of Postmortem Interval: Determination of Hypoxanthine in Vitreous Humor by Mass Spectrometry**

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After attending this presentation, attendees will understand the potential contribution of a new method for confirmation and quantification of hypoxanthine in vitreous humor for determining the postmortem interval.

This presentation will impact the forensic science community by showing this a method and how it can increase the accuracy to calculate the postmortem interval, creating new possibilities, especially if a combined study is done taking into account the weight and the temperatures of the body and the environment.

Currently there are a number of procedures for determining the postmortem interval (PMI). Numerous methods have been used, some pseudoscientific, others merely descriptive, many without practical value, and most of them without any corroborating mathematical support. Others suffer from poor reproducibility and consequent uncertainty of their results exacerbated by failure to carry out field tests. Those using a methodology based on body cooling rate, although accompanied by statistical support, include certain clearly subjective factors.

The best results derive from the biochemistry of the vitreous humor (VH) and are based on the simultaneous determination of potassium (K) and hypoxanthine (Hx), correlating the value of both substances with postmortem interval. New statistical approaches have led to more precise and robust methodological processes which have helped clarify the interpretation of results. However, the analytical methodology for the determination of hypoxanthine has remained unchanged and therefore subject to certain limitations of validity and quantification in particular.

**Objective:** The goal of this presentation was to develop and fully validate a new method for confirmation and quantification of hypoxanthine in vitreous humor, applying liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

**Methods:** Vitreous humor samples, taken in fresh bodies, were subjected to solid-phase extraction. Chromatographic separation was performed using an (2.1x100mm, 3µM) analytical column, working in gradient mode, with acetonitrile and ammonium acetate 10mM (pH=4.5) as mobile phase. A tandem mass spectrometer was employed for the detection of hypoxanthine. Mass spectrometer worked in electrospray positive mode (ESI+) and MRM

mode, monitoring two precursor-product transitions:  $137 > 110$ , and  $137 > 119$  for identification and quantification of hypoxanthine. For method validation, linearity, precision, accuracy, limit of detection and quantification, matrix effect, extraction efficiency, and process efficiency were studied. All the studied validated parameters were within the accepted criteria, and LOQ of hypoxanthine was  $10 \mu\text{M}$ .

**Discussion:** It is a well known fact that hypoxanthine alone cannot provide an estimate of the time of death with any more accuracy than the potassium. However, it is necessary to use different variables to lend precision to the estimates. Hypoxanthine is one of the variables validated as an estimator for postmortem interval. The improvement in the statistical and analytical methods must be used in estimation methodology, reducing the confidence interval and increasing the accuracy and thereby provide the forensic pathologist with an objective validated method for postmortem interval estimation. With this method new studies could be made, for example taking into account the weight and the temperatures of the body and the environment.

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**PMI, Hypoxanthine, LC-MS/MS**

## **G79 Fire, Flies, and Wasps: PMI Estimation of Burned Remains**

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After attending this presentation, attendees will have novel information about the advantages of the practical applications of Forensic Entomology (FE) on burnt remains. In this particular condition FE could be the only method for PMI estimation.

This presentation will impact the forensic science community by validating the entomological approach in the PMI estimation on burnt bodies. The two reported cases, based on experiments, statistically confirm the applicability of the entomological approach based on the fly developmental rate for PMI estimation.

Different methods can be used in order to destroy a body or make its identification impossible: mutilation, burning, burying, acid dissolution, and concealment into concrete blocks. Burying and burning are perhaps the most common. Cases of burnt bodies found in different locations such as open fields, cars, and indoors have been reported regularly in literature. In burnt bodies, arthropod specimens may be the only tool useful in the estimation of the minimum time of death (mPMI). In fact, burning prevents the use of the classical thanato-chronological techniques (e.g., body temperature, livor, rigor, algor mortis, [K+]) for mPMI estimation.

This work deals with PMI estimation of two cases of burned bodies found during the summer of 2009, in Northern Italy, in the region around Milano. The two cases show several common characters, and in both cases the PMI estimation was (and could only be) performed using an entomological approach. This was possible thanks to data collected during experiments carried out during the previous years, in the same region (Vanin, Cattaneo, in prep). Insects were collected both from the crime scene and during the autopsy following standards and guidelines proposed by the European Association of Forensic Entomology (Amend et al., 2007).

Late June 2009, the burned body of a man was found along a river in a suburban area. The body was lying on the ground in a mostly skeletonized stage, with some areas of putrefied skin still surviving on forearms, lower limbs, and head which appeared dry due to heat and air exposure. The torso and hip regions had been completely burnt and presented as charred osseous remains. The Diptera samples were composed by larvae (maggots), pupae and puparia of three species *Lucilia sericata*, *Phormia regina* (Calliphoridae) and *Stearbia nigriceps* (Piophilidae). Several puparia of the blowflies were parasitized by the wasp *Nasonia vitripennis*. Among the Coleoptera, larvae and adults of *Dermestes frishii* (Dermestidae) were sampled, whereas all the other coleopters (Cleridae, Histeridae, Silphidae, Staphylinidae) were collected only at the adult stage. The entomological evidence allowed for

estimation of a mPMI between 24 and 31 days. This value was obtained adding the time of development of the host, *Phormia regina*, and the time of development of the parasitoid, *Nasonia vitripennis*.

In July 2009, the body of a woman was found in the garden of an abandoned house in a village. The body, almost completely skeletonized, with some signs of burning to the extremities of arms and legs, was lying on the ground in a supine position and was covered by a plastic sheet. The Diptera samples were composed by larvae (maggots), pupae and puparia of three species *Lucilia sericata* (Calliphoridae), *Hydrotea capensis* (Muscidae), and *Fannia canicularis* (Fanniidae). Several puparia of the blowfly *L. sericata* and one of the muscid *H. capensis* showed the typical holes of a parasitoid infestation. The parasitoid presence was confirmed by the emergence in the rearing chamber of several *Nasonia vitripennis* adults.

Coleopteran species were collected both at the adult and larval stages. In particular, several larvae and adults of the dermestid *Dermestes frishii*, *Necrobia rufipes*, and *N. violacea* were collected. Histeridae, Staphilinidae, Nitidulidae, and Tenebrionidae were collected only at the adult stage. The entomological evidence allowed for an estimation of a mPMI between 57 and 63 days. This value was obtained adding the time of development of the host, *H. capensis*, and the time of development of the parasitoids, *Nasonia vitripennis*. This estimation was confirmed by the developmental rate of the beetle *Dermestes frishii*.

These two cases demonstrate, in agreement with previous experiments (Vanin, Cattaneo in prep), that in burned remains the first waves of insect colonization (Calliphoridae, Muscidae, Sarcophagidae) are the same as in the case of “fresh” bodies. This supports the application of the entomological approach, based on the developmental rate, for PMI estimation. In contrast the composition of the community cannot be used due to the occurrence at the same time of insects belonging to different “colonization waves.”

It is worth mentioning that the presence of the parasitoid *Nasonia vitripennis* offers additional information for PMI estimation.

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**PMI, Burned Remains, Forensic Entomology**

## **G80 The Use of Forensic Messenger RNA (mRNA) Analysis to Determine Stain Age**

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After attending this presentation, attendees will understand the principles of forensic mRNA analysis and learn how it may be used as a method to determine the age of biological stains found at crime scenes.

This presentation will impact the forensic science community by proposing a novel method for determining the age of biological stains. The proposed method could prove crucial for forensic casework and could run parallel to the predominant DNA analysis method. Where DNA analysis is used to identify a suspect, mRNA analysis could be used to determine the age of biological stains, that in turn could place the suspect at the scene of a crime at a specific time.

RNA has been presumed to be very unstable, as one of its features is that it degrades rapidly as it is broken down by ribonucleases. In previous literature however; it has been shown that RNA may be extracted from biological stains up to 16-years-old. For stain age determination, it has been suggested that using a decay rate ratio, derived from two endogenous controls differentially expressed within the body fluid stain, should eliminate the effect of any external decomposition factors. This should then be expressed as a linear change in mRNA expression over time.

The majority of previous research concentrates on determining the age of bloodstains. In this study, blood and saliva stains were used. The goal of the study was to try to determine the age of these biological stains by isolating mRNA and quantifying the expression of housekeeping genes 18s, GAPDH, and ACTB also known as  $\beta$ -Actin. This study aims to demonstrate proof of principle and explore any limitations to a decay rate ratio, to see if there is any correlation between the age of a stain and the amount of genetic material present.

Blood and saliva samples have been collected on a regular basis over the past two years. Bloodstains were prepared using the finger prick method and depositing blood on to a sterile filter paper. Saliva stains were prepared by swabbing the inside of the cheek with a buccal swab. All samples were taken from healthy individuals. The samples were stored at room temperature and protected from sunlight. Both blood and saliva samples were extracted using Qiagen RNeasy Mini Kit with the appropriate modifications for each body fluid. The extracted samples then underwent RNA quantitation, DNase digestion, and reverse transcription using M-MLV reverse transcriptase and random hexamers. The resulting complimentary DNA (cDNA) was then quantified using absolute quantitation on a 7500 Fast Real-Time PCR System, using pre-designed Taqman Gene Expression Assays for human housekeeping genes 18s, GAPDH, and ACTB ( $\beta$ -Actin). Only two housekeeping genes were run together at one time, this was to compare the differences between all three, for example the decay rate ratio between 18s and GAPDH as well as 18s with ACTB and then GAPDH with ACTB. The two values from quantifying the two housekeeping genes were then expressed as a ratio. The proof of principle would be demonstrated by a linear downward expression of the two genes over time.

The results from initial experiments show that mRNA can be extracted from stains up to two years old. Further experiments are being carried out to obtain enough data to determine the proof of principle.

Future work will involve optimising the protocols and identifying the shortest stain age per body fluid as well as exploring the use of more housekeeping genes. With bloodstains being the current focus in most of the literature, experiments will be carried out to determine the expression levels of human housekeeping genes in semen to see if it is possible to identify the time of deposition. Identifying the time a semen stain was deposited in a sexual offence case could prove to be vital when trying to convict or exonerate an individual.

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#### mRNA, Stain Age Determination, Housekeeping Genes

## G81 Death Due to an Acute Cocaine Intoxication and a Man With a Precedent Violent and Aggressive Behavior: Is it an Excited Delirium?

*Gabriela Perilli, MD\*, Carmela Fiore, MD, Santina Cantatore, MLT, Margherita Neri, MD, PhD, and Irene Riezzo, PhD, University of Foggia, Department of Forensic Pathology, Osp D'Avanzo, Viale degli Aviatori, 1, Foggia, 71100, ITALY*

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The goal of this presentation is to show an unusual fatal case of cocaine related death in a long-term abuser with symptoms of excited delirium. Postmortem findings with a complete histological, immunohistochemical, and proteomics study (Western blotting) on brain and heart and toxicological analysis of cocaine distribution in fluids and tissues are discussed.

This presentation will impact the forensic science community by describing the importance of a careful crime scene investigation, toxicological, and histological examinations in every drug-related death in order to clarify the exact mechanism of the death.

Excited delirium is a state of mental and physiological hyperarousal commonly associated with the use of cocaine. The actual pathophysiology of patients who have been previously identified with signs and symptoms of excited delirium syndrome is complex and poorly understood. Patients suffering from excited delirium can be extremely hostile and violent. The fundamental manifestations are delirium with evidence of psychomotor and physiologic excitation. There are several different potential underlying associations or causes, including stimulant drug abuse especially cocaine. The literature shows a high risk of developing psychosis in psychotropic substance abusers; psychotic symptoms, and experience of paranoia and suspiciousness are reported during the use of cocaine.

**Case Report:** April 24, 2011, at 11.00 a.m., a woman called the police and said that she found her husband dead at his home. A crime scene

investigation was performed by forensic pathologist and police, who found the body of a 34-year-old Caucasian man in the kitchen. The man lived alone after a quarrel with his wife. The corpse was lying supine on the floor, wearing blood stained clothes. Close to the head, the floor was covered in blood. On the floor near the corpse, the following was found: a plastic handle length of 11.5cm; broken knife blade total length of 18.7cm; scissors with the blades length of 8.5cm; a bloodstained pillow; a spoon smeared with white powder; and, broken glasses. In the bedroom, next to the bed, a surgical patch and gauze were found. The entire house was in disarray, the furniture was upturned and broken. The man was known for his cocaine use as well as for his violent and aggressive behavior. The wife told to police about previous episodes of mental and physiological hyperarousal, anxiety, exaggerated startle responses, psychomotor agitation, destruction of inanimate objects, insomnia, and fatigue.

A complete autopsy was performed 48-hours after death. Multislice computed tomography (MSCT) was performed prior to autopsy. External examination recorded multiple superficial and parallel incised wounds to the neck and the flexor surface of left arm and were interpreted as hesitation marks. Abrasions, contusions, and lacerations were noted on the head and face and on both hands. Extensive loss of bilateral ala, destructive lesions of nasal septum, and hard palate perforation were observed. Section of the neck revealed thin hemorrhages in the subcutaneous tissues and muscles; but neck blood vessels were intact. Internal examination revealed cerebral edema, the brain weighed 1,522g, measured 18.5x16.5x7.8cm; the heart weighed 380 g, measured 12.5x11.5x5.5cm. The coronary arteries, the myocardium and the valvular apparatus were normal. Into the esophagus surgical gauze was found. An ectopic formation of pharyngeal tissue was showed. The other organs did not showed specific alterations except for an intense vascular congestion.

Routine histological investigations, applying hematoxylin and eosin staining, were performed on all organs samples. Brain samples presented subarachnoid and perivascular hemorrhages, vasogenic edema; lungs showed a massive pulmonary edema. Myocardium presented foci of fragmentation of entire myocells in anomalous cross bands formed by segments of hypercontracted sarcomeres and myofibrillar rhexis, pach-fibrosis, loss of the usual parallel alignment of myocardial cells, with a star-like disposition, defined as disarray. Between the disarrayed myocytes an increased interstitial matrix and a myofibrous hyperplasia of arterioles was observed. Liver showed a microvesicular liver steatosis. The ectopic neoformation of pharyngeal tissue was constituted by skin, fat and hair. The examination of other organs was unremarkable except for generalized hemostasis. To characterize and identify excited delirium "markers," an immunohistochemical study was confirmed using the Western Blotting analysis, performed on brain and heart tissue using the principal markers of programmed cellular death, apoptosis (NF- $\kappa$ B, bcl-2, bid, Smac/Diablo, TNF- $\alpha$ , Tunel assay), alteration of dopaminergic transmission (antibody anti - Tryptophan hydroxylase), Heat Shock Proteins, and  $\beta_1$  -  $\beta_2$  cardiac receptors.

Cocaine was detected in the subject's urine through immunoenzymatic screening. Toxicological analysis by solid-liquid extraction and gas chromatography/mass spectrometry (GC/MS) analysis, was carried out to identify and quantify the individual substances present in the biological fluids and organs. Cocaine concentration was as follows: blood 23.76mcg/mL/g, liver 332.95mcg/mL/g, urine 230.42mcg/mL/g, and kidney 49.38mcg/mL/g. Ecgoine methyl ester concentration was: blood 12.56mcg/mL/g, liver 18.37mcg/mL/g, urine 154.82mcg/mL/g, and kidney 4.82mcg/mL/g. Benzoylcegoine concentration was: blood 6.57mcg/mL/g, liver 58.34mcg/mL/g, urine 737.27mcg/mL/g, and kidney 3.28mcg/mL/g. Examination of nasal swabs was performed and it was positive for cocaine. No other drugs or alcohol were detected.

According to the crime scene data, autopsy, histological and toxicological findings, death was attributed to an acute cocaine intoxication in a subject with symptoms of excited delirium.

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#### Cocaine Abusers, Oxidative Stress, Excited Delirium

## **G82 Homicide by Strangulation With Postmortem Dismemberment of the Victim: Looking for Injuring Tool**

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The goal of this presentation is to provide insight into various aspects of this type of postmortem injury due to desmemberment, including new diagnostic means, which may help the forensic community with the identification of injuring tool.

This presentation will impact the forensic science community by increasing awareness of the importance of completing extensive examinations and tests to establish the type of the lesions and their vitality, the cause of death, the effect of drugs on the victim, the characteristics of the possible injuring tool. Many morphological and technical details concerning the victim, the used instruments for killing, and dismembering are presented.

Criminal dismemberment or mutilation, with regard to all legal autopsies, has an average frequency of 1:500. The classification of the different types of postmortem dismemberment/mutilation includes defensive, offensive dismemberment of bodies and necrophilous dismemberment. But also intravital decapitation or mutilation (e.g., transection during accidents) may occur. Victims may be of all ages, but primarily between 20 and 60 years old and generally are female. Apart from an unfavorable personal life situation at the time of the offense, essential predisposing factors were: poor integration in society and family; occupational problems; drug abuse; and, mental diseases. As the interactions between these factors differ in every single case and may be combined with other factors, the analysis of such homicides should always be based on the assessment of the individual case. Apart from rare cases of necrophilia, the victim of dismemberment is always a victim of homicide. Perpetrators are most commonly person close to, or at least acquainted with the victim, between 30 and 40 years of age. The psychiatric expertises often classify the perpetrators as "normal," infrequently as abnormal or even as psychotic. Dismemberment is performed at the site of homicide, generally in the place inhabited by the victim, the perpetrator or shared by both.

In cases of postmortem dismemberment, the main forensic task, apart from the identification and assignment of body parts to one or several individuals, is the determination of the cause of death and the identification of the injuring tool. Notably in cases of offensive postmortem dismemberment, the findings on the victim may be of special importance which also provide investigators with objective means to reconstruct the course of events and get to the killer.

**Case Report:** A man who was walking along a road of the suburbs was alarmed by the foul odor coming from a garbage bag and a rucksack along the road. When the plastic was cut human lower limbs were revealed among the decomposition. The alerted detectives found three other body parts wrapped in layers of plastic bags, also contained in two buckets, not far from the first discovery site. The head was intact, allowing classical identification, in accordance with other external findings (tattoos, scars, lesion of the left foot, etc.). The victim was identified as a 49-year-old woman, who was a known drug addict, affected by HCV infection. She had been seen alive two days prior wearing a dark shirt and black pants, and limping with a bandage on the left foot. The investigation inside the woman's home was unremarkable. The victim's former boyfriend was suspected of the murder.

The medical examiner performed an autopsy and opened the garbage bag revealing five dismembered body parts: head-chest, upper limbs from the shoulder down, and lower limbs from the buttocks down were cut off using a sharp object. The whole body, as well as the inner organs were very well preserved. Maggots were present on the hairs.

Multi-slice-3D-CT on the five body parts indicated the use of a sharp tool, especially on the basis of the witness marks produced on bone, typically found on the kerf wall or floor. A horizontal ligature mark encircling the neck was clearly visible, it was consistent with a rope. There were multiple petechiae in the skin. The autopsy showed also deep hematomas of the scalp, the rachis severed at L5 intervertebral disk level, and a necrotic ulcer at the left heel. The long bones displayed an extreme degree of splintering and bending breakage. Histological investigation revealed cerebral edema, acute emphysema, alveolar hemorrhages and edema, contraction band necrosis to the heart, hepatic cirrhosis and steatosis, dermoepidermal detachment, flattening of the epidermal layers, and massive hemorrhages in the cutaneous and subcutaneous tissues of the neck. No hemorrhages were visible in the cutis samples from limbs and chest cuts. Also the spinal cord and the cartilage of the intervertebral disk at L5 level didn't show erythrocytes. Immunohistochemical studies (Fibronectin,  $\alpha$ 1- antichymotripsin, antitriptyase, CD 31, and collagen type IV) performed on the cutis specimens collected from the neck lesion, chest and limb cuts confirmed the vitality only of the neck lesion. Toxicological analyses were positive for morphine, methadone, and EDDP. DNA typing by PCR amplification established that tall remains belonged to the same woman. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), an analytical technique used for the detection of trace metals, was performed on the cutis and cartilage specimens to determine characteristics of the sharp tool used to dismember. The death was classified as a homicide by ligature strangulation with postmortem dismemberment. Dismemberment and bone breakage was accomplished by cutting, blunt force, and, in the case of long bones, manual bending.

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### **Dismemberment, Ligature Strangulation, ICP-AES**

## **G83 Severe Hemorrhagic Pancreatitis in Forensic Autopsies: Report of Four Unequivocal Cases and Two Equivocal Cases**

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After attending this presentation, attendees will recognize factors from history, autopsy, and histology which may assist in identifying severe or hemorrhagic pancreatitis as a cause of death in forensic cases.

This presentation will impact the forensic science community providing case presentations showing how severe pancreatitis may be identified as a cause of death in forensic autopsies; differentiating unequivocal hemorrhagic pancreatitis from postmortem autolysis; and, providing consideration for difficult cases in which equivocal pancreatitis is a "red herring" which is not substantiated as the cause of death.

Pancreatitis is a natural disease which varies in severity from mild to fatal. Severe hemorrhagic pancreatitis may present at forensic autopsy, where it may represent the cause of death. Pancreatitis may be related to gallstones, to the abuse of alcohol, and to some prescription drugs. With this, toxicology is important in fully examining a death from pancreatitis. Pancreatitis may also represent a confounding or "red herring" contributor to death; it may also be suspected when it is not present.

A series of four forensic cases of fatal hemorrhagic pancreatitis from the Tidewater experience illustrates these findings, contrasted with cases which show how suspected pancreatitis (whether present or not) may present pitfalls to the medical examiner when assigning the cause of death.

Acute pancreatitis can arise from a variety of etiologic factors, but in most cases the specific cause is unknown. In some instances chronic

alcoholism or toxicity from some other agent, such as glucocorticoids, thiazide diuretics, or acetaminophen, can bring on an acute attack of pancreatitis. In about half the patients a mechanical obstruction of the biliary tract is present, usually because of gallstones in the bile ducts. Viral infections also can cause an acute inflammation of the pancreas. Acute hemorrhagic pancreatitis is an acute inflammation of the pancreas accompanied by purulent peritoneal fluid, the formation of necrotic areas on the surface of the pancreas and in the omentum, and, frequently, also accompanied by hemorrhages into the substance of the gland. Some of the postmortem findings are discussed and potential pitfalls in diagnosing acute hemorrhagic pancreatitis on postmortem examination.

**Case 1:** EL, a 32-year-old male with a history of hypertension and chronic alcohol use complained of recent onset abdominal pain associated with nausea and vomiting was found unresponsive in bed. He was pronounced dead in the emergency department. Medications included hydrocodone, cetirizine, sildenafil citrate, and metoprolol. Internal examination revealed red discoloration and hemorrhage into an enlarged and partially necrotic pancreas, distended pancreatic duct with inspissated material, 200cc of purulent peritoneal fluid, hepatomegaly (4,000g), and cardiomegaly (438g). Histology revealed extensive acute inflammation with patchy necrosis superimposed on diffuse fibrosis, inspissated material in the ducts, and peripancreatic fat necrosis. The liver showed marked steatosis. The cause of death was due to hemorrhagic pancreatitis due to acute and chronic alcohol use.

**Case 2:** EW, a 42-year-old male with history of chronic alcohol and recurrent chronic abdominal pain unrelieved by over the counter stomach relief medications became incoherent before having a witnessed collapse. He died in the emergency department. On internal examination, the pancreas has multifocal areas of hemorrhage, partially necrotic, a pseudocyst in the head, inspissated material in the ducts, and peripancreatic fat necrosis and saponification. There is 200cc of purulent peritoneal fluid, marked fatty change of the liver, and mild splenomegaly (184g). Histology reveals extensive mixed inflammation, necrosis, pseudocyst, severe fibrosis (especially in the tail), and hemorrhage of the pancreas. The liver shows marked steatosis and regenerating nodules with severe chronic inflammation. The cause of death is hemorrhagic pancreatitis due to chronic alcoholism.

**Case 3:** SF, a 66-year-old female with a history of chronic alcohol use and gallstones was hospitalized for a subdural hematoma associated with an unwitnessed fall. She died approximately two months later while in a rehabilitation center. On internal examination, the head and body of the pancreas were firm with inspissated material in the main duct and the tail of the pancreas contained an abscess surrounded by necrosis. There was also six liters of ascites, severe jaundice, cardiomegaly (500mg), splenomegaly (327g), gallstones, and a fatty liver. Histology showed broad tract of fibrosis in the pancreas with amorphous cellular material in the ducts and advanced necrosis with saponification. The cause of death was attributed to multi-organ failure due to acute pancreatitis.

**Case 4:** MC, a 55-year-old male with a history of gastroesophageal reflux disease, hyperlipidemia, and hypertension complains of abdominal pain for a day associated with vomiting and diarrhea before being found dead on the living room floor in his home by his mother. Medications include fenofibrate. Internal examination shows severe hemorrhage into the pancreas with focal areas of necrosis, moderate amount of retroperitoneal hemorrhage, 400cc of serosanguinous fluid in peritoneal cavity, 100c of serosanguinous fluid in the right pleural cavity, and peripancreatic necrosis. Histology reveals diffuse fibrosis of the pancreas with multifocal areas of hemorrhage. Toxicology is currently pending. The cause of death was attributed to hemorrhagic pancreatitis. (however, this is a recent case that hasn't been officially signed out yet).

**Case 5:** LW, a 33-year-old female with a history of pancreatitis, hypertension, gout, foot pain treated with tramadol and recent shortness of breath was found unresponsive at home and died in the emergency department despite resuscitation. Her medications included tramadol, temazepam, and zolpidem. On internal examination, the pancreas was unremarkable but there was hepatosplenomegaly (1854 and 217g), cardiomegaly (495g), pulmonary edema (1579g combined weight), small left

lower lobe pulmonary embolus, and a deep vein thrombosis in the right lower extremity. Histology showed a small focus of bronchopneumonia. When toxicology showed elevated levels of hydrocodone (0.07mg/L) and tramadol (5.2mg/L), death was attributed to the combined respiratory depressive effects of the two drugs.

**Case 6:** PC, a 49-year-old institutionalized woman with a history of mental retardation, recurrent renal stones, and gastric ulcer complained for two days of abdominal pain. Her medications included valproic acid for seizure disorder. She was treated with morphine and found unresponsive in a chair hours later. On internal examination, the tail of the pancreas showed sharply demarcated patchy areas of hemorrhage, and histology revealed diffuse fibrosis of pancreas with patchy areas of necrosis and hemorrhage. When toxicology showed elevated levels of morphine (0.2mg/L) and thioridazine (0.9mg/L), death was deemed due to overdose, although abdominal pain was probably due to acute pancreatitis, which is a recognized complication of valproic acid administration.

In these six cases, hemorrhagic to purulent fluid was always present in the lesser sac when hemorrhagic pancreatitis was the unequivocal cause of death. It was not identified when pancreatitis was either absent, or not the primary cause of death. Other findings common to fatal cases included extensive neutrophilic inflammation, and extension into peripancreatic fat. The absence of these findings suggests prudence in waiting for the toxicology results to determine the cause of death.

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#### **Hemorrhagic Pancreatitis, Forensic Pathology, Cause of Death**

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## **G84 DNA Persistence in Soft Tissues**

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After attending this presentation, attendees will become familiar with the relationship between Accumulated Degree Days (ADD) and DNA degradation and will have developed an understanding of how this relationship can be used in order to estimate the time frame during which it is still possible to amplify a complete DNA profile in temperate climates.

This presentation will impact the forensic science community by delivering results from a controlled decomposition experiment on animal muscle tissue providing a stimulus for the refinement of the guidelines for soft tissue collections in Disaster Victim Identification (DVI) cases, as well as discussing the feasibility of using DNA degradation as an alternative means of assessing the postmortem interval (PMI) of decomposing remains.

DNA plays a vital role in the process of disaster victim identification; DNA analysis can provide the identification of an individual, aid the re-association of remains which are fragmented, and be of assistance in ongoing legal or medical investigations. In many cases, the collections of samples for DNA based victim identification needs to be part of the first response plan. Depending on the condition of the body, Interpol has given recommendations on which samples are best to extract for DNA typing. Blood and buccal swabs are the preferred source for DNA from non-decomposed cadavers; however, where neither blood nor buccal cells are available other samples need to be collected. The Interpol recommended sample for decomposing remains is compact bone, whereas the use of muscle tissue is only proposed in cases of non-decomposed, mutilated remains. However, what is regarded as the onset of decomposition is not further specified. While it is undisputed that DNA in bone and teeth experiences greater protection against degrading environmental influences and hence exhibits superior preservation to DNA in soft tissues, the processing of hard tissues for DNA analysis can be quite labor intensive and time consuming. Depending on the rate of DNA degradation in soft tissues, it could be possible to utilize muscle tissue for DNA based identification in DVI cases for a longer time postmortem, which could significantly increase the sample throughput and speed up the identification process.

The decomposition of tissues is a process dependent on accumulated temperature rather than time itself. Accumulated Degree Days (ADD), the

cumulative total of daily average temperatures, are successfully being used as a quantitative measure to estimate the PMI in the fields of forensic entomology and anthropology. Recent research has also suggested ADD to be a measure of predicting DNA degradation in soft tissues.

The present study monitored DNA degradation in muscle tissue of common rabbits (*Oryctolagus cuniculus*) and domestic pigs (*Sus scrofa*). This research was conducted at the Taphonomic Research in Anthropology Centre for Experimental Studies (TRACES) in the Northwest of England. A total of 120 tissue samples were cut into pieces weighing 5g and placed into open 50ml plastic tubes. They were suspended on a mesh plateau about 3cm from the bottom of the tube into which a hole was drilled. This allowed rain water to drain, preventing the samples from becoming submerged. Thirty samples of each species were left open to insect access and 30 were covered by a mesh preventing insect activity, accounting for four different experimental groups. Tissue samples were collected in triplicates per experimental group roughly every 50 ADD and frozen immediately after collection in order to stop any enzymatic or bacterial DNA degrading activities. DNA extractions were carried out using a standard blood and tissue Kit, according to the manufacturer's protocol. The extracted DNA was then visualized on a 1.5% (w/v) agarose gel and quantified fluorometrically using a dsDNA assay kit. The samples were genetically profiled using a species specific PCR multiplex (4-plex) which simultaneously amplifies genomic DNA amplicons of 70 bp, 194 bp, 305 bp and 384 bp.

Results show that the total amount of extractable DNA of all samples decreases from death to around 200 ADD (this interval broadly equates to two weeks in a temperate environment, e.g., 14-15°C) and then exhibits a continuous increase. This can be accounted for by an increase in foreign bacterial DNA which coincides with the tissue samples losing their physical structure and integrity. There is no statistically significant difference between the different species ( $p=0.33$ ) or samples to which insect access was enabled and those to which it was not ( $p=0.93$ ).

Partial profiles can be obtained up to 200 ADD, which correlates with the minimum amount of extractable tissue DNA present. Increasing amounts of foreign DNA outcompete the tissue-specific DNA after this point. Studies at the University of Central Lancashire show that full profiles can be obtained up to 173 ADD for individual tissue samples.

The initial results of this study indicate that, depending on the climate, a full DNA profile can still be obtained from muscle tissue after several days. These results suggest, that the current guidelines for soft tissue sampling in DVI cases are very conservative, and the time for victims to be identified could potentially be significantly reduced by expanding the postmortem interval in which to sample muscle tissues.

**DNA Persistence, Accumulated Degree Days (ADD), Disaster Victim Identification (DVI)**

## **G85 Association of Chronic Methamphetamine Use and Idiopathic Pulmonary Arterial Hypertension**

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After attending this presentation, attendees will gain insight into the prevalence of pulmonary hypertensive changes in a metropolitan methamphetamine/amphetamine user population, and learn the implications of this finding on cause and manner of death determination.

This presentation will impact the forensic science community by evaluating the association between methamphetamine/amphetamine use and the development of idiopathic pulmonary hypertension (IPAH) in the forensic setting and increases awareness of this potential relationship in the forensic community. Identification of increased rates of IPAH among methamphetamine users would provide the forensic community with further insight into a potential mechanism of death in this population, especially in

autopsies showing minimal cardiac and cerebral pathology, very low methamphetamine levels in the blood, or in cases with only anecdotal evidence of methamphetamine use and no other identifiable cause of death. Additionally, identification of IPAH in decedents with no known risk factors might suggest the need for additional toxicological studies to include evaluation for exposure to amphetamines/methamphetamines or other stimulants. In the clinical setting, knowledge of the increased risk for IPAH among methamphetamine users would alert physicians to consider this disorder as well as to screen for amphetamine/methamphetamine use in IPAH patients with no identifiable risk factors.

Increased rates of amphetamine use are reported among IPAH patients with no other risk factors. Indeed, one study found that patients with pulmonary hypertension and no recognizable risk factors were 10 times more likely to have a history of stimulant use than patients with known risk factors. Methamphetamine was the most commonly used substance among IPAH patients in this study. Case reports of IPAH in association with illicit stimulant use, including methamphetamine and cocaine, have recently been published. Additionally, there are some reports in the literature suggesting a link between fatalities associated with stimulant use and IPAH. To date there is minimal data linking the development of IPAH to chronic methamphetamine use and even less published on the gross and histologic findings of pulmonary arterial hypertension at autopsy.

The pathologic effects of chronic methamphetamine use are incompletely understood. Given the similarities between cocaine and amphetamines in mechanism of action, it is plausible that methamphetamine and cocaine users would develop similar cardiopulmonary sequelae. There are many reports describing the cardiac and cerebral effects of methamphetamine. In contrast, there is limited discussion in the literature on pulmonary pathology in chronic methamphetamine users. Most reports have highlighted the association of methamphetamine with acute pulmonary edema and/or development of pneumonia; very few studies have specifically evaluated for histologic changes of IPAH in methamphetamine fatalities. There are, however, multiple reports of pulmonary hypertension in association with prescription amphetamine use, and methamphetamine likely produces some of the same adverse effects as prescription amphetamines.

The case records of Office of the Medical Examiner, City and County of Denver identified 166 deaths where methamphetamine was detected and 10 deaths with amphetamine alone detected on toxicological screen. The gross and histologic findings of these cases will be evaluated retrospectively to determine if evidence of IPAH is present. Correlation with the cause and manner of death, toxicological analysis, and where possible the length of use and route of administration of methamphetamine and/or amphetamine use will be discussed. Where available, medical records will also be reviewed for evidence of a prior history of pulmonary disease, specifically IPAH. The proposed mechanisms for the development of arterial changes in the lungs will be discussed.

**Methamphetamine, Pulmonary, Hypertension**

## **G86 Crossing Through the Borderland — Fatalities Due to Attempted Illegal Crossings Between the Mexico and United States Border Along the Rio Grande River in Southwest Texas 2007-2011**

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After attending the presentation, attendees will be able to: (1) recognize the hazards faced by illegal immigrants while attempting to cross the border; (2) become familiar with federal laws regarding immigration and the scope and role of the United States Border Patrol; and, (3) recognize the most common causes and manners of deaths in those immigrants who die while illegally crossing.

This presentation will impact the forensic science community by shedding a light on the extent of the problem of illegal crossers in regard to identification, public health issues and the financial burden placed upon the counties in which they cross.

Thousands of immigrants from Central and South America, as well as Cuba, China, and other countries, attempt to illegally cross the Texas-Mexico border every year. Many are successful. Many are apprehended. A significant number die—succumbing to drowning, heat stroke, trauma, and natural death. Due to the vast amount of ranchland along the southwestern border of Texas, many succumb to the effects of the environment as they walk for days in extreme environmental conditions without adequate food, water, or other supplies.

During a five-year period from January 2007 through December of 2009, the Webb County Medical Examiner's Office has examined the remains of over two hundred illegal crossers. Although the most common cause of death in the earlier years was drowning, the majority of deaths that are seen now are due to heatstroke and dehydration. Other causes seen include rattle snake envenomation, blunt force injuries (pedestrians and motor vehicle drivers and passengers), and natural deaths due to heart disease.

Not all illegal crossers who die are sent for autopsy. The Webb County Medical Examiner's Office services seven additional counties that are under the jurisdiction of Justices of the Peace. Because of the cost associated with sending these individuals for autopsy, many are identified based on rudimentary means and returned to their families or buried at the counties' expense in pauper graves. Although Texas law mandates that all unidentified deaths have DNA submitted for analysis, compliance with this law is low in the smaller rural counties.

All unidentified crossers that are sent for autopsy are radiographed, photographed, and autopsied, including dental charting. Information is entered into the National Missing and Unidentified Persons System (NamUs) and samples for DNA analysis are sent to the University of North Texas Health Science Center. Skeletal remains are sent for complete anthropological analysis by a forensic anthropologist.

Open communication with the Mexican Consulate's offices as well as the other consulate offices is imperative. During this period, the office has experienced between a 65% to 75% identification rate. Identified Mexican nationals are returned home at the expense of the Mexican government. Others do not fare as well as the funds are not available to assist the families. Both unidentified crossers, as well as those identified but unclaimed, are buried in a pauper's grave at the expense of the respective county. If a decedent is identified at a later date through DNA analysis, the decedent can be disinterred at the family's expense.

New and pending legislation, especially with health care reform, may change the numbers of crossers that are attempting to illegally gain entry into the United States. In the meantime, educating this population on the dangers associated with illegal crossing must continue and use all means in attempting to identify them so that they may be returned home must be used.

**Border, Illegal, Crosser**

## **G87 Retinal and Optic Nerve Sheath Hemorrhages Associated With Non-Traumatic Subarachnoid Hemorrhage: Two Cases of Terson Syndrome in Young Children**

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After attending this presentation, attendees will learn to recognize Terson syndrome and understand the direct relationship between rapidly increased intracranial pressure and ocular and optic nerve sheath hemorrhages.

This presentation will impact the forensic science community by demonstrating the direct relationship between rapidly increased intracranial pressure and intraocular and optic nerve sheath hemorrhages; and by reinforcing the need for thoroughness in investigation and postmortem medical examination of all sudden childhood deaths.

Ocular and optic nerve sheath hemorrhages in children are frequently attributed to inflicted traumatic brain injury; and when seen in conjunction with subarachnoid hemorrhage, are often mistakenly considered pathognomonic of Shaken Baby Syndrome. Two cases of nontraumatic subarachnoid hemorrhage with ocular and optic nerve sheath hemorrhage (Terson Syndrome), from Miami-Dade County are presented.

**Case 1:** A 2-year-old child was with his mother and her boyfriend when he complained of sudden ear pain and became hysterical. She was able to calm him and he fell asleep. When the mother returned to check on him, he was unresponsive. At autopsy diffuse subarachnoid hemorrhage, subdural hemorrhage and hematoma (75 grams) of the brain and spinal cord, and optic nerve sheath and retinal hemorrhages were identified. The cause of death was certified as Shaken Baby Syndrome with the manner recorded as a homicide. Subsequent investigation and review of the autopsy material revealed an intracerebellar hemorrhage with numerous, irregular blood vessels consistent with a ruptured arteriovenous malformation; and the death certificate was amended.

**Case 2:** An 8-month-old child whose parents observed her to be weak and lethargic with increased crying and loss of appetite for approximately two days. She was seen by her primary care physician, diagnosed with a stomach virus and sent home with instructions for supportive care. Her parents became concerned when she did not improve, and she was taken to the hospital. A preliminary CT scan showed basilar subarachnoid hemorrhage with possible mass effect and a ventriculostomy was placed. Despite maximum support, she died later that evening. At autopsy a ruptured giant aneurysm of the posterior communicating artery with diffuse basilar subarachnoid, retinal, and optic nerve sheath hemorrhages was identified.

Initially described in the early 20<sup>th</sup> century, Terson Syndrome referred to vitreous hemorrhage associated with subarachnoid hemorrhage. Today, the definition includes any degree of intraocular hemorrhage associated with intracranial hemorrhage and rapid elevations intracranial pressure. Although the findings in both of these cases mimic those described in cases of inflicted traumatic brain injury, they illustrate the importance of first excluding a natural disease process and of thorough examination of the brain in all pediatric cases.

**Terson Syndrome, Retinal Hemorrhages, Non-Traumatic**

## **G88 Polymorphic Salivary Glycoproteins Recognized by the Carbohydrate-Binding Protein Peanut Agglutinin**

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The goal of this presentation is to characterize inter-individual variation (polymorphisms) in a subset of salivary glycoproteins that bind to the galactose binding protein peanut agglutinin. This research was undertaken because there is: (a) lack of knowledge on buccal cell glycoproteins; and, (b) characterization of both buccal cell and salivary fluid glycoproteins will facilitate testing these proteins as biomarkers for pathological conditions and forensic applications such as indicators of exposure to toxins and identity testing through protein polymorphisms.

This presentation will impact the forensic science community by showing how lectins such as peanut agglutinin may be used to detect and

characterize salivary fluid and buccal cell glycoproteins. The identified glycoproteins may be further tested for applications as forensic and biomedical markers.

Methods used in this study included: (1) SDS gel electrophoresis and tests of Peanut agglutinin (PNA) binding to proteins electrophoretically transferred to nitrocellulose sheets (blotting); (2) treatment of samples with the enzyme neuraminidase which removes terminal sialic acid from oligosaccharides; (3) microtiter plate binding assays; and, (4) fluorescence microscopy of buccal cells by the use of fluorescent PNA.

An initial analysis was performed to determine the nature of salivary glycoproteins that might be recognized by the galactose-binding protein peanut agglutinin (PNA). Different samples of cell free salivary fluid were analyzed by electrophoresis and blotting with PNA. A protein of molecular mass around 150,000 Daltons bound to PNA in six out of eight salivary fluid samples. This approximately 150,000 Dalton protein had slightly different electrophoretic mobilities in the various samples, being close to 150,000 Daltons in two samples, greater than 150,000 Daltons in three samples and less than 150,000 Daltons in one sample. In the one sample, this approximately 150,000 Dalton protein consisted of two closely spaced bands. The staining intensity of this protein also varied among samples suggesting that different amounts of this protein bound to PNA in different saliva samples. No proteins in human serum bound to PNA. In additional analyses, 45 salivary fluid samples were analyzed for PNA reactivity and 39 of the 45 samples contained the 150,000 Dalton PNA-reactive protein, but six samples were negative. Twelve of the positive samples reacted very strongly with PNA compared to the others. Thus in a total of 53 salivary fluid samples, 84.9% (45) contained a 150,000 Dalton PNA-reactive protein and 15.1% (eight) did not under native conditions.

In another analysis, three matched pairs of buccal cell preparations and corresponding salivary fluid samples from the same donors were compared for PNA-binding proteins. Buccal cells and their corresponding salivary fluid fractions contained proteins of similar electrophoretic mobility that migrated at positions of molecular mass between 200,000 to 150,000. The buccal cell PNA-binding proteins were slower in mobility and of slightly larger molecular mass than the corresponding salivary fluid proteins.

PNA binds to terminal galactose residues on oligosaccharides that are attached to glycoproteins by O-glycosidic linkages. Galactose is sometimes terminated by sialic acid (neuraminic acid sugar residues) which prevent binding of PNA. To further examine the nature of the 150,000 Dalton salivary protein, the binding of PNA was tested with and without treatment of electrophoretically transferred (blotted) salivary proteins with a *Clostridium perfringens* enzyme neuraminidase which removes sialic acid from oligosaccharides. For untreated samples of salivary fluid, only three out of eight samples had 150,000 Dalton proteins that bound PNA and one of these bound very weakly. After treatment with neuraminidase, seven out of the same eight samples had 150,000 Dalton proteins that bound PNA and the staining of the three samples that were previously reactive with PNA was increased. For a serum sample that was also tested in this experiment, no binding of PNA was observed without neuraminidase treatment, but after neuraminidase treatment very high molecular bands became reactive with PNA.

These results indicated that a salivary fluid protein of approximately 150,000 Dalton varies in reactivity with PNA because individuals vary in how much terminal sialic acid is present on oligosaccharides that are attached to this protein.

An experiment was performed to determine if inter-polypeptide disulfide bonds were involved in the structure of the 150,000 Dalton PNA-binding salivary fluid protein. Four different salivary fluid samples were subjected to SDS gel electrophoresis under non-reducing and reducing conditions and electrophoretic transfers of the gels were analyzed for PNA binding. No shift in electrophoretic mobility was observed for the 150,000 Dalton protein in reducing conditions compared to non-reducing conditions.

The results indicated that this protein did not form inter-chain disulfide bonds with any polypeptide that was large enough to cause a significant shift in electrophoretic mobility. The results also suggested that the 150,000 Dalton PNA-reactive salivary fluid protein did not contain extensive intra-

polypeptide bonds of a sort that would cause changes in electrophoretic mobility.

Two different samples of buccal cells were also analyzed by PNA binding after gel electrophoresis under both reducing and non-reducing conditions and electrophoretic transfer to nitrocellulose sheets. The PNA-reactive protein migrated as a very diffuse band whose mobility was the similar under both conditions. This result indicated that the PNA-reactive buccal cell protein did not form inter-chain disulfide bonds with any polypeptide sufficiently large to alter its electrophoretic mobility.

The presence and relative amounts of PNA-binding proteins in salivary fluid and buccal cell was also confirmed by microtiter plate binding assays which showed that PNA bound to both buccal cells and salivary fluid coated onto microtiter plate wells. PNA binding to salivary proteins produced higher absorbance readings than certain other lectins such as soy bean agglutinin and galanthus nivalis agglutinin which recognize different oligosaccharides than PNA. However other lectins such as artocarpus integrifolia bound in greater amounts than PNA to salivary proteins and produced higher absorbance readings. Fluorescence microscopy by the use of fluorescent PNA or streptavidin and biotinylated PNA confirmed that all buccal cells tested bound PNA to their surfaces.

From the results of this study, it was concluded that buccal cells and salivary fluid contain glycoproteins of molecular mass between 200,000 to 150,000 that vary in amount of terminal galactose and molecular mass among individuals. These proteins may be further examined for use as forensic and biomedical biomarkers.

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#### **Saliva, Glycoproteins, Biomarkers**

### **G89 Use of Proteins to Obtain Measure of Identity in the Absence of Usable DNA**

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After attending this presentation, attendees will have a greater appreciation of the potential use of proteins in developing random match probabilities. Attendees will also have greater insight into additional uses of proteomics in forensics.

This presentation will impact the forensic science community by exploring how DNA can be wonderful if it is present in the sample. In the absence of usable DNA, protein can be a potential source of genetic information. This presentation focuses on extracting genetic information from a protein sample after DNA has been degraded or is contaminated. This information can be used to develop random match probabilities.

The use of nuclear DNA to provide matches between a forensic sample and an individual has revolutionized forensic science. DNA matches may have less than a 1 in  $10^{13}$  probability of occurring randomly in the population. Unfortunately DNA is chemically and physiologically labile. In certain contexts, such as in hair shafts, degraded forensic samples, or in contaminated samples such as rape kits, amplification of DNA and subsequent DNA-typing is difficult or not possible due absence of usable DNA. Protein however can theoretically substitute for DNA: the sequence of amino acids is a record of the template DNA and incorporates in the primary amino acid sequence non-synonymous single-nucleotide polymorphisms (SNPs). There are 185,000 non-synonymous SNPs in the human genome (an average of nine amino acid changes per protein). 65,000 of these SNPs have a calculated allelic frequency based on genomic sequencing of bio-geographic populations. Many of these single amino acid polymorphisms (SAPs) can also be detected through tandem mass spectrometry. For this study it is hypothesized that SAPs will be detected in hair protein samples and that enough will be detected to provide a basis for calculating random match probabilities. To test this hypothesis three hairs from one individual were ground and digested with 20 $\mu$ g trypsin overnight. Three aliquots of 0.5% of the sample were then applied to a LC/MS/MS (UHR-qTOF) mass spectrometry instrument. A total of 18,154 peptides were detected, 1,694 of which were unique and not redundant. This corresponded

to 285 proteins, 120 of which contained a total of 291 unique and non-redundant polymorphic peptides. In addition, data from the initial application was submitted to the Robust Accurate Identification (RAID) portal operated by the Yu group of the Computational Biology Branch at the National Institutes of Health ([www.ncbi.nlm.nih.gov/CBBresearch/Yu](http://www.ncbi.nlm.nih.gov/CBBresearch/Yu)). These algorithms are able to search for SAPs. A total of 361 polymorphic peptides were identified using this method. When the results from both searches were combined, polymorphic peptides with corresponding allelic dbSNP frequencies from the Utah and Northern European populations were identified in 80 proteins. Using the product of all phenotypic frequencies the probability that one person would have that combination of markers was calculated at  $5.5 \times 10^{-5}$ , or one in 18,800. Using loci from 14 of these proteins the chance that one person would have that profile was calculated at  $5.8 \times 10^{-5}$  (or one in 17000). Both major and minor alleles were detected at 4 loci, two of which were identified using the RAID algorithm. These 14 loci provide a basis for developing random match probabilities using peptide information alone. Naturally use of these loci requires additional levels of scrutiny, such as comparison with synthetic peptide standards, confirmation of inferred SNPs using a SNP-chip, confirmation of the robustness of the analysis by repetition, and analysis with hair samples from additional individuals. Higher levels of discrimination are probable with the use of custom reference protein databases, which contain all known SAPs. The precise relationship between amount of hair and level of information, or sensitivity, also needs to be established. While not as genetically powerful as DNA-typing, proteotyping has the potential to significantly contribute to the probative information gained from protein forensic samples in the absence of usable DNA template. This methodology, developed for hair shaft protein, has the potential to be applied to all protein samples that are found in a forensic context.

**Single Amino-Acid Polymorphism, Mass Spectrometry, Random Match Probability**

## G90 Inborn Errors of Metabolism Explain a Suspected SIDS Case

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The goal of this presentation is to underline the difficulty of diagnosis of SIDS. According to San Diego SIDS diagnostic criteria, a clinical, anamnestic, circumstantial, and autptic investigation must be performed. In most of cases death remains unjustified after the diagnostic procedures and SIDS is unresolved. This presentation concerns a case of resolved SIDS, in which the postmortem investigation led to diagnosis of dilated cardiomyopathy (DCM) with endocardial fibroelastosis, associated to tubular hypoplasia of the aorta and inborn errors of metabolism.

This presentation will impact the forensic science community by showing the difficulty to diagnose clinical silent cardiomyopathy in children younger than one year old, and so an accurate clinical, anamnestic, circumstantial and instrumental investigation can lead to a timely diagnosis of DCM in life.

A 10-month-old female infant was born full term (41+2 gestational week) at a weight of 3,800g. She had no neonatal complications. She was exclusively fed with formula and her mother had familiar history of Celiac disease. At 4-months-old, growth retardation and several generalized convulsive seizures, without fever, appeared. Laboratory investigation and electroencephalogram were unremarkable. When she was 10 months-old, the infant reported recurrent convulsive crisis (twice a night) and she was admitted into the hospital, where, on clinical examination, she appeared a very small infant (weight 6720g, length 70cm, CC 43cm). Electroencephalogram revealed paroxysmic activity and decelerated rhythm on right occipital area. Laboratory blood values demonstrated reduced red

cells count, respiratory acidosis (pH 7.333, pO<sub>2</sub> 37.1 mmHg), increased liver enzymes. Celiac disease HLA haplotypes examination was negative. Aminoacids dosage in blood revealed above all high citrulline levels. The ECG showed inverted T-waves in all derivations. In the following days the infant returned home to continue anticonvulsant therapy, but in the night she had recurrent convulsive crisis and she was admitted to the Emergency Department for blue lips, generalized hypotony, and cardiorespiratory arrest. Cardio-pulmonary resuscitation was unsuccessful and the infant was declared dead.

The external examination was unremarkable. The internal examination revealed heart increased in size (7.5x7.5x3.8cm) and weight (161g). The left ventricle showed a marked dilatation with an anterior wall thickness of 1.2cm, a lateral wall thickness of 1.4cm and an interventricular septum thickness of 1.2cm. At the transverse section the myocardium showed white areas, extended from atrio – ventricular plane to apex, with increased consistence. The right ventricular chamber was dilated with increased wall thickening. The left and right ventricular endocardium was shining and brightness. The descending aorta showed a short tract of tubular hypoplasia with vascular constriction to a minimum vascular diameter of 0.2cm, at 2.5cm from the left subclavian artery. Macroscopical examination of other organs was unremarkable, although liver showed hepatomegaly (size 15x9x4cm and weight 256g).

Histological examination of the heart revealed diffuse fibrosis mainly interstitial and with an undulating aspect, and fields with patchy fibrosis, rare foci of contraction band necrosis, disarray and disappearance of myofibrils with intramyocardial oedema resulting in empty sarcolemmal tube and with any type of reaction (colliquative myocytolysis grade 1) in the subendocardial layers. Left ventricular examination revealed a diffuse and considerable thickening of the endocardium resulting from proliferation of fibrous and elastic tissue representing a diffuse subendocardial fibroelastosis. Liver histological findings were suggestive of diffuse microvesicular steatosis. Non-identified metabolic disease was characterized by the following laboratory data: citrulline 38 mmol/l (VN 9.50-33.61). The urinary acid dosage offered many alterations: methylmalonic acid 12 (VN <7). The complete aminoacidogram presented: taurine 202.763 (vn 38-127), aspartic acid 29.335 (vn 3-9), serine 185.407 (vn 92-181), cystine 5.645 ↓ (vn 25-62), ethanolamine 7.841 (vn 0-6). The urinary amino acid dosage presented: “aspartic acid 41.524 (vn 3-10), glutamine 66.581 ↓ (vn 74-197), glycine 96.764 ↓ (vn 114-445), valine 5.770 ↓ (vn 6-19), cystathionine 0.141 (vn 0-0), leucine 3.564 ↓ (vn 4-16), fenylalanin 8.754 ↓ (vn 11-28), 1-metyl-histidine 17.889 (vn 0-0), histidine 69.135 ↓ (vn 92-278).”

These data show the close correlation between cardiomyopathy and metabolic-genetic disease, supported by the presence of hepatomegaly and reduction in cranial diameters.

The dilated cardiomyopathy is a myocardial disorder characterized by dilated left ventricular (LV) chamber and systolic dysfunction that commonly results in congestive heart failure. DCM shows an incidence of 0.34 cases per 100,000 children and it represents the half of all the pediatric cardiomyopathies. Age younger than one year is the most common age at diagnosis of DCM. In the most severe cases, the affected children are clinically silent until they suddenly present signs and symptoms of heart failure (breathlessness at rest, orthopnoea, early onset fatigue, abdominal pain, pallor). DCM is prevalent with 66% in the first year of life and it is correlated with metabolic diseases in 9% of cases. The main characteristics of this pathology are: increasing of size and weight of the heart, expansion of cardiac cavities and fibrosis. Metabolic diseases cause many alterations that involve mainly the brain, the hearth and multiorgans deficit generically leading to hepatopathy, kidneys, ocular and cutaneous alterations.

In conclusion, the cause of death was a dilated cardiomyopathy associated with tubular hypoplasia of the first tract of descendant aorta in a small female infant affected by inborn errors of metabolism.

**Dilated Cardiomyopathy, Inborn Errors of Metabolism, Sudden Infant Death Syndrome**

## **G91 A Methodological Approach in Deep Venous Thrombosis Fatal Cases: Clinical Diagnosis, Therapy, Genetics, and a Histopathological Approach**

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After attending this presentation, attendees will see how clinical diagnosis of pulmonary embolism (PE) is notoriously inaccurate, with many cases either wrongly diagnosed (overdiagnosed) or missed (underdiagnosed), and autopsy is still considered as the diagnostic gold standard. The accuracy of antemortem diagnosis of pulmonary embolism is within the range of just 10–30%, so representing one of the most frequent missed diagnoses in sudden, unexpected death. Abnormalities within the gene loci encoding for natural anticoagulants (antithrombin, protein C, and protein S) and for fibrinogen have been shown to be rather uncommon risk factors for VTE. The goal of this study was to verify the systematic search for the most common genetic thrombophilias (Factor V Leiden (G1691A)) and FII ((G20210A) gene variants) and dating the thrombus.

This presentation will impact the forensic science community by showing how in a case of death from PE, autopsy dissection, documentation and studies concerning pulmonary emboli are relevant for the evaluation of such fatality. The criteria to determine the chronological changes in the venous sites of thrombosis are supported by many technologies and methodologies available to forensic scientists, but are unfortunately very rarely applied in daily judicial practice autopsy findings, histology and genetic studies are essential to analyze various thrombotic risk factors and etiologies; thus a reliable protocol is needed for their investigation. The review of polymorphisms associated with thrombotic disease has highlighted the considerable variability of clinical associations with the various polymorphisms. Histological age determination of thromboses is a valid aid to date the DVT phenomenon and the chronological changes of the thrombus, so the forensic pathologist can objectively determine the causal relationship between a previous trauma and the following fatal PTE episode.

Fourty-three fatal cases of pulmonary embolism as confirmed by postmortem examination are described. The selection was carried out on the basis of some criteria including completeness of patients' medical history diagnosis of certainty of the cause of death, identification of macroscopic thrombus, identification of the thrombotic site, availability of formalin-fixed paraffin-embedded tissues for genetic and histochemical studies. In all these cases postmortem examination confirmed the diagnosis of PE. PE was recorded as the cause of death only when the necropsy stated that embolism was the main contributing cause of death and when emboli were identified either in the main pulmonary trunk or in the proximal right or left pulmonary arteries formed from the bifurcation of the main trunk. Emboli found in the distal pulmonary arteries after further division of the right and left pulmonary arteries were included too. In each case, five tissue samples were obtained from lungs; one piece from each lobe. Cross sections in each segmental pulmonary artery were prepared and microscopically examined. The dissection of the deep veins of the pelvis and legs was performed to search for the starting point of venous embolism. In the venous sites of thrombosis, the histological assessment was performed in conjunction with the surrounding vascular wall of uncut blood vessel with at least three to six different transverse incisions. Pathologic features were estimated using histological sections stained by hematoxylin–eosin, (H&E), trichromic stains (Masson, Azan, Mallory, PTAH, Van Gieson) and Von Kossa for calcium

salts. Perl's stain for hemosiderin was used to confirm the presence of iron. Immunohistochemical investigation of thrombus and embolus samples was performed using polyclonal anti-fibrinogen antibodies, CD61, CD45, CD15, CD68. To investigate whether the FV Leiden and FII mutations increase the risk of fatal PE, we investigated their presence in pathology material. The spectrophotometric analysis of the quality and quantity of DNA which was extracted led us to choose the heart as the best specimen to be used as a reference sample. DNA was extracted from a total of 43 paraffin wax embedded tissue specimens of heart. For DNA extraction "Tissue and Hair Extraction Kit (for use with DNA IQ)" protocol was used. The spectrophotometric analysis of DNA was performed. DNA samples were genotyped by real-time quantitative polymerase chain reaction for factor V Leiden and FII A20210 alleles.

As a whole, 41 patients (95.3%) had at least one risk factor. Pre-existing symptoms are described just before fatal embolism in 18 (41.9%) out of 43 patients. In 18 out of 43 (41.9%) it was not possible to find the thrombotic site. In 24 out of the remaining 25 cases the involvement of the deep veins of one leg was shown; in one case the thrombus was localised in the inferior caval vein, 10 (41.7%) were iliac vein thromboses, seven (29.1%) femoral, two (8.3%) popliteal, three (12.6%) posterior-tibial, one (4.1%) anterior-tibial and one (4.1%) peroneal vein thromboses. In our cohort of patients, four (10%) out of 40 cases carried the 20210A prothrombin gene variant in heterozygosis. One (2.5%) out of 40 carried the Factor V Leiden (G1691A) gene variant in heterozygosis. Patients carrying these gene variants in homozygosis or carrying both were not present in our case-series.

This study strongly underlines the relevance of a complete methodological approach, integrating clinical data by means of autopsy findings and histological study. On the contrary, investigating common inherited thrombophilia is not warranted. This observation strengthens the concept that thrombophilia screening is indicated when VTE occurs in young subjects in absence of malignancy, major trauma or surgery.

### **Factor V Leiden, Fatal Venous Thromboembolism, Prothrombin**

## **G92 Ehlers-Danlos Syndrome Type IV Revealed by Sudden and Unexpected Death: A Novel Point Mutation in the COL3A1 Gene**

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The goal of this presentation is to describe the case of a 25-year-old man who presented with sudden death, consecutive to acute and extensive arterial dissection. Postmortem genetic analysis revealed heterozygosity for a novel point mutation in the COL3A1 gene leading to a diagnosis of Ehlers-Danlos syndrome type IV.

This presentation will impact the forensic science community by showing the importance of considering Ehlers-Danlos Syndrome (EDS) diagnosis when confronted with spontaneous arterial rupture and then of alerting the family members of this hereditary and potentially fatal condition. Many health professionals are not familiar with EDS type IV syndrome, which is sometimes mistaken for coagulation disorders, Silverman syndrome, or physical abuse in children. Vascular Ehlers-Danlos syndrome can also be confused with other types of EDS, Marfan syndrome or Loeys-Dietz syndrome in adulthood.

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders characterized by tissue fragility, excessive skin extensibility and joint mobility. Its prevalence in the general population varies between

1/10,000 to 1/25,000, with no ethnic predisposition. Type IV, also known as the vascular type represents 5% to 10% of all EDS types. It is an autosomal dominant disorder resulting from mutations in the gene for type III procollagen (COL3A1). The clinical diagnosis is made on the basis of four clinical criteria: easy bruising, thin skin with visible veins, characteristic facial features (acrogeria), and rupture of arteries, uterus, or intestines.

A 25-year-old white male died at home after sudden collapse and dyspnea. He had neither medical history nor cardiovascular risk factors except for tobacco consumption. Family members reported an addiction to cannabis.

A complete postmortem examination was performed. This man was 180cm tall and thin, and presented with dysmorphic facies (elongated face and acrogeria), elongated upper limbs and prematurely aged skin of the extremities, without evidence of recent trauma. The autopsy showed a massive hemo-pericardium of 200ml and a hemothorax of 330ml complicating a complete thoracic aortic dissection involving both the ascending and the descending aorta (type I of De Bakey, type A of Stanford). The dissection originated from the ascending aorta and extended into the abdominal aorta. The distal end of the dissection was situated in the superior mesenteric artery. Furthermore, aneurysms of both renal arteries and the celiac trunk without dissection were noted, with recent thrombosis in the left renal artery and the celiac trunk. Histological examination showed a complete aortic dissection localized to the media, the false channel being filled out by blood without any sign of organization or inflammation. There was also fibro-muscular dysplasia of the renal arteries and celiac trunk with thickening of the media due to hyperplasia, along with irregular arrangement of the smooth-muscle fibre. The diagnosis of EDS syndrome type IV was suspected and genetically confirmed. Molecular analysis of blood samples revealed heterozygosity for a causative mutation in exon 40 of COL3A1 gene. In research, this mutation has not been reported before.

The Villefranche classification identifies six clinical types of EDS, among which EDS type IV accounts for about five to 10% of cases. Complications were rare in childhood. Twenty-five percent of the patients had a first complication by the age of 20 years, and more than 80% had at least one complication by the age of 40. The mean life expectancy is between 48 and 54 years, most deaths resulting from arterial dissection or rupture with uncontrolled bleeding. The types of complications are not associated with specific mutations. Because of the autosomal dominant nature of this condition, family members should be informed of this genetic condition and submitted to genetic testing if they wish to. Based on this information, a particular check-up can be done with respect to obstetric complications and safety in everyday activities. Novel therapeutic modalities are under development.

**Sudden Death, Ehlers-Danlos Syndrome, Aortic Dissection**

## **G93 Maternal Congenital Antithrombin-III Deficiency in an Intrauterine Fetal Death**

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The goal of this presentation is to show how fetal autopsy and a careful placental examination as well as a complete genetic study, on both mother and fetus in this case, play a substantial role not only in identifying the cause of death and in serving justice, but also as a means of helping clinical and forensic practice.

This presentation will impact the forensic science community by showing how fetal autopsies should be performed following recommended protocol and should include a careful placental examination and genetic analysis.

Thrombophilia is a multigenetic disorder caused by an inherited and acquired defect and has been described as a predisposition to thrombosis.

There has been growing interest in thrombophilia and its connection to the pathogenesis of certain pregnancy complications (such as gestational losses) because of the prothrombotic state it creates. In normal pregnancy, thrombotic risk increases and has been characterized as an evolutionary advantage against severe blood loss after delivery and placenta separation. A recent hypothesis has suggested, however, that maternal thrombotic predisposition could interfere with the initial development of an adequate uteroplacental circulation and may result in the production of microthrombosis in the placental vessels. Massive perivillous fibrin deposition (MPFD) and the related lesion maternal floor infarction (MFI) are rare but serious causes of placental insufficiency, occurring in 0.03 e 0.5% of deliveries.

The presented case concerns about an intrauterine fetal death at 30-weeks gestation in a 24-year-old woman who was affected by congenital antithrombin-III deficiency, with previous family history of venous thrombosis and venous thromboembolism, and who was receiving anticoagulant prophylaxis during pregnancy. During her pregnancy, a reduction of physiological protein S and antithrombin were reported and the woman was subsequently treated pharmacologically by her gynecologist.

At 30-weeks gestation, the woman was admitted to the department of gynecology after noting a few days with no movement from her fetus. After a rapid clinical and echographic study showed a typical image of "Spalding" (overlap of the fetus's cranial bones), the woman was induced and then vaginally delivered the lifeless fetus.

A complete autopsy of the fetus was performed 48-hours after death. The external examination revealed the presence of maceration over up to 40% of the fetal surface. The autopsy excluded the occurrence of acute and significant abnormalities in all fetal organs.

A complete examination of the placenta (size: 16x15x4cm, weight: 560 grams) was performed after fixation in buffered formalin. Upon external examination, a series of voluminous, fluid-filled serous cysts were noted to be occupying a total area of 12x10x5cm. On coronal sections, the peripheral portion of the placenta showed multiple whitish-bluish, hard areas, 16x4cm wide. At the point of insertion of the umbilical cord, two cysts were observed, each 1.5x1.0cm in size.

The etiopathogenetic definition was outlined by histological examinations, which were performed on placenta tissue samples using haematoxylin-eosin (H&E), Trichrome stain and Perl's, and revealed an intraluminal thrombosis of placental vessels with signs of villa ischemia and massive perivillous fibrin deposition (MPVFD).

The intrauterine fetal death was consequently attributed to acute respiratory failure (hypoxia fetal) by placental thrombosis secondary to maternal thrombophilia. A careful placental examination as well as a complete genetic study, on both mother and fetus in this case, therefore played an important role in identifying the possible cause of intrauterine fetal death. These practices remain important so that such data may help to advise parents who are considering whether or not to consent to a postmortem examination.

**Intrauterine Death, Antithrombin-III Deficiency, Placental Examination**

## **G94 The Coming "Omics" Revolution and Forensic Pathology**

*Victor W. Weedn, MD\*, Office of the Chief Medical Examiner, 900 West Baltimore Street, Baltimore, MD 21223*

After attending this presentation, attendees will gain an appreciation for the quickening of genomics, transcriptomics, proteomics, and metabolomics research that will likely revolutionize clinical medicine.

This presentation will impact the forensic science community by increasing recognition and raising awareness of why the "omics" revolution is closer than generally appreciated and what this may mean for forensic pathologists.

The medical community has been hearing about the importance of clinical genetic testing for some the last couple of decades, but the promised tsunami has not materialized. Nonetheless, advances have progressed. The convergence of cheaper technologies, the ability to data mine the information,

and the greater understandings that have been achieved set the stage for transformative or disruptive new approaches to medicine. Specifically, the intersection of the informatics, robotics, microfluidics, and mass spectrometry has enabled the creation of vast data sets from single laboratories that are permitting ever deeper comprehension of integrative biologic systems. The human genome project began in 1990 and was completed in 2003 at a projected cost of \$3 Billion dollars. Today there are several commercial companies founded on the anticipation of whole genome sequencing for \$1,000.00 and the Archon Genomics X \$10M Prize has been recently offered for the first team to sequence 100 genomes in 10 days. Clinical efforts perhaps need only focus on the set of genes that comprise only a small part of the whole genome. Off the shelf gene chips can now analyze all 25,000 human genes. Clinical genetics laboratories are moving from single target assays to multiplex systems and high-throughput systems interpreted using massive parallel processing. In addition to DNA gene targets, the global expression of those genes thorough the messenger RNA in the transcriptome is now routinely tested and is more informative about what is going on in the cells because it is a measure of the gene regulation. Microarray chips have become cheaper, more ubiquitous and more consistent and robust. They are used in clinical labs now for leukemia classifications. The RNA is translated into proteins. The proteome is affected by post-translational processes, not seen in gene or expression arrays. Because proteins are present in higher concentrations, they offer some advantages to testing. Eventually, the proteins, as the work horses of the cell, result in metabolic functions which can be assessed through metabolomics methods. Through recognition of complex patterns, recognizable disease patterns emerge. Pathology, the study of disease, stands to be transformed by such interrogations. This has been recognized by the College of American Pathologists (CAP) and launched a multiyear campaign to transform the specialty and adapt to the new realities. The reintegration of clinical and anatomic pathology is a central concept of this effort. The CAP has created a Transformation Program Office which "facilitates, coordinates, and integrates Transformation-related activities across the College." As Osler famously stated "as goes pathology, goes medicine" and "personalized medicine" is a goal that is becoming possible and even, perhaps, affordable. Accordingly, pathology residents are increasingly being exposed to these newer diagnostic technologies. If the health of a live individual can be so thoroughly described through omics testing, then surely it will have an impact on the diagnostics of those who succumb to such maladies. Yet there has been little talk of such these in forensic pathology. Genetic channelopathy testing and pharmacogenomics have garnered some discussion. Should the possibility of a molecular autopsy be considered in our armamentarium? Could a blood sample distinguish between an MVA caused by an MI or an MI cause by a MVA? Should we continue to cling to a procedure perfected in the 18<sup>th</sup> century as the main diagnostic tool?

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#### **Omics, Transformation, Genomics**

### **G95 Microbial Analysis of Bitemarks by Sequence Comparison of Streptococcal DNA**

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After attending this presentation, attendees will have an appreciation of an alternative, objective approach to bitemark analysis.

This presentation will impact the forensic science community by demonstrating the feasibility of linking a suspect to a crime by comparing the streptococcal DNA derived from the teeth with that from a bitemark.

Human bitemark analysis can be a vital component in the investigation of violent offenses, providing crucial physical and biological evidence in criminal prosecutions. Variability in mechanical properties related to anatomical location, ageing and ethnicity of the skin undermine morphometric bitemark analysis. While the recovery of human DNA from bitemarks may provide extremely compelling evidence, the presence of enzymes, especially deoxyribonuclease I, in saliva compromise the recovery of exposed DNA.

The scientific rigor applied to the establishment of nuclear DNA analysis has highlighted the deficiencies in evidence underpinning other forensic disciplines, including bitemark analysis. Such inconsistencies now challenge the value and objectivity of morphometric bitemark evidence. Consequently, investigation in our laboratory pursued an alternative method of analysis, based on bacterial genotyping.

More than 700 bacterial taxa have been detected in the human oral cavity. The predominating species are of the genus *Streptococcus* and comprise the principal bacteria colonising the surface of the teeth. Streptococci exhibit extensive genetic diversity, which provides the premise for research aimed at exploring the forensic value of matching teeth to bitemarks by bacterial genotyping. Streptococcal profiles may be distinctive among individuals to the degree that genotypic comparisons of isolates from bitemarks and teeth can provide a correct match with a high level of confidence.<sup>1,2</sup>

The current research extends this approach by applying high-throughput sequencing to obtain streptococcal DNA sequences amplified directly from bitemarks and teeth. Comparison of the sequences from the two sample types was used to establish the probability of matching a bitemark to the teeth responsible.

With ethical committee approval, ten participants consented to producing self-inflicted bitemarks on the bicep region to transfer bacteria from the teeth to the skin, in a benign fashion. The area of skin to be bitten was swabbed immediately prior to the generation of the bite. The bite and anterior teeth of each participant were swabbed three hours following the generation of the bite. Skin, bite and teeth samples from each participant were processed to extract the bacterial DNA. This provided the template for PCR using streptococcal-specific primers for three different regions of genomic DNA, to evaluate which region offered maximal discrimination between participant samples. The PCR products were elucidated using high-throughput sequencing technology (GS FLX, Roche) and the sequences from each bitemark were compared to those generated from each of the ten teeth samples. Statistical modeling, using the proportions of overlapping identical sequences (i.e., those detected in both sample types), indicated the predictive power of each region of DNA to correctly match a bitemark to the teeth responsible.

The highest proportion of overlapping sequences occurred between a bite and the teeth responsible in seven, eight and nine out of ten combinations for the three respective DNA regions. No DNA fragments were generated from the unbitten skin samples indicating that all amplified products had originated from the teeth and not the skin.

Statistical modeling to assess the predictive value of each of the three DNA regions revealed that while two were capable of correctly matching a bitemark to the teeth responsible with 92% and 96% accuracy, the third achieved 99% accuracy.

In conclusion, these findings indicate a very high likelihood of matching bacterial DNA amplified directly from a bitemark with bacterial DNA from the teeth responsible, constituting an objective method for analyzing bitemarks in situations where the perpetrator's DNA cannot be recovered.

#### **References:**

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- <sup>2</sup> Rahimi, M., Heng, N.C.K., Kieser, J.A. and Tompkins, G.R. (2005) Genotypic comparison of bacteria recovered from human bitemarks and teeth using arbitrarily primed PCR. *Journal of Applied Microbiology*, 99, 1265- 1270.

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**Bitemark, Bacterial DNA, Streptococcus**

## **G96 Fatal Diabetic Ketoacidosis and Antipsychotic Medication**

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After attending this presentation, attendees will understand the relationship between antipsychotic medications and the development of diabetes mellitus, diabetic ketoacidosis (DKA), and hyperosmolar syndrome.

This presentation will impact the forensic science community by demonstrating the relationship between antipsychotic medication administration and diabetes, and identifying those decedents most at risk. This understanding may affect the way that these deaths are certified, not only in terms of cause, but also manner, depending on the jurisdiction.

Schizophrenic and bipolar patients have been found to have an increased predisposition to the development of type 2 diabetes mellitus, independent of psychotropic medication administration, and after adjusting for other known risk factors. Within this susceptible population, however, poor glucose control, exacerbation of pre-existent diabetes mellitus, and new onset diabetes also have been widely described in association with the administration of certain antipsychotic medications, primarily second generation (atypical) antipsychotics, but, to a lesser degree, chlorpromazine, a low-potency first generation (typical) antipsychotic. This association has not usually been described with haloperidol, a high-potency first generation antipsychotic. The ushering in of these newer second generation (atypical) antipsychotic medications has rapidly rendered first generation antipsychotics all but obsolete due to the extreme efficacy of the former in treating psychotic symptoms, coupled with their greatly diminished negative motor and sexual side effects relative to the older medications. Their potential for sequelae of glucose dysregulation, however, has become a topic of much clinical literature review and analysis with recommendations for patient selection and monitoring. Theoretical mechanisms of dysregulation include insulin resistance, decreased insulin secretion, weight gain, hypertriglyceridemia, and elevated low-density lipoprotein cholesterol; however, a scientific consensus around mechanism has not been reached. While the atypical antipsychotics include quetiapine, clozapine, olanzapine, risperidone, aripiprazole, and ziprasidone, those that are thought to carry the highest risk for such metabolic side effects are clozapine, olanzapine, and quetiapine, with risperidone having the lowest.

In forensic practice, fatal diabetic ketoacidosis is the initial presentation of diabetes in some of these patients, who fall under the purview of the medical examiner due to their often precipitous and out-of-hospital deaths. This is the first large series describing such fatalities. Seventeen deaths were reported due to diabetic ketoacidosis in psychiatric patients treated with second generation antipsychotic medications.

Death certificates and toxicology data from January 2005 to December 2009 were searched for instances of diabetic ketoacidosis and hyperglycemia. The medical examiner records were reviewed which included the autopsy, toxicology, police, and medical examiner investigators' reports. Of all persons with DKA-related deaths (what is denominator/), 17 had a psychiatric history and were under current therapy with a second generation antipsychotic medication. The cause of death, contributing conditions, age, race, sex, co-morbidities, toxicology results, and BMI were extracted. Postmortem toxicologic analysis was performed on all decedents by the Forensic Toxicology Laboratory at the Office of Chief Medical Examiner. The decedents ranged in age from 32 to 57 years (average 48 years). There were fifteen men and two women. There were eleven Black, four Hispanic,

and two White decedents. In all 17, the immediate cause of death was DKA. The psychiatric disorders included: ten schizophrenia, three bipolar/schizophrenia, two bipolar, and two major depression. The most frequent atypical antipsychotic medications were quetiapine and olanzapine (six each) followed by risperidone (three). Only one of the 17 had a medical history of diabetes. The decedent's BMI ranged from 20 to 60 (average 33). In 10 of these deaths, the use of the antipsychotic medication was recognized as the cause of death and the psychiatric disorder and/or medication were reflected in the cause and manner of death statements.

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**Diabetes, Antipsychotic Medications, Fatal**

## **G97 Initial Studies Into Effects of Moderate Heat on Soft Tissue and Bone**

*Karl Harrison, PhD, Cranfield University, Defence Academy of the United Kingdom, Shrivenham, SN6 8LA, UNITED KINGDOM; and Brooke L. Webster, MSc\*, and Victoria Martin, MSc, Cranfield University, Shrivenham, SN6 8LA, UNITED KINGDOM*

After attending this presentation, attendees will acquire valuable insight into the response of soft tissues, specifically brain tissue, to relatively low temperatures that may dominate in protected areas within modern compartment fires. They will observe that these soft tissues can be preserved through carbonization while still maintaining a degree of chemical structure therefore viable for use in further forensic analysis, e.g. tissue identification, PMI, etc. This research also describes the application of modern fire techniques being used in an archaeological setting.

This presentation will impact the forensic science community by providing proven information that brain tissue can maintain chemical structure after visual carbonization of the material has occurred, it is evident that these types of samples are viable for use in further forensic research into the reaction of postmortem human remains to a burning event. This method of preservation can also potentially impact future archaeological methods of analysis when dealing with burned human remains.

This study uses evidence from the archaeological record to initiate experimental observations of the behaviour of brain tissue when exposed to moderate temperatures that might be expected at a low level within a modern compartment fire. The site of Çatalhöyük in central Turkey preserves remains of an extensive Neolithic settlement that has been noted for the preservation of the remains of burnt buildings, beneath some of which have been found the graves of buried individuals.

In one such burnt structure on the site, five sets of remains have exhibited evidence of intense carbonization, caused by heating in a reduced atmosphere whilst the remains were buried. The remains of each individual showed varying levels of carbonisation to their skeletal structure along with revealing carbonised organic material within their crania that is thought to be charred yet preserved brain tissue. The assumption is that radiating heat from the burning of the overlying structure has caused these changes; however, the complete image of this burning event and the correlation between it and the condition of the remains is not yet fully understood.

The studies discussed here have attempted to model and recreate the burning event and its effects on the buried remains by using forensic fire investigation techniques to analyze the thermal characteristics of the building materials of the settlement and comment on the amount of fuel and duration of burning that the characteristics of thermal alteration might suggest. If it can be proved analytically that the carbonised organic material is brain tissue it could potentially be the oldest surviving human brain material yet identified, as well being one of the few subject to such a peculiar means of stabilization.

Studies conducted on skull fragments from the remains indicate the skull reached temperatures of less than, or equal to, 500°C and were exposed to this heat for an extended period of time. No existing research was found that considered the effects of such relatively low temperatures and it is generally assumed that brain tissue would not survive this, or any type, of burning event. Experimental burning of porcine brain material, coupled with

the use of micro-CT and FTIR analysis, were used to assess the capacity of brain tissue to carbonise at these temperatures over different durations of time and attempt to identify any remaining chemistry within the sample after this exposure.

The results of this work have not only succeeded in using the techniques of fire investigation to quantify the thermal energy required to create the necessary conditions to carbonise buried human remains, but in addition analysis of the preserved brain tissue has revealed preserved organic traces still present. This work not only demonstrates the adaptation of forensic techniques for archaeological applications, but also provides a valuable insight into the response of soft tissues to the relatively low temperatures that may dominate in protected areas within modern compartment fires.

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#### **Fire, Brain, Human Remains**

### **G98 Age Estimation of Wounds Using the Proximity Ligand Assay**

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After attending this presentation, attendees will learn how a novel immunohistochemical technique can improve the interpretation of histological samples from injuries.

This presentation will impact the forensic science community by showing how the proximity ligand assay has an advantage over other immunohistochemical methods by providing reactivity only when two marker proteins bind to each other, or are in close proximity, which may be an indication of a transient reaction after injury, not seen in uninjured tissue.

In the forensic pathology routine casework determining the time of infliction of wounds may be a critical issue. Conventional histological methods allow for a rough age estimation, but suffer from high imprecision. Platelet-selectin (P-sel) shows rapid dynamics in the early phase of an injury. Under normal conditions, it is stored in Weibel-Palade bodies in endothelial cells and in  $\alpha$ -granules in platelets. After an injury, a degranulation occurs in these cells and P-Sel is transferred to the cell membrane; however, only for a short time, implying that membrane-bound P-Sel will disappear within hours after an injury. Deciding to take advantage of the possible co-localization of P-Sel and the Platelet Endothelial Cell adhesion molecule (PECAM-1), abundant in the cell membrane of both endothelial cells and platelets, by applying the proximity ligand assay (PLA) technique. This assay uniquely produces a reaction only when two secondary antibodies are physically very close. Further, von Willebrand factor (vWF) is also stored in the same granules as P-sel. This co-localization should therefore be expected to produce a PLA reaction under normal conditions. Degranulation due to vessel injury should reduce the positivity when these cells release P-Sel to the cell membrane and vWF to the circulation. Other combinations of antibodies, to factors involved in the coagulation and complement systems, as well as some early inflammatory markers were also investigated. The positivity of P-Sel - PECAM-1 showed a discrete time window. PSGL-1 is the natural ligand to P-Sel when exposed as a receptor in the membrane. P-Sel- PSGL-1 also showed a limited, and earlier, time window. A number of reactions in the coagulation cascade were further examined. The reactivity of thrombin and fibrinogen showed a reactivity in the early phase of an injury, but the reactivity remained for a prolonged period. Antibodies against a number of other factors in the coagulation cascade were also examined, but the results were difficult to interpret. The methodology requires that each antibody shows a reasonable staining pattern in normal and injured tissue, and that their close co-localization occurs during a limited time period after an injury. Collagen III and Glycoprotein VI each showed a decent reactivity in normal tissue when applied separately, but still failed to produce a distinct reactivity in the early phase after injury when tested by the PLA technique. This may either be explained by a too large distance between the epitopes on

each protein not allowing for the antibodies to produce a reaction. Alternatively, it might indicate that these factors do not interact, and that observation would then constitute a novel finding. The appearance of coagulation factor complexes of various kinds showed variable time patterns that need further studies. Having stated that, the reactivity of the different antibody combinations of the coagulation and complement systems consistently produced a negative reaction in uninjured samples, as well as in injuries of older age. It is believed that this methodology, using suitable combination of antibodies, will improve the age estimation of injuries, and conclude that the technique can be used by unexperienced users, since the all-or-none response that this method provides allows for an easy interpretation.

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#### **Wound Age Estimation, Immunohistochemistry, Postmortem**

### **G99 Confined Space Asphyxia: An Unrecognized Hazard in a Plasma Fractionation Tank**

*Lisa A. Scheinin, MD\*, and Lakshmanan Sathyavagiswaran, MD, Los Angeles County Coroner's Office, 1104 North Mission Road, Los Angeles, CA 90033*

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After attending this presentation, attendees will understand how to recognize potential oxygen-deficient atmospheres in the workplace and the hazards they pose to rescuers as well as workers.

This presentation will impact the forensic science community by increasing awareness of potentially hazardous workplace situations that can lead to rapidly fatal deaths. This awareness, in turn, will allow for correct assessment of at-scene conditions by death investigators and aid the medical examiner in diagnosing the correct cause of death.

Confined spaces are often unrecognized as potentially lethal workplace hazards. Confined spaces are defined by the National Institute for Occupational Safety and Health (NIOSH) as spaces not designed for continuous occupancy by workers but large enough to allow persons to enter, and which have limited means of access; they also often have unfavorable natural ventilation. The Occupational Safety and Health Administration (OSHA) defines a "permit-required confined space" (permit space) as a confined space that has one or more of the following characteristics: contains or has the potential to contain a hazardous atmosphere; contains material that could engulf someone who enters; has inwardly converging walls or sloping floors that taper into a smaller area that could trap the entrant; or contains any other recognized safety or health hazard. Examples include tanks, tanker trucks, silos, pits, sewers, and underground vaults.

Confined spaces may not outwardly appear hazardous and may have been entered at other times without incident. They may contain flammable, toxic, or oxygen-deficient internal atmospheres. Oxygen-deficient atmospheres, i.e., those that contain less than 19.5% oxygen, can pose an immediate danger to life and should not be entered without NIOSH-approved self-contained or supplied-air breathing apparatus. In particular, atmospheres with less than 10% oxygen may cause extremely rapid loss of consciousness and death to those who enter them. Oxygen-deficient atmospheres can be caused by consumption of oxygen or its displacement by another gas.

The leading cause of death in cases involving confined spaces is asphyxia, usually from exposure to oxygen-deficient atmospheres. The victim often had not been properly trained and did not recognize or understand the hazards involved. More than half of workers who die in confined spaces were trying to rescue other workers.

A case history involving a worker at a pharmaceutical company who died inside a plasma fractionation tank after entering it inappropriately and without protective apparatus is presented. Two workers who attempted to rescue him were also injured, one severely. Death and injuries were felt to be due to an oxygen-deficient atmosphere of which the workers were most likely not aware. The case will serve as an illustration of the potential hazards of confined spaces to both workers and their rescuers. It will also assist forensic investigators and forensic pathologists in recognizing and correctly assessing such hazards during scene visits and death investigation.

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#### **Asphyxia, Oxygen-Deficient, Tanks**

## G100 Unknown Body Found in a Rug — Unraveling the Answers

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After attending this presentation, attendees will understand challenges in death investigations to determine identity, cause of death, manner of death, time of death, and place of death require cooperation between a wide range of different disciplines.

This presentation will impact the forensic science community by showing how the importance of cooperation between different forensic disciplines can determine the questions surrounding a death investigation.

**Introduction:** Death investigation seeks to answer several important questions—who, what, where, when, and how death occurred. This process often requires the concerted efforts of a wide range of specialized individuals. The gravity is even more pronounced in those cases which are suspicious for criminal activity. Death investigation is not directly related to the legal outcome of these cases, but does provide evidence which is used by the court of law in their independent assessment of the guilt or innocence of accused individuals.

**Case Presentation:** An unidentified, largely decomposed body was discovered wrapped in a rug and partially concealed in a public park in Wilkesburg, Pennsylvania in September 24, 2009. The circumstances of the discovery of the body, and the advanced state of decomposition with extensive insect activity significantly interfered with identification of the deceased; as well as determination of the time of death; the place that death actually occurred; and the cause and manner of death.

**Discussion:** The average person is unable to wrap themselves in a rug and tape the rug closed by themselves prior to their own death, suggesting that the deceased was placed in this position by additional person(s), who may or may not, have played a direct role in the death. At the very least, the actions of this additional person (or persons) led to the concealment of several points of interest within this investigation.

Identification of the cause of death could not be determined by autopsy, or toxicological studies of the physical remains. No bony injuries were visible either grossly or by radiological scans of the remains.

The identity of the deceased was successfully ascertained and required the efforts of a range of individuals. A chance event spared identifying features from decomposition on the left hand and wrist including a portion of a tattoo and a single intact fingerprint. The deceased had finger print impressions on record. Additionally, the identity was corroborated by forensic anthropology, and forensic dentistry. The details were complicated by incomplete dental records, lack of documentation of a wrist tattoo and a delayed missing persons report.

The time of death was investigated by the work of a forensic entomologist based on evidence found on the body and at the scene. The individual was last documented to be alive in February of 2009, and a missing person report was issued on July 4, 2009.

The location of death (and the eventual determination of both the cause and manner of death) required police investigation and trace evidence comparison. A vehicle was obtained with carpet fibers in the box compartment. These fibers were definitively matched to the rug in which the deceased was discovered and led to the residence of the owner of the vehicle. The residence contained a similar carpet with a portion removed which also matched the rug in which the deceased was found. A bullet hole was found in the floor area corresponding to the missing area of carpet.

**Conclusion:** The final report on the death involved a 36-year-old Black female found wrapped in a rug was determined to be due to a perforating gunshot wound of the chest with a manner of homicide. The identity of deceased was discovered, the place of death and an approximation of the time of death were found. The police investigation and trace evidence matching provided the final cause and manner of death.

The police report information on the case involves two men who hired a female sex worker. They claimed she robbed them and they re-acquired her, where upon she was shot. The body remained in the home for an unspecified period of time before she was taken to the park.

Multiple resources and specialists were required for the successful resolution of this difficult case.

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### Homicide, Identification, Trace Materials

## G101 Autopsy Findings in the Morbidly Obese

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After attending this presentation, attendees will gain a more detailed understanding of the spectrum of pathological changes in persons with a body mass index greater than 35kg/m<sup>2</sup> who have undergone medicolegal autopsy.

This presentation will impact the forensic science community by providing details of pathology and normality in a population of obese individuals who have died both of and with their disease.

Obesity is an increasingly prevalent problem in the United States, Australia and internationally. In both the U.Ss and Australia, in excess of 25% of the adult population are obese (body mass index, BMI, greater than 30kg/m<sup>2</sup>). In all, in excess of 60% of adults are presently either overweight (BMI, between 25kg/m<sup>2</sup> and 30kg/m<sup>2</sup>) or obese, and these levels have been increasing over the last decade. Obesity is linked to a wide range of chronic and acute disease, and previous studies have shown a strong association between obesity and premature death. However, these studies have almost invariably involved acceptance of clinical diagnoses on death certificates and in medical records, and are generally not based on confirmed autopsy observations. Autopsies are frequently not performed in cases of morbid obesity, with many reasons proffered, including a pre-existing assumption as to the cause of death, a general unwillingness to perform autopsies in such cases and a concern about the occupational safety and health of pathologists and mortuary personnel.

In this study, a cohort of 1,000 deaths reported for medicolegal examination in Sydney, Australia were retrospectively studied, in Class II (BMI 35 to 40kg/m<sup>2</sup>) and Class III (BMI greater than 40kg/m<sup>2</sup>) obese adult autopsy subjects. Full autopsies were performed in all cases, with histologic examination performed on representative tissues in all, and toxicology testing in relevant cases.

Of the 1,000 subjects, 59.2% were male, 40.8% were female, and the median age of the study population was 57 years (range 18 to 91 years). There was an inverse relationship between age at death and BMI. The median BMI in the study population was 38.74 and ranged from the minimum inclusion BMI of 35kg/m<sup>2</sup> up to 94.81kg/m<sup>2</sup>.

The manner of death was natural in 80.1% of cases, and the most common cause of death was cardiovascular disease in 49.1% of all cases, followed by pulmonary causes which accounted for 13.7% of all deaths. Neoplastic diseases were given as the cause of death in only 1.2% of cases. Homicide was the manner of death in 1.3% of cases and 4.4% were suicides. 14.2% of deaths were considered accidental. Positive toxicology was recorded in 46.9% of cases and drug toxicity was the direct cause of death in 8.8% of cases. Obesity was given as the direct cause of death in 2.4% of cases, with an additional 2.0% of subjects having obesity listed as an antecedent cause and 13.6% as a significant condition contributing to death. Cause of death was unascertained or undetermined by the autopsy pathologist in 3.0% of cases.

Obesity is known to have many serious effects on the cardiovascular system. Cardiac pathology in 88.8% of cases was identified, with significant coronary artery atherosclerosis the most common finding. Severe, potentially lethal coronary atherosclerosis was observed in 37.5% of cases. Paradoxically, and as reported previously, those cases with a BMI greater than 50 kg/m<sup>2</sup> were found to have less severe atherosclerosis than the less

severely obese. High rates of pathology were also identified in the respiratory system (87.7%) and in the liver (77.8%) with hepatic steatosis identified in two thirds. Renal pathology, predominantly nephrosclerosis and other forms of chronic renal disease, was observed in 60.5% of cases.

The autopsy investigation of cases of morbid obesity can provide important information to pathologists, law enforcement and public health authorities on the nature of this disease and events surrounding death. As probably expected, there is a high rate of lethal heart disease, and pathology of other major organ systems is very common, which together result in high rates of premature death. A detailed description and characterisation of the lethal form of this disease can only assist in designing effective death prevention strategies in a very common condition which is increasing in frequency. Finally, we note the low number of cases where obesity is given as a cause of death or a contributor to the death in this study – both pathologists and clinicians should be encouraged to include obesity in the cause of death formulation in those cases where appropriate.

#### Autopsy Pathology, Obesity, Sudden Death

## G102 The Genetic Identification of United Kingdom Calliphoridae – A Multi-Gene SNaPshot® Approach to Species ID

Helen Godfrey, MSc\*, and Judith A. Smith, PhD, University of Central Lancashire, School of Forensic and Investigative Sciences, Preston, Lancashire PR1 2HE, UNITED KINGDOM

After attending this presentation, attendees will understand some of the problems associated with current identification methods and the advantages of molecular identifications. The sequencing results of a multi-gene approach and the development of a SNaPshot® multiplex for species identification will be presented.

This presentation will impact the forensic science community by providing a novel approach to Calliphoridae species ID. The developed SNaPshot® multiplex will provide a more time and cost effective way of analysing samples compared to the traditional Sanger sequencing. Multiple regions are analysed simultaneously, whilst the short amplicons make it suitable for degraded and comprised samples frequently found in forensic cases.

A dead body is an attractive habitat for many insect species but it is the members of the Blowfly family (*Calliphoridae*) that are usually the first to arrive, using the body as an oviposition site. The stage of larvae found on a body can be a useful indicator of time since death, but in order for species specific life cycle data to be applied, accurate species identification is critical. Damaged, unviable or immature specimens can be difficult to identify morphologically and recent work has focused on the genetic identification of Blowfly species.

The aim of this study is to assess the potential of various genetic regions to differentiate between United Kingdom Blowfly species of forensic importance. Nine genetic regions, including both nuclear (ITS2, 28s rRNA, CAD, Bicoid and Elongation factor 1 alpha) and mitochondrial DNA (Cytochrome oxidase I and II, cytochrome b and 16s rRNA), have been sequenced for six UK species commonly used in forensic investigations (*Calliphora vicina*, *Calliphora vomitoria*, *Lucilia sericata*, *Lucilia illustris*, *Lucilia caesar*, and *Photophormia terranova*).

Existing sequences were downloaded from the sequence database and aligned. Primers were either previously published primers or manually designed based on the sequence alignments. Genomic DNA was extracted using a mini kit, from wild-caught specimens collected from nine locations throughout the United Kingdom.<sup>1-3</sup> PCR amplifications were performed as single plexes for each gene and purified before DNA sequencing. Sanger sequencing was conducted in-house using a cycle sequencing kit. Sequence reactions were ran on ABI 310 and 3500 genetic analysers and analysed using Sequence Analysis software v5.4 (Applied Biosystems).

Results show that while most regions are suitable for distinguishing between species, problems still exist when identifying closely related species such as *Lucilia illustris* and *Lucilia caesar*. Analysis revealed that the mitochondrial regions Cytochrome Oxidase I and II (COI and COII) and Cytochrome b are capable of distinguishing between all species examined. These regions exhibit higher levels of inter versus intra species variation making them ideal for species ID.

The nuclear markers appear more conserved, having levels of inter species variation. Each of the nuclear markers sequenced can only differentiate samples to the genus level, failing to distinguish between *Lucilia illustris* and *Lucilia caesar* at each marker (ITS2 – 400 bp, 28s rRNA – 2.2 Kb, CAD – 700 bp, Bicoid – 350 bp and Eflα – 1 Kb).

As the rapid identification of species is advantageous in forensic investigations, a SNaPshot® approach to species ID was investigated. SNPs from the following regions were chosen to differentiate between the six UK species: 28s rRNA (4 SNPs), Eflα (4 SNPs), COI (2 SNPs), COII (2 SNPs), Cytochrome b (2 SNPs) and 16s (1 SNP). Nuclear markers were included so that any possible hybridisation between species (with the exception of *Lucilia illustris* and *Lucilia caesar*) could be detected. This SNaPshot® multiplex gives a unique haplotype for each species. Each species can be differentiated based on between 4-12 SNPs.

#### References:

1. Z. Song, X. Wang, G. Liang, Species identification of some common necrophagous flies in Guangdong province, southern China based on the rDNA internal transcribed spacer 2 (ITS2), *Forensic Sci. Int.* 175 (2008) 17-22.
2. J. Stevens, R. Wall, Genetic relationships between blowflies (*Calliphoridae*) of forensic importance, *Forensic Sci. Int.* 120 (2001) 116-123.
3. F.A. Sperling, G.S. Anderson, D.A. Hickey, A DNA-Based Approach to the Identification of Insect Species Used for Postmortem Interval Estimation. *J. Forensic Sci.* 39(2) (1994) 418-427.

#### Forensic Entomology, DNA, Calliphoridae

## G103 The Microbial Ecology of Carcass Decomposition Between Habitats and Through Time

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After attending this presentation, attendees will have a better understanding of the role of microbial ecology that drives the rate and biological dynamics of decomposing remains during different seasons and between years. Attendees will also be introduced to a growing knowledge foundation on how this information can be used to better understand and predict the stages of carcass decomposition, with direct application to estimates of a minimum postmortem interval (mPMI) using microbial communities. Attendees will learn how changes in the metabolic use of different carbon sources by the microbial community during decomposition succession can be utilized to estimate the stages of decomposition.

This presentation will impact the forensic science community by providing an introduction to the potential use microbial communities in crime scene investigations.

Microbial communities are fundamental to decomposition ecology of carrion and human remains. Studies in both aquatic and terrestrial systems have shown that microbial communities follow a pattern of succession by metabolizing and modifying resources in a way that makes them usable or unusable to other subsequent colonizing organisms. While there have been studies describing the succession and diversity of microbial communities involved in carrion decomposition, none have evaluated their potential use for making estimates of the mPMI in criminal investigations. In this study an economical method was employed for understanding changes in environmental microbial communities using Biolog EcoPlates™, microarray plates with 31 different carbon sources that are differentially used by microbial communities, providing a metabolic signature that acts as a surrogate for the functional diversity of the communities being evaluated. These profiles are often called microbial community level physiological profiles (MCLPPs) and can be calibrated with temperature and genomic sequencing to provide ecological data for predicting the duration of body decomposition.

The objectives of this study were to describe microbial community metabolic changes during decomposition (i.e., succession) in two different natural settings during multiple seasons and between years. The hypothesis was tested that MCLPPs from decomposing remains, the soil beneath and 1m away would change during decomposition as a function of microbial community succession, and that these changes would vary depending on season and year, but not with habitat for the carcass communities. It was predicted that successional changes in MCLPPs could be validated with the 454-pyrosequencing of the microbial communities.

Microbial samples were taken from carrion (swine) (N = 3–9), the soil underneath (treatment soil) and 1.0m away from each carcass (control soil). To understand microbial community structure differences on the carcass, swabs of the buccal, urogenital and shoulder skin were evaluated, and MCLPPs were described using Biolog EcoPlates™. Three experiments were performed to test the above hypothesis: (1) seasonal decomposition of swine carcasses during 2009 in a rural forested lot (Habitat 1) in Ohio; (2) a decomposition experiment in July/August 2010 in a different rural forested lot (Habitat 2) about 25 km away from Habitat 1; and, (3) a repeated 2010 experiment in July/August 2011 to provide an assessment of annual variation in succession on swine carcasses. In the 2010 experiment, matched samples of each individual sample were taken and evaluated using the bacterial tagged encoded FLX amplicon pyrosequencing (bTEFAP) method with a pyrosequencing platform.

Using a tiered multivariate statistical analysis approach we found significant differences in the microbial communities both on the carcass and in the soil beneath and 1.0m away from carcasses, with significant differences in decomposition succession between seasons and habitats. There were not significant differences in MCLPPs among body regions or among replicate carcasses within a season, habitat or year, indicating consistency of MCLPPs among carcasses within the same habitat during the same time of year. However, within each season, habitat and year there were significantly different daily MCLPPs during decomposition that corresponded with established stages of decomposition described in the literature. Although more studies are needed to verify our findings, these results indicate that microbial metabolic profiles on carcasses have excellent potential for use in estimating stages of decomposition, and thus, time since death in localized habitat conditions and within specific timeframes. However, the data indicate that comparisons of metabolic profiles among locations or among seasons and years would be difficult or unrealistic. Validation and comparisons of MCLPPs with metagenomic sequencing is ongoing.

**mPMI, Period of Insect Activity, Nocturnal Oviposition**

## **G104 Blowfly Oviposition Dynamics on Liver Bait and Swine Carcasses Exposed at Dusk**

*Maureen Berg, BS\*, 300 College Park, University of Dayton, Science Center 211, Dayton, OH ; Andrew J. Lewis, BS, University of Dayton, Department of Biology, 300 College Park, Dayton, OH 45469-2320; Megan Shoda, MS, University of Dayton, 300 College Park, Dayton, OH 45409; and Tiffany Blair, BS, and M. Eric Benbow, PhD, University of Dayton, Department of Biology, 300 College Park, Dayton, OH 45469-2320*

After attending this presentation, attendees will better understand nocturnal and diurnal oviposition (i.e., egg laying) behavior of blowflies, and the possible implications to estimates of the minimum postmortem interval (mPMI) and period of insect activity (PIA). Attendees will learn how environmental conditions affect blowfly oviposition dynamics, and how this can impact insect colonization throughout decomposition.

This presentation will impact the forensic science community a broader view of decomposition ecology, specifically insect colonization, and allow the attendees to evaluate how to refine methods of estimating the PIA, thus a more accurate minimum postmortem interval.

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One aspect of forensic entomology concerns the use of arthropod evidence at crime scenes to estimate a mPMI based on the species that colonize and develop on the remains. The major assumption that blowflies do not oviposit at night can influence the length of the PIA and thus entomologically-based mPMI estimates. Previous field studies have indicated either an absence of nocturnal oviposition or highly reduced activity that may have been associated with artificial lighting. The objectives of this study were: (1) to test for nocturnal blowfly oviposition and evaluate the effects of different lighting conditions (i.e., artificial and natural) on oviposition using liver baits as a resource; and, (2) monitor oviposition on replicate swine carcasses exposed to the environment at dusk, in different habitats and temporally (i.e., three years).

In the first experiment (Liver Experiment), blowfly oviposition of liver baits was evaluated under experimental light treatments in wooded lot. We hypothesized that nocturnal oviposition would not occur, but initial diurnal oviposition would be correlated with abiotic conditions (i.e., temperature, humidity, precipitation). In a wooded habitat near Xenia, OH, from July-October, 2009, 35.0±2.0g pieces of beef liver bait (N=3/treatment) were placed under three artificial lighted conditions: high (6 lx), low (3 lx), and no light (0 lx). Different bait locations, 1m off the ground and on the ground, were also evaluated to understand the effect of height above ground on oviposition. Oviposition was monitored for 24 hours beginning with bait exposed two hours prior to sunset (determined by NOAA). At each time point, approximately 25% of collected eggs were reared for identification, while the remaining were weighed. A regression analysis for *Phormia regina* was developed to predict the number of egg/larvae from egg mass ( $F=1775$ ,  $df=43$ ,  $p<0.0001$ ,  $R^2=0.97$ ). Temperature and humidity were monitored.

From all treatments and replicates only 90 eggs were collected within two hours of sunset and no oviposition was documented during the nighttime hours. There were statistically significant effects of light ( $F=27.86$ ,  $df=3$ ,  $p<0.0001$ ) and height above ground ( $F=15.8$ ,  $df=2$ ,  $p=0.0004$ ) on diurnal oviposition in August 2009. August was significantly the warmest month as statistically determined using one-way ANOVA, with a mean nighttime temperature of 20°C. There was less diurnal oviposition during the other months when average nocturnal temperatures ranged from 10-20°C, while average nighttime temperatures <10 °C were associated with no oviposition

the following day. Further, the greatest diurnal oviposition was associated with high artificial light and location (height-above-ground) treatment effects.

In the second experiment, replicate swine carcasses were exposed to the environment two hours before sunset and monitored for oviposition as in the liver experiment described above. During the summer of 2009 this was done in a small forested lot using six replicate carcasses, while in 2010 and 2011 identical trials were conducted in a different forested lot about 25km from the first location and using four and six carcasses, respectively.

Similar to the liver experiment, little to no nocturnal oviposition was documented on most carcasses in 2009, 2010 and 2011; however, there was notable exception with one carcass in 2009. On an evening with temperatures only between 15-20°C and rainfall, active oviposition was observed by one female *Lucilia* spp. approximately 0.5 hours before sunset under 0 lux conditions in the mouth of one out of the six carcasses. These are environmental conditions that are never expected to be associated with blowfly oviposition and could be important to estimates of PIA (and thus mPMI) in criminal investigations. Although it requires additional study, it is reported here that there is approximately a 17% chance that early nightfall oviposition can occur during rainfall under relatively mild temperatures during mid-summer in southwest Ohio. Diurnal oviposition in these trials was similar to the Liver Experiment. In 2009, 2010, and 2011, oviposition occurred no later than sunset and no earlier than two hours after sunrise. The average humidity, temperature, and lux that initial oviposition occurred during the three years was 71%, 25°C, and 30lx, respectively. This study demonstrates that early sunset/sunrise oviposition by blowflies varies greatly even within the same habitat, and the variability due to location, temperature, humidity, and light should be taken into consideration when making estimates of the PIA and mPMI using insect evidence.

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**Phormia Regina, Oviposition, Decomposition**

## **G105 Invertebrate Species Diversity, Richness, and Evenness Indicate Delayed Colonization of Remains**

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After attending this presentation, attendees will be able to comprehend the utilization of multivariate statistical approaches for analyzing adult insect data associated with decomposing carrion in order to predict a delay in colonization (i.e. pre-colonization interval); specifically, adult insect species richness associated with decomposing human remains can be used to determine the pre-colonization and post-colonization interval and thus provide a more accurate minimum postmortem interval (mPMI).

This presentation will impact the forensic science community by showing how insects are commonly used to estimate the period of insect activity (PIA), which may correspond to minimum postmortem interval. However, forensic entomologists currently are not able to offer a quantified pre-colonization interval estimate. Consequently, estimates based on succession or development more accurately represent the mPMI which could be much shorter than the actual postmortem interval.

Adult primary colonizers, specifically blowflies (Diptera: Calliphoridae), are rarely used by forensic entomologists to predict PIA because of a high level of variation and unpredictable nature of utilizing a resource. The adults are most commonly used to confirm species identifications of larval specimens. Blowfly larval length and weight are almost exclusively used to estimate the age of a larva which can be used to predict the amount of time since eggs were laid on a body. The PIA consists

of two intervals: the pre-colonization and the post-colonization intervals. During the pre-colonization interval remains are made available in the environment, insects will detect and subsequently accept the remains. An oviposition event occurs which indicates the beginning of the post-colonization interval. Insect activity including insect succession during the post-colonization time interval has long been the cornerstone of forensic entomology. Current research focusing on the pre-colonization interval will better understand species interactions and mechanism controlling colonization of remains.

This study used passive insect-trapping methods to assess primary colonizer species diversity, richness, and evenness on three swine carcasses that initially had access to insects (ACC) and three swine carcasses that were excluded from insect access (EXC) for five days using insect exclusion cages. After five days, EXC carcasses were exposed to insects via removal of the exclusion cages. Insects were collected every 12 hours for both treatments of carcasses and then identified to the lowest taxonomic level. Ecological parameters including Simpsons index, Shannon-Weaver index, species richness and species evenness were analyzed with univariate and multivariate statistical approaches. Succession patterns of forensically important insect species were similar for ACC and EXC carcasses with *Phormia regina* being the initial and most abundant blowfly species followed by *Lucilia coeruleiviridis* and *Cochliomyia macellaria*. Coleopterans from the families Staphylinidae, Trogidae, Histeridae and Dermestidae were the next wave of insects utilizing the carcasses. Despite similarities between succession patterns with each treatment, Student's t-tests indicated that ACC carcasses had significantly fewer insect taxa than EXC carcasses exposed on each day insects. A multivariate approach was taken to determine any insect community differences between the ACC and EXC carcasses. Nonparametric multidimensional scaling analysis coupled with multiple response permutation procedure indicated a significant difference between ACC and EXC carcasses ( $p < 0.0001$ ).

These data are important because of their implications in forensic entomology. Mainly, if a body is excluded from insect activity for five days and insect collections are made using standard entomological evidence collection protocols there is a possibility that the PIA will be underestimated by up to five days. However, the the limitations of these data as only a single time frame was used to exclude insect was understood. More studies are ongoing to determine if these results would be consistent for multiple days of insect exclusion, but at this time collections of adult insect communities present at a body are promising as an indication of whether or not there has been a delay in insect colonization of a body.

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**Decomposition Ecology, Entomology, Delayed Colonization**

## **G106 Species Interactions Between Forensically Important Blowfly Species and the Invasive Hairy Maggot Blowfly (Diptera: Calliphoridae)**

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After attending this presentation, attendees will become familiar with species interactions of native and invasive calliphorid species observed at decomposing animal and human remains.

This presentation will impact the forensic science community by demonstrating the importance of understanding biological and ecological aspects of forensically important blowfly species commonly collected at the crime scene.

**Introduction:** Native to tropical regions of Australia and the Orient, the hairy maggot blowfly, *Chrysomya rufifacies* (Macquart), was first reported in the New World in 1978 (Costa Rica) and then in the United States in 1980

(Texas). As an aggressive species, *Chrysomya rufifacies* has disrupted the natural balance of insect communities associated with corpses and carcasses in the continental United States. First-instar larvae are necrophagous, while second and third-instar *Ch. rufifacies* are facultatively cannibalistic and predaceous on other blowfly larvae.

The secondary screwworm fly, *Cochliomyia macellaria* (F.), is abundant throughout the New World and is often the first arrivers at human and animal remains. Due to overlapping niches, species interactions between *Ch. rufifacies* and *C. macellaria* not only affect their life histories but also impact forensically important predatory beetles. Prior to arrival of *Ch. rufifacies* in Louisiana in 1995, Tessmer and Meek (1996)<sup>1</sup> study seasonal abundances of adult Calliphoridae. They determined that >95% of the emerged adults in summer and fall 1992 were *C. macellaria*, as well as, approximately 52% of *C. macellaria* larvae migrated <0.9 m away from carcasses, and 65% moving in a SE/SW direction.

**Material and Methods:** Five experiments were conducted during summer and fall seasons in 2008-09 in a grassland habitat in Hammond, Louisiana. Each experiment included three fresh swine carcasses (55-70 kg), placed 30 m apart, with heads facing north. Two research phases per experiment: (1) carcass utilization by calliphorid larvae; and, (2) larval dispersion and adult emergence. Phase 1 sampling protocol: all carcasses manually sampled daily until majority of blowfly larvae migrated and/or pupated, with emphasis on species interactions within five regions of carcass (head, anterior portion, anterior limbs, posterior portion, and posterior limbs) and monitoring of resource quality per region (high, medium, low). Phase 2 sampling protocol: collection method designed to resemble Tessmer and Meek (1996). Nine emergence cages were constructed from PVC pipe and fiberglass screening: one center cage (1 x 1 x 0.6 m) placed directly over each carcass, eight cages (1 x 0.6 x 0.6 m) placed 60 cm away from center cage and each other. Emerged flies aspirated daily until no calliphorid adults were observed for two consecutive days. Temporal and spatial models were determined using logistic regression analyses (Proc Glimmix, SAS 9.1).

**Results:** Fifteen carcasses studied in 2008-09 were analyzed for spatial and temporal patterns of calliphorid larvae within five carcass regions (Phase 1). Seven species of blowfly larvae were collected until 10-15 d of decomposition, with majority of occurrences being *C. macellaria* and *Ch. rufifacies* larvae. Logistic regression models clearly demonstrated behavioral, spatial and temporal patterns observed in nature, including: delayed oviposition by *Ch. rufifacies*, relocation of *C. macellaria* to lower quality resources (limbs) to avoid predation, early migration of *C. macellaria* larvae away from carrion, and increased probability of *Ch. rufifacies* at all regions of carcass with favorable resources. All Type III tests were highly significant ( $P > F$ ,  $P < 0.05$ ) for time and carcass region, as well as, Tukey-Kramer pair-wise comparisons for species and region. Nine swine carcasses studied in 2009 were analyzed for spatial and temporal patterns of migrating post-feeding larvae (Phase 2). A total of 90,112 adults were aspirated between 11-19 d of decomposition: *C. macellaria*: 62,470 (69%), *Ch. rufifacies*: 27,568 (31), and *Lucilia sericata*: 74 ( $> 0.0008$ ). Predicted adult emergence for *C. macellaria* and *Ch. rufifacies* peaked on days 13 and 16-17, respectively. All Type III tests were highly significant ( $P > F$ ,  $P < 0.05$ ) for time, cage position (north-south, east-west), and all interactions. Tukey-Kramer pair-wise comparisons were also significant for species and carcass region. Majority of *C. macellaria* were collected in the center cage (~89%), with less larvae migrating south and west. Whereas, *Ch. rufifacies* larvae migrated predominantly south and east.

**Conclusions:** In comparison to Tessmer and Meek (1996), a decrease in abundances of adult *C. macellaria* were documented. However, *C. macellaria* remained the predominant blowfly species collected in emergence cages (69%) despite the presence of *Ch. rufifacies*. In addition, the majority of *C. macellaria* larvae did not migrate a notable distance away to pupate as was hypothesized. Understanding species interactions of forensically important blowflies at a crime scene is of utmost importance for postmortem estimations.

## Reference:

1. Tessmer, J.W., and C.L. Meek. 1996. Dispersal and distribution of Calliphoridae (Diptera) immatures from animal carcasses in southern Louisiana. *J. Med. Entomol.* 33(4): 665-669.

## Forensic Entomology, Blowfly Species Interactions, Hairy Maggot Blowfly

### G107 Developing Genomic Tools for Forensically Important Flies to Improve Forensics

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After attending this presentation, attendees will learn that developmental and quantitative genetics has an improving forensic entomology. Attendees will also gain an appreciation of how to develop genomic tools and implement genomic techniques to improve forensic entomology.

This presentation will impact the forensic science community by showing how recent technological advances have allowed non-model organism researchers to conduct genomic research which means that genomics can be used to improve problems in forensic entomology, which is dominated by non-model organisms. The data presented here represent the first set of genomic tools for a common forensically important blowfly species.

Recent research indicates that quantitative and functional genetic principles will be useful in improving the accuracy and precision of arthropod derived postmortem interval estimates. However, such endeavors require genomic tools, which are lacking in non-model species. Next-generation sequencing provides the necessary capabilities to develop genomic tools for blowflies and other forensically relevant species. For many species whole genome assembly is not feasible, but it is possible to assemble sequences that represent the transcriptome: the subset of the genome represented by transcribed genes. However, transcripts possess alternative splices, which must be accounted for in the assembly process (or ignored at the cost of losing information). The *de novo* assembly of the *Lucilia sericata* (Diptera: Calliphoridae) transcriptome is described here, outlining computational and molecular efforts to identify and confirm alternative splice, allelic, and gene expression information in a species important to forensic entomology.

An algorithm that enables the identification of alternative splices in assembled transcripts through the use of de bruijn networks was developed. This tool, ASplice, also identifies putative SNPs and reports library specific gene expression estimates (expressed as reads per million mapped reads per thousand bases of transcript; or RPKM). Before assembling blowfly data, *Drosophila melanogaster* data was assembled in this manner, revealing false positive and false negative rates for transcript and splice identification. The performance of ASplice was also compared to other algorithms, demonstrating the ability of the program to be conservative (unlikely to identify false positives) and/or less memory intensive than competing *de novo* assembly software packages.

After validation of the assembly methods, *Lucilia sericata* RNA derived from embryonic, larval, pupal, and adult samples was sequenced using a combination of Illumina and 454 sequencing. All developmental stages were sequenced using Illumina, producing >6 billion bases of raw useable sequence. In addition, reciprocal subtractive hybridizations were performed between larval and pupal samples in a manner that would enrich for transcripts that are differentially expressed between: (1) feeding and postfeeding third instars; and, (2) early and mid pupation. Samples derived from subtractive hybridizations and salivary gland RNA were sequenced using 454 sequencing, yielding tens of millions of bases of sequence data.

Results indicate that the authors have identified hundreds of transcripts (and isoforms) that are strong candidates for use in predicting blowfly developmental age in a manner that increases precision compared to traditional forensic entomology approaches. Many of these loci also have *Drosophila* homologs that are expressed in developmentally regulated patterns commensurate with expression patterns observed in *Lucilia*, while others are unique to the species. Thousands of putative SNPs and splices have been identified, which will be useful in quantitative and population genetic studies. Sanger sequencing has been used to confirm assembled sequences in seven genes, including several alternatively spliced sex determination genes. Results from the analysis of the transcriptome will be presented and the audience will be exposed to several lines of research that can be employed once genomic tools are available for forensically important species.

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**Postmortem Interval, Genomics, Gene Expression**

## **G108 Improving Postmortem Interval (PMI) Estimations Through Curvilinear Development Modeling of the Blowfly *Lucilia Sericata* (Meigen)**

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After attending this presentation, attendees will have a better understanding of the importance of forensic entomology in estimating postmortem interval (PMI) and the advantages of using curvilinear development data for postmortem interval calculations.

This presentation will impact the forensic science community by vastly improving on the current developmental data available for *Lucilia sericata* and increasing the accuracy of PMI estimations in human death investigations.

The blowfly *Lucilia sericata* is among that group of insects that can occur rapidly on decomposing animals. When found on a human body, the developing eggs, larvae, or pupae of *L. sericata* could be used as an index pointing to the initial time of death (postmortem interval or PMI). Estimating the PMI is crucial in most human death investigations, because time of death is needed for properly reconstructing events before and after death. To use the insects, like *L. sericata*, in estimating PMI we must be able to determine the insect age at the time of discovery and backtrack to time of oviposition. Consequently, understanding temperature-specific development rates is essential. Unfortunately, existing development models of forensically important insects are only linear approximations.

Here, experiments and findings for building a curvilinear developmental model for *L. sericata* are reported. Experimental considerations include diet, humidity, light cycle, temporal patterns of stage transitions, and temperature measures. Experiments were conducted over ten temperatures (10°C, 12.5°C, 15°C, 17.5°C, 20°C, 22.5°C, 25°C, 27.5°C, 30°C, and 32.5°C). Twenty eggs (collected immediately after oviposition) were placed on 25g (0.05lb) of beef liver that was on a 5cm<sup>2</sup> (2in<sup>3</sup>) moist paper towel in an 88mL (3z) plastic cup. The cup was placed in a 9cm<sup>3</sup> (3.5in<sup>3</sup>) plastic container that had 2.5cm (1in) of wood shavings in the bottom. A thermocouple was placed in the containers to monitor the internal

temperatures. Measurements were taken at intervals calculated from accumulated degree hours (ADH). Each life stage had five measurement points: at the beginning, one-quarter mark, one-half mark, three-quarter mark, and the end. Each point was replicated four times, for a total of 20 measurements per life stage. During each measurement, the cups were pulled from the chamber and the stage of each maggot documented with a microscope using the posterior spiracle slits of each maggot (the number of slits corresponds to the life stage).

The *L. sericata* data illustrate the advantages of curvilinear models in describing development at environmental temperatures near the biological minima and maxima, and the practical significance of curvilinear models over linear approximations. Results here represent the first in a series of larger studies modeling development of key forensically important blowflies of North America.

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**Blowfly Development, Decomposition, Human Death Investigation**

## **G109 Can *Lucilia Sericata* Change Gravesoil Microbial Community Structure?**

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After attending this presentation, attendees will understand that there is potential in using fatty acids to characterize gravesoil microbial community with the ultimate goal of estimating postmortem interval (PMI). Attendees will also understand the effect of the blowfly *Lucilia sericata* on gravesoil microbial community structure.

This presentation will impact the forensic science community by the development of an additional method to determine extended PMI and increasing the understanding of cadaver decomposition. This knowledge can be used in conjunction with other methods to estimate PMI, such as forensic entomology.

Death investigations heavily rely on accurate estimations of PMI to assist in identification of victims and suspects as well as determining the validity of alibis. Currently, the most reliable method to estimate PMI at an outdoor death scene is forensic entomology. In cases where active blowfly larvae have already migrated away from the body, estimating PMI becomes difficult.

A controlled laboratory experiment was conducted to determine if the presence of the blowfly *Lucilia sericata* (Diptera: Calliphoridae) can affect the structure of the gravesoil microbial community. To do this Petri dishes were placed (150 mm x 25 mm) filled with 150 grams (g) of washed sea sand inoculated with 150 g of Pawnee clay loam soil in growth chambers. Soil was collected from Nine Mile Prairie, a natural tall-grass prairie ecosystem, which is located approximately nine miles northwest of Lincoln, Nebraska. Inoculated soil was calibrated to a water holding capacity of 55% and left to equilibrate for seven days in growth chambers.

A mouse carcass (killed with carbon dioxide) was then placed on its left side on the inoculated sand within 30 minutes of death. Fly eggs (10 per carcass) were counted and placed on the right eye of selected carcasses shortly after placement on soil and were monitored daily to prevent desiccation.

The temperature was kept at approximately 22°C during the experimental period and the water content of the inoculated sand was maintained at 55% every 3-4 days by adding distilled water. Carcass decomposition was monitored every 24 hours for 35 days using a decomposition scoring system. In addition, carcass mass loss was measured at 7, 14, 21, 28, and 35 days postmortem. A destructive harvest design was used to avoid the influence of carcass disturbance on the rate of decomposition. Following carcass harvest, inoculated sand was collected and analyzed for lipid phosphorus, fatty acid methyl esters, pH, and electrical conductivity. This experiment was replicated four times and controls (inoculated sand with no carcass) were used.

Results indicated that overall, carcass treatment had minimal effect on mass loss. There was no significant difference (P = 0.058) between the

treatments, however the overall percent of mass loss between treatments varied. Mass loss of carcasses on Petri dishes reached approximately 35% ± 3.38 of total body weight after 35 days, carcass on soil reached approximately 62% ± 5.80, and carcasses on soil with insects reached approximately 60% ± 5.44. A higher total body score was observed for carcasses on soil with insects than carcasses on soil, which were both higher in total body score than carcasses on Petri dishes. The highest possible total body score for all treatments was 21. Carcasses associated with insects had only their face consumed and therefore it was observed that the remainder of the body decomposed in a similar fashion to carcasses on soil not associated with insects. Lipid phosphorous analysis indicated that soil associated with carcasses had a much greater total microbial biomass than control soils. There was no significant difference between gravesoils regarding the presence or absence of insects for this analysis. Results from the analysis of fatty acid methyl esters will be presented.

**Forensic Taphonomy, Extended Postmortem Interval, Ecology**

## **G110 Hypostasis and Time Since Death: State of the Art and Proposal of an Operative Instrumental Protocol**

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After attending this presentation, attendees will understand that in evaluating time since death the study of parameters derived from the three abiotic signs (hypostasis, rigor mortis, and body cooling) is still the most commonly used in practice. This is due to its simplicity and rapid execution and because it allows immediate deductions to be made at the crime scene, thus rapidly orienting the investigations. However, some of these methods, especially the evaluation of hypostasis, are highly subjective, being largely based on the personal experience, skill and scientific knowledge of the operator. This makes standardization very difficult and the final result may not be entirely satisfactory in estimating the time of death, as compared to witness reports and circumstantial data, that are sometimes very accurate.

This presentation will impact the forensic science community and society. In fact, it is known that the lividity that occurs after death has certain qualitative and quantitative features, but although these findings can contribute to estimate the time of death, the evaluation is still subjective and other parameters are often more accurate. For this reason, a standard method for studying hypostasis to estimate the time of death is needed.

An extensive volume of “archeological” references, covering a period since 1700, in which hypostasis was underestimated as a medicolegal phenomenon will be analyzed. Initially, it was considered only to prevent misdiagnosis of death. Then various authors set chronological limits for the appearance of lividity. Since then, changes of hypostasis characteristics have always been used for a basic orientation in evaluating the postmortem interval. The main problem is that several external and internal factors, often difficult to recognize, can affect the evolution of the phenomenon, in relation to conditions that change the qualitative and quantitative status of blood. Only in the 1930s was the advisability of “testing” hypostasis “mobility” suggested, although it was never specified exactly how to perform these operations. The small evidence provided in this regard is evidently based on personal experience and it is therefore difficult to propose these “guidelines” in concrete cases to pathologists with little practice and experience.

A test of hypostasis as it is usually done in daily practice was performed. A map of the back of the body regions, where hypostasis appears if the body is in supine position, was made. A questionnaire was administered to 35 experts and residents training in forensic medicine. The results of these tests confirmed that no standardized method is used in daily practice, although the population sample consisted of coroners coming from the same school. Then, some internal, external, or subjective factors were considered (cause of

death; ambient temperature; humidity; anatomical site to test; intensity and duration of the compression; assessment of color intensity), that can cause variations in hypostasis formation and evolution. These factors and their incidence in the evaluation are the basis of this experimental study, based on measurement of these same parameters using specific equipment (a colorimeter, a dynamometer, and a thermometer set also for moisture measurement) selected for its ease of use.

In conclusion, the results of this research are illustrated and discussed. They show that in time since death evaluations based on hypostasis, a more objective method is needed. It must be remembered that many factors may invalidate the most accurate analysis made using traditional parameters.

**Hypostasis, Standardization, Time Since Death**

## **G111 Frozen-Thawed vs. Freshly Killed: A Comparison of the Volatile Organic Compounds Detected From Decomposing Remains**

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After attending this presentation, attendees will have enhanced their understanding of the scent of death that is released during the decomposition process of a human cadaver analogue.

This presentation will impact the forensic science community by expanding upon the limited knowledge of volatile organic compounds (VOCs) that evolve from decomposing remains, as well as assessing the impacts that freezing and then thawing the remains may have on the VOCs generated.

The decomposition process, which starts soon after death (Vass 2001), has been studied under an array of conditions using a variety of animal models, such as pigs (Payne 1965), squirrels (Johnson 1975), rabbits (Johnson 1975), rats (Micozzi 1986) and humans (Mann et al. 1990). In the latter study, the effects of temperature on the decomposition process of human remains were investigated; it was found that when temperatures were cold or near freezing, the rate at which the cadavers decomposed reduced or ceased entirely. In the 1986 study conducted by Micozzi, the effects of temperature on the decomposition process were also evaluated. Within this study, the effects on rats that were frozen after being euthanized and then thawed versus those that were freshly killed were compared; it was determined that those rats that were frozen and then thawed decomposed from the “outside-in” while those that were freshly killed decomposed from the “inside-out.”

Alive, the body consists of proteins, carbohydrates and lipids, and at death, these compounds breakdown into simpler molecules generating a putrid odor which has come to be known as the *scent of death*. Several research groups have evaluated the VOCs that are released during the decomposition process of human remains. Vass et al. (2004, 2008) conducted a two-part study: in part one, the authors discovered over 400 volatile organic compounds associated with the decomposition process of buried human remains and in part two, 30 compounds were selected as important markers in human burial decomposition and 19 out of those 30 compounds were identified in non-buried decomposing human remains. In a study conducted by Statheropoulos et al. (2005), volatile organic compounds that were released during the decomposition process of two cadavers were evaluated and over 80 compounds were detected. In another study conducted by Statheropoulos et al. (2007) over 30 decomposition-associated VOCs were detected. Hoffman et al. (2009) conducted a study evaluating the VOCs released from 14 separate tissue samples that were previously used as victim-recovery canine training aids; their study revealed over 30 volatile organic compounds. In a recent study performed by DeGreeff (2010), over 30

compounds were detected from human remains samples collected at a morgue and crematorium.

A method optimization study was performed, using a standard mixture of previously reported compounds for decomposing remains, to critically evaluate two different extraction techniques: Activated Charcoal Strip (ACS) and Solid-Phase Microextraction (SPME). In addition, different gas-chromatographic column chemistries were also explored to determine the best chromatographic stationary phase suitable for decomposition volatiles. The optimization study revealed that the use of SPME in combination with GC/MS equipped with a Sol-Gel Wax column provided the best response and selectivity for the target analytes. The optimized methods to evaluate the volatile organic compounds emanating from the decomposing remains of eight human cadaver analogues were utilized: four were frozen upon euthanizing then thawed prior to analysis and the remaining four were freshly killed. A variety of compounds were detected and included the following classes: aldehydes, ketones, carboxylic acids, and sulfur-containing compounds. A comparison between the human cadaver analogues that were frozen-thawed vs. freshly killed will highlight the similarities and differences between each set of specimens, as well as demonstrate the significant impact that freezing of the remains has on the VOCs detected.

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**Scent of Death, Decomposition, Volatile Organic Compounds (VOCs)**

## **G112 Applications of Social Network Services in Medicolegal Death Investigation**

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After attending this presentation, attendees will have an understanding of how online social network services can provide valuable information relevant to medicolegal death investigation. This may include information leading to identification of unidentified remains, time of death determination, manner of death, and other relevant issues. Attendees will be aware of specific case examples in which social media has been helpful, and will understand some of the limitations and pitfalls associated with use of these sites for such information.

This presentation will impact the forensic science community by introducing the concept of using social network services to gather information crucial to medicolegal death investigation, and furthermore, will illustrate cases in which these media have already been useful.

With the increased popularity of social networking sites such as Facebook®, LinkedIn, Twitter, and Google®, a wealth of new resources are available to aid in medicolegal death investigation. Individuals are utilizing these social networking services (SNS) to share public information with the world and, in a sense, are creating a database of profiles that can be searched by anyone having an internet connection. While the information available does depend on the level of security the user has placed on his/her profile page(s), there are a large number of users who post publicly and have little or no security limitations on their information.

The medicolegal death investigator will immediately recognize the advantage of such a searchable public database. Information commonly available on user profiles includes photographs of the user (the owner of the profile page), past education and work history, and a list of friends and family. Pictures on a user's profile on such sites as Facebook®, MySpace, LinkedIn, and Google® can be used for preliminary identification purposes. Users may also have family members and significant others on their Facebook® "friends list," Twitter "followers," or Google "circles," which could aid in establishing the next of kin, and finding a way to contact them (via clicking on their profile, and sending a message). The "friends list" can also, of course, provide an idea as to the types of people with whom the decedent chose to socialize, which might be relevant in certain circumstances.

The actual content posted by the user may also be of interest. Suicide threats can be made via a Facebook® wall post, Twitter "tweet," or Google

"share", just as they have been sent via text message on cell phones in the past. These "tweets" and "shares," updated activity lists on Facebook® profile pages, and even comments made by the user on other people's pages ("walls" in the case of Facebook®) can help in determining when a user was last at their computer (and/or, last known alive). All of these potential findings can aid in pinpointing a more precise time of death.

Furthermore, the comments left by the decedent user can give a sense of their state of mind, while the comments left by others on the decedent's "wall" or page can reveal aspects of the relationships the decedent had with others. Through the use of comments on profile pages, a user can hypothetically leave a public electronic suicide note while a murderer could also be found to make remarks on a victim's profile page, either before or after death.

There are limitations to the information gleaned from SNS. Some users may share their passwords with others, making it possible that the "user" is not the one updating their profiles or posting comments. Additionally, password-saving features in some computer programs may make it possible for another individual to post to a decedent's account by simply using the decedent's computer. Another issue is that information can be removed at any time by anyone who has access to the account. Furthermore, the "friends list" should be considered with caution, as it may consist of any range of constituents, from just a few closest friends and family for the most conservative of users, up to hundreds of acquaintances and strangers for other users with no concerns for privacy. Finally, it behooves one to remember that people are capable of lying over the internet just as they are of lying in person, and perhaps even more so because of the distancing and semi-anonymity.

This presentation will increase awareness of the usefulness of SNS to the medicolegal death investigator and/or forensic pathologist; describe specific applications to death investigation; and encourage attendees to consider how they might utilize the information available on these sites in their own investigations, while recognizing its limitations and potential pitfalls. Specific casework examples from the Onondaga County Medical Examiner's Office will be provided, with screen captures of relevant pages where available. The presentation will also cover what happens to a user's profile page after the user dies.

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**Social Network Services (SNS), Death Investigation, Facebook®**

## **G113 Skeletal Trauma Observed in Exhumed Skeletons Compared With Trauma Recorded in the Corresponding Forensic Autopsy Reports**

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After attending this presentation, attendees will understand important recommendations for medical examiners when performing autopsies in violent death cases where bone structures are compromised. This information is important for the medical examiner and forensic anthropologist alike for the determination of the mechanisms and causal agents of injuries during autopsies and analysis of skeletonized bodies or skeletal remains.

This presentation will impact the forensic science community by demonstrating particularly to anthropologists and medical examiners the importance and the advantages of interdisciplinary, simultaneous and integrated casework in cases where hard tissues have been affected.

The purpose of this paper is to determine the consistency between the analysis of trauma obtained from exhumed skeletons and the findings described from the corresponding fresh body autopsy reports. One hundred and thirty seven skeletons from the modern skeletal collection curated by the

National Institute of Legal Medicine and Forensic Sciences (INMLCF) in Colombia were analyzed. The presence of traumatic injuries to bone was observed on 42 individuals. Of this sub-sample, all individuals died between 2005 and 2006 and there were thirty-three males and nine females, with an age range of 18-84 years old. The following features were documented: (1) compromised bone structure and location; (2) type of traumatic injury; (3) fracture characteristics; (4) possible lesion mechanism and causal agent; and, (5) number of gunshot wounds when applicable, including number of entrance and exit wounds. The review of the autopsy reports for these individuals included a search for description of these same five features.

The results of the analyses and the comparison of the information will be presented in terms of consistency between the findings described on autopsy reports and the findings of the examination of skeletonized remains, particularly in terms of the affected bone structure, type of fracture and its characteristics, mechanisms of the injury and causal agent, number of gunshot impacts, and number of entrance and exit wounds.

Regarding the exhumed cases examined, in 30 (71%) cases gunshot trauma was observed, sharp trauma in one (2%) case, blunt trauma in six (14%) cases and in five (11%) cases mechanism of trauma could not be determined with sufficient degree of certainty. The information obtained from the autopsy reports in these same cases stated that in 27 (64%) cases death was due to gunshot wounds, in three (7%) cases due to stab wounds, in 10 (24%) cases due to blunt force trauma and in two (5%) cases the cause of death was not determined and trauma to the skeleton was not described.

After comparing both groups of results, in 32 (76%) cases the information regarding mechanism of trauma was consistent with the information registered in the autopsy reports. However, in five (12%) cases, the information was not consistent and in five (12%) cases trauma was observed but the mechanism was not determined. Considering the number of impacts in gunshot cases and sharp trauma (30 in total), in 19 (63%) cases the number of impacts observed on the dry bones was not consistent with that recorded in autopsy reports. In eight (27%) cases the information of number of impacts is consistent with the information registered by the medical examiner in the autopsy report.

The accurate determination of traumatic bone injuries and the causal mechanism during the autopsy of fresh bodies or bodies with a large percentage of soft tissue remaining requires extensive dissection and good medical knowledge. On the other hand, when examining skeletonized remains, medical examiners and anthropologists must consider the physiopathology of aspects of trauma and anatomic relationships in order to make adequate interpretations of trauma, the number of impacts, and cause of death. The simultaneous and integrated participation of the anthropologist in the morgue, together with the medical examiner, is strongly recommended for cases where bony structures have been compromised.

**Bone Trauma, Medico-Legal Autopsy, Forensic Anthropology**

## **G114 Characteristics of Medical Examiner/Coroner Offices Currently Accredited by the National Association of Medical Examiners**

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After attending this presentation, attendees will become familiar with the characteristics of medical examiner and coroner (ME/C) offices that have attained Full or Provisional Accreditation by the National Association of Medical Examiners (NAME), such as budget, area and population served, staffing levels in key personnel positions, and the breakdown of specific types of cases.

This presentation will impact the forensic science community by providing an awareness of the profile characteristics of offices that are currently accredited by NAME.

Medicolegal death investigation in the United States is highly variable from state to state, and sometimes even within a single state. In 2004, the only comprehensive survey of the ME/C system in the United States was performed, and the Bureau of Justice found 16 states with a centralized statewide medical examiner, 14 states with a county coroner system, seven states with a county medical examiner system, and 13 states with a mixed coroner and medical examiner system.<sup>1</sup> At present there is no national oversight of medicolegal death investigation, with each state deciding on the minimum standards and criteria that must be attained. The National Association of Medical Examiners (NAME) has created inspection and accreditation standards that they believe should apply to all offices, large and small. The current NAME Inspection and Accreditation program was approved in 1997, although NAME has had inspection and accreditation programs for decades before that. As of July 21, 2011, there are 57 American offices/systems that are either fully or provisionally accredited (as well as one office in Singapore and one in Puerto Rico), and an additional nine offices/systems for whom inspection is in progress.<sup>2</sup>

Thus far, there has been no characterization of these offices/systems that have successfully attained accreditation. As part of the inspection and accreditation process, each office/system must fill out a detailed survey<sup>3</sup> In what ways are these offices similar and in what ways do they differ? By analyzing and presenting the data from these 58 accredited offices/systems (the office in Singapore has been excluded from the analysis) this presentation will help to partially answer those questions. Broadly, this presentation will look at jurisdictional, financial, and personnel characteristics of the NAME accredited offices. Basic operational issues such as the presence or absence of in-house toxicology and histology, an office's own dedicated medicolegal death investigators, and access to radiology, forensic odontologists, and forensic anthropologists will be presented. More specifically, we will present measures such as the average physical size of an accredited office, population served, total budget, funding per person served, and age of their facilities. Other administrative questions to be addressed include the number of pathologists (including board-certified) and support personnel (such as autopsy assistants, investigators, toxicologists, administrators, and clerical support). This presentation will also look at total deaths in the jurisdiction, autopsies performed (including external examinations and partial autopsies), and breakdown of those autopsies by manner of death. For each of these measures, are the answers fairly uniform from one office/system to another, or is there a degree of variability between successful applicants? The data will be presented both statistically and visually via graphs, charts, and tables. Statistical analysis of the self-provided survey responses that each successfully accredited office/system submitted at the time of their inspection for accreditation will be provided. This study will provide computed data utilizing the provided survey responses, such as investigators per total reported deaths, investigators per scene attended, toxicology staff per autopsy, forensic pathologists per capita, and autopsies per pathologist full-time equivalent, and other measures about the staffing and financial resources available to these successfully accredited offices. Preliminary review of a subset of the data to include offices accredited since 2009 shows a mean population served of 1.8 million  $\pm$  2.1 million (range: 0.16 to 10.47 million). The mean annual budget is \$4.1 million  $\pm$  \$4.9 million (range: \$0.47 to \$26.83 million). The mean budget per capita is \$2.80  $\pm$  \$1.88 (range: \$0.47 to \$10.22). The mean number of deaths investigated is 2179/year  $\pm$  2680 (range: 253 to 14,910). The mean number of autopsies performed is 1051/year  $\pm$  973 (range: 76 to 3978). Mean number of pathologists (excluding residents) is 5  $\pm$  6 (range: 0 to 26; median 3); the mean number of autopsies per pathologist is 215  $\pm$  118 (range: 28 to 616; median 207). These early values varied widely and were not normally distributed, but rather were skewed by a few very large and a few very small offices. More detailed and complete analysis will be presented, including visual aids to facilitate appreciation for the full range of the data.

Considering the cost and effort of an accreditation application, this information could be extremely valuable to offices/systems preparing to

undergo inspection, or ones that are considering whether they ought to do so. Coroners, chief medical examiners, and the administrators will be well-served by the information to be presented.

#### References:

1. Bureau of Justice Statistics Special Report: Medical Examiner and Coroners' Offices, 2004; Hickman, M, Hughes, K, Strom, K, and Roper-Miller, JD.
2. [http://thename.org/index.php?option=com\\_content&task=view&id=67&Itemid=69](http://thename.org/index.php?option=com_content&task=view&id=67&Itemid=69)
3. [http://thename.org/index.php?option=com\\_docman&task=doc\\_download&gid=41&Itemid=26](http://thename.org/index.php?option=com_docman&task=doc_download&gid=41&Itemid=26)

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**Accreditation, National Association of Medical Examiners, Administration**

## **G115 Literature Search for Journal Articles Authored by Board Certified Forensic Pathologists**

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After attending this presentation, attendees will understand the scope and type of peer-reviewed journal articles authored or co-authored by board certified forensic pathologists since the board certification was first offered in 1959.

The presentation will impact the forensic science community by providing a summary of the number and types of articles written by board certified forensic pathologists and by having available a readily searchable database of such articles written and published between 1959 and 2010.

Although typical on-line research resources such as Medline, PubMed, and other medical journal databases provide for easy searching of published articles by author or subject, it is much more difficult to search and retrieve articles written by specified types of authors. Further, a specified group of authors may publish articles that are not captured by a single key word or even large group of keywords.

In February 2010, a research grant from the AAFS Pathology/Biology Section to conduct a literature search was received. Shortly thereafter, an on-line survey was conducted of forensic pathologists that provided data which was extrapolated to suggest that the total number of journal articles authored or co-authored by the nearly 1,400 persons board certified in forensic pathology since 1959 could be estimated to fall between 10,000 and 20,000.

Using a master list of board certified forensic pathologist names, an initial search of Medline and PubMed disclosed more than 182,000 articles containing the last names and first initials of the authors. A search was constructed using author last name and all permutations of first name, first initial, middle name, and middle initial. It was apparent in reviewing these articles for subject matter that the large number of articles was due mainly to author names that included that same first initial but were different people. Attempts to narrow the list down by using keywords such as "forensic pathology" or MeSH headings had some impact on reducing the number of articles, but further study of board certified forensic pathologists with known numbers of articles showed that such filtering eliminated articles that should be included in the study results. Similarly, limiting the search to a relatively large group of selected journal titles also resulted in elimination of articles that should be included. A different approach was then taken to filter the number down by eliminating articles written 10 years or more before a person with a given last name and first initial was certified in forensic pathology, then eliminating articles that did not contain one or more of 238 key words in the title or abstract, then by eliminating articles that were not published in one of 645 selected journals with two or more published articles possibly published by a board certified forensic pathologists and having a journal title that seemed potentially relevant to forensic pathology subject matter. After these filters were applied, the group of remaining articles numbers 33,469.

The titles and abstracts of these articles were reviewed manually and articles were eliminated if the subject matter did not involved forensic pathology-related issues. Ultimately, at the time this abstract was prepared, there were 27,052 articles remaining, although further review and filtering was still underway.

In short, this research project has been more complicated and time consuming than the authors had originally envisioned. Because many board certified forensic pathologists since 1959 have died, retired, or are no longer active in the field, and because there are nearly 1,400 such persons over time, it was not feasible to collect the curriculum vitae of individual forensic pathologists and combine their publication lists into a database.

Between August 2011 and the AAFS Annual Meeting in February 2012, it is anticipated that the target group of published articles will be further refined by manual review of publications. The plan is to categorize published articles into groups including Letters and Editorials, Case Reports, Case Series, Review Articles, Original Research, and others which do not fit into the preceding categories. A draft database will probably be made available for review by forensic pathologists to gauge how effective the search was in identifying appropriate articles, with an opportunity to add articles that were not located by our search procedures. The finalized database will be available on-line for review and searching by those who are interested. It is conceivable that yearly updates to the database could be made. Options will be explored to facilitate retrieval of such articles in the future, perhaps by suggesting that the board certification status of forensic pathologist authors be noted somehow in the published manuscript.

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**Forensic Pathology, Publications, Journals**

## **G116 Standards of Practice in Forensic Pathology — Initial Outcomes From a Training Program for Medical Examiners and Coroners**

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After attending this presentation, attendees will be informed about a novel NIJ-sponsored medicolegal death investigation training program aimed at educating medical examiners and coroners (ME/Cs) in nationally established best practices and standards in forensic pathology. Additionally, attendees will learn about further training resources for implementing policies and guidelines based on those standards in their offices.

This presentation will impact the forensic science community by alerting participants to the design of a NIJ sponsored regional training program intended to inform ME/Cs of current forensic pathology standards of practice and providing them with toolkits to translate the training into office-specific best practices and policies. Educating all ME/Cs in forensic autopsy practice standards is the first step in establishing national compliance with those standards. Adherence to standards of practice will create more uniformity and consistency across jurisdictions and improve the quality of death investigation. Dissemination of this training methodology has the potential to vastly improve the quality of ME/C services in the United States.

The National Association of Medical Examiners (NAME) defines best practices for forensic pathologists, medical examiners, and coroners in the United States through their forensic autopsy performance standards and voluntary inspection and accreditation program, along with incorporating guidelines established by NIJ. Qualifications for ME/C vary, but the majority of these decision makers have little or no forensic pathology experience and lack the essential instruction their roles demand. Many ME/Cs are unfamiliar with NAME practice standards, resulting in substantial discrepancies in the quality and consistency of forensic pathology across jurisdictions. Additionally, many offices have no jurisdiction-specific policies concerning scene investigation, selection of cases for autopsy, record keeping,

identification, or handling mass fatalities. The lack of awareness of and establishment of policies and practices negatively impacts criminal justice practice throughout the United States, as specifically noted in the National Academy of Sciences Report on forensic science.

In 2011, through a NIJ-sponsored grant, two training programs were developed in nationally established best practices and standards in death investigation for ME/C and death investigators. Feedback from the first session was used to augment the second training program. Topics included: standards in death investigation; standards in selecting cases falling under ME/C jurisdiction; investigative requirements; selection of cases for postmortem examinations; standards in forensic autopsies, autopsy reporting, documentation of significant postmortem examination findings; effective use of ancillary tests, forensic consultants, and support services during investigations and postmortem examinations; interpretation and the use of scientifically based opinions in death investigation; the unidentified body, and NamUs; mass fatality plans; scene and morgue safety; organ and tissue donation laws and organ procurement organizations; morgue operations; collection of evidence; chain of custody procedures; sexual assault and bite mark evidence; issues in toxicology, specimen collection, and interpretation of results; personnel and staffing of the ME/C office; laboratory requirements, reports, and record keeping; annual reports; computerized storage options; and accurate death certificate completion.

Beyond educating the trainees regarding best practices for forensic pathology, the training program provides participants with an extensive take-home *Toolkit* of materials, including modifiable generic policies and forms for implementation in their local jurisdictions. A website was developed that also contains the toolkit materials and other resources, as well as the opportunity to submit questions regarding policies to forensic pathologists from a NAME accredited office. Further, feedback on the needs and concerns of the trainees regarding their ability to put best practices into place, particularly given the very different problems and needs each ME/C office faces based on their location, the population size serviced, and available resources was used to improve the program. Finally, post-training changes in ME/C policies and practices with regard to incorporating NAME standards was explored and documented.

Although recommendations to improve the ME/C system have been made in the past, to our knowledge, this is the first training program that gathered together key forensic pathology decision makers, trained them in best practices and standards for death investigation based on forensic pathology practice standards, and supported them (via website and peer-coaching) in effecting change and quality improvements. It also uniquely provides the ME/Cs with template policies, procedures, and forms, as a *Toolkit* to allow easy implementation of policies in line with forensic pathology practice standards.

**Best Practices for ME/Cs, NAME Standards, Medical Examiner/Coroner Offices Training**

## **G117 Iatrogenic Death: A Review of Cases From 1990–2000 Investigated at the Department of Forensic Medicine, Vienna**

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After attending this presentation, attendees will have an increased awareness for the importance of addressing the problem of malpractice in order to relativize the hysteria propagated by the media.

This presentation will impact the forensic science community in terms of knowledge and awareness by addressing the problem of iatrogenic deaths.

**Introduction:** Regulations in Austria mandate, that every deceased person must undergo an external examination before burial, which is usually performed by general physicians. In the event that death occurs in a public hospital, the clinical pathologist must perform this examination. The objectives of this examination are to issue a death certificate. Furthermore, the Austrian Physicians Law stipulates that in any suspected case of a criminal offense, death or physical injury, a report has to be made to the police. In general, such cases of death are followed by a postmortem performed by a forensic pathologist.

Medical malpractice is a global problem which can have considerable financial and legal consequences for the community and personal consequences for those involved. Because of scarce country-specific data, media reports have been giving rise to somewhat of a public hysteria in Europe. Austria, a small country in central Europe with slightly over eight million inhabitants and its capital, Vienna, with about 1.6 million inhabitants, are deficient in providing statistical and epidemiological data concerning medical malpractice cases. Although relevant epidemiological and statistical data have not yet been determined, the media, especially in Vienna, persists in claiming that medical malpractice causing iatrogenic deaths occurs frequently.

**Results:** Due to the fact that there are no reliable data about medical malpractice and iatrogenic deaths in Austria, the data from a total of 7,211 autopsy reports filed between 1990 and 2000 at the Department of Forensic Medicine in Vienna have been retrospectively analyzed. In 2,074 cases, deceased individuals underwent medical treatment before death. Out of these 2,074 cases, only 55 deaths were relevant for this study. Thirty cases of surgical adverse events, 19 cases of negligence and six medication-related incidents could be found and were included in this analysis.

In those cases where court files could be obtained, the outcome was also included in this analysis. Out of a total of 40 cases from available court files, 36 cases were dropped, there were two acquittals and two convictions. In other words, in less than one in a hundred cases (2.75%) charges were pressed against the treating medical doctors.

Conclusively, the number of medical malpractice practices leading to iatrogenic deaths in Austria seems to be very low in comparison to other countries, where, during the same time period, 4.5 in a hundred cases (4.5%) were prosecuted. Although the figures claimed by the media do not appear to be legitimate and lack credibility since they are too high, the possibility that many cases go unnoticed in Austria, needs to be taken into consideration.

**Iatrogenic Death, Medical Malpractice, Vienna**

## **G118 Overview of Current Clinical Forensic Medical Practice in Beijing, China**

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After attending this presentation, attendees will learn the basic structure and jurisdiction of clinical forensic medicine in China. In addition, the education, training, and qualification of clinical forensic physicians will also be presented.

This presentation will impact the forensic science community by providing participants with a deeper understanding of the role and scope of the specialty of clinical forensic medicine globally. This presentation will also demonstrate the interrelationship of the roles of clinical forensic medicine and forensic pathology.

Clinical forensic medicine refers to that subspecialty of forensic medicine that involves a wide range of activities at the interface of medicine and law. It generally involves the assessment and interpretation of the physical and mental conditions of living individuals. Clinical forensic medicine has become widely practiced in China in the last two decades.

A study was conducted on the current system of clinical forensic medical practice in Beijing, China. This study showed that there are 158 clinical forensic physicians certified by the Bureau of Justice in 14 offices in Beijing within five relatively independent agencies: private clinics, hospitals, government sponsored societies, department of forensic medicine in a university, and a forensic medical center. In addition, the police agency has its own clinical forensic physicians. The qualifications of clinical forensic physicians vary widely from agency to agency. The basic requirements for certifying as a clinical forensic physician are: (1) being a Chinese citizen with good moral standards, not having any felony convictions; and (2) having a bachelor's degree of medicine majoring in forensic medicine (BM Foren. Med) or Bachelor's degree in forensic sciences with on-the-job training in clinical forensic medicine for five years, or having a bachelor's degree of general medicine (BM) with working experiences in various clinical specialties and 240-hour special training in forensic medicine. In general, clinical forensic physicians do not deal with the deceased, and forensic pathologists do not deal with living individuals. However, in China, there are forensic pathologists who are involved in both the medico-legal death investigation and clinical aspect of the forensic medicine.

In 2010, a total of 38,759 cases were investigated by the clinical forensic physicians in the police agency and 16,107 cases were investigated by the clinical forensic physicians within the other 14 offices in Beijing. The services provided by the clinical forensic physicians mainly include: (1) documentation and interpretation of injuries due to physical assault, motor vehicle accident, and work; (2) examination of victims of sexual assault and the alleged perpetrators, (3) evaluation of the interaction of trauma and disease, (4) assessment of disability due to injuries, (5) investigation of personal damage due to medical malpractice, and, (6) examination of physical and mental status to determine the fitness to be interrogated by police or detained in police custody. Clinical forensic physicians also provide expert opinions/advice to court on the compensation of personal injuries in civil litigations. This presentation gives a general overview of current clinical medical practice in Beijing, China.

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**Clinical Forensic Medicine, Injury Assessment, Civil Litigation**

## **G119 The New INFOR (International Network for Forensic Research) Classification of Asphyxia: Towards an International Agreement**

*Anny Sauvageau, MD\*, Office of the Chief Medical Examiner; 7007 116 Street, Edmonton, AB T6H 5R8, CANADA; and Duarte N. Vieira, PhD, MD, Rua Antonio Jose de Almeida, No 117, Coimbra, 3000-044, PORTUGAL*

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After attending this presentation, attendees will know about the new INFOR Classification of Asphyxia.

This presentation will impact the forensic science community by standardizing the classification of asphyxia.

**Introduction:** The classification of asphyxia and the definitions of subtypes are far from being uniform, varying widely from one textbook to another and from one paper to the next. Unfortunately, similar research designs can lead to totally different results depending on the definitions used. Closely comparable cases are called differently by equally competent forensic pathologists/medico-legal doctors.

In response, a unified system of classification was recently proposed. This standardized classification was pieced together by drawing mainstream definitions from a thorough review of forensic textbooks and literature. In the present study, an international consultation on this unified classification was undertaken in an attempt to achieve a global agreement on a standardized classification of asphyxia.

**Material and Methods:** A questionnaire was designed to evaluate which parts of the standardized classification and which definitions the international forensic community is ready to adopt and which parts need to be revised.

**Results:** Two hundred and three surveys were compiled: 110 from North America, 65 from Europe, 10 from form Asia, 5 from Oceania, four from Central and South America, four from Africa, and 5 from Middle-East.

There is a large majority in favor of adopting the overall standardized classification and the following definitions: suffocation (79%), confined spaces/entrapment/vitiated atmosphere (79%), strangulation (85%), hanging (84%), ligature strangulation (79%), positional asphyxia and traumatic asphyxia (74%), and drowning (78%).

The epiglottis as the anatomical landmark between smothering and choking is agreed with a small majority (57% worldwide, 75% in Europe, 80% in North-America).

There are two elements of the classification that will have to be further worked on. First, there is no consensus if the category of asphyxia labelled confined spaces/entrapment/vitiated atmosphere should be further subdivided (37% worldwide, 28% in Europe, 39% in North-America) or not (48% worldwide, 62% in Europe, 43% in North-America) (note: the sum of the % for yes and no do not ad to 100% because of a small % of abstentions). Second, it is not clear if a hanging accompanied by a fall from height should be part of the classification of asphyxia (46%) or not (47%). Third, it is not clear if mechanical asphyxia is a broad term encompassing several types of asphyxia caused by various mechanical means (33% worldwide, 58% in Europe, 11% in North-America) or is a term that designates asphyxia by restriction of the respiratory movements either by the position of the body or by external chest compression (60% worldwide, 31% in Europe, 84% in North-America).

**Discussion:** Mutual concessions are going to be necessary to achieve international agreement; but as a scientific community, we cannot continue to use different definitions and classifications depending on our geographical location or depending on our favourite textbook. As a scientific community, forensic pathologists and medicolegal doctors need to agree on a standardized classification of asphyxia. The practice of forensic pathology and legal medicine has been for a long time part art and part science. To grow as a scientific discipline, an effort has to be made to shift away from art and move toward a more scientific approach, and the standardization of classifications and definitions is an important step in that direction.

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**Asphyxia, Classification, Forensic**

## **G120 When Hospitals Fail to Report Deaths in Medical Examiner Jurisdiction: What Are We Missing?**

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After attending this presentation, attendees will be able to identify categories of deaths that hospitals often fail to report to medical examiners/coroners (ME/C). Attendees will understand the impact that the lack of reporting may have on the legal and public health systems.

This presentation will impact the forensic science community by allowing death investigators to recognize which types of deaths are often not reported to the medicolegal death investigation system; understanding these categories will allow death investigators to develop targeted educational

outreach within community healthcare facilities in order to maximize reporting of appropriate deaths.

Although laws governing medicolegal death investigation vary among jurisdictions, reportable deaths generally include deaths of children, sudden and unexpected deaths, and deaths resulting from physical or chemical injury. However, some hospital deaths that should come within ME/C jurisdiction are not reported, either because of lack of knowledge on the part of healthcare providers, or in some instances because of religious or cultural beliefs and misconceptions about the consequences of reporting. When hospitals fail to report deaths to the ME/C, the cost is delayed law enforcement investigation, inaccurate death certification, and missed opportunities to collect evidence from decedents.

This study undertakes this prospective to ascertain the types of deaths that are not reported and to identify areas for targeted quality improvement efforts, e.g., education and development of guidelines. The study is a five-year longitudinal descriptive analysis of non-reported deaths that were in medical examiner jurisdiction. It is impossible to determine the total number of such deaths in any jurisdiction; however, we were able to identify 71 cases. The deaths were discovered after days to years' delay. The deaths were often discovered because of calls from family or lawyers regarding the death certificate, calls from funeral homes, from police, or from the bureau of vital statistics when a community physician completed the death certificate. Almost 60% were decedents over 60 years of age; not surprisingly, this category included those who died from injuries sustained in falls (e.g., hip fractures and subdural hematomas). In some cases, elements of neglect were also suspected. Smaller percentages of cases were identified in which cultural or religious factors may have influenced the medical facility's decision to not report. Unexpectedly, in 7% of the cases, the manner of death was ultimately either classified as homicide or classified as undetermined where homicide was suspected but investigative elements were lacking. The majority of the cases, 82% were accidents, and 11% were suicides. That none of these deaths were ultimately classified as natural may reflect relatively less cases in this category (i.e., that most natural deaths were appropriately reported), or may reflect a lack of means of identifying these cases. In 21% of these cases the death came to the attention of the medical examiner before the body was buried or otherwise disposed, and therefore direct examination was possible. If each of the 71 deaths had been reported in a timely fashion 44% would have been autopsied, while the other 56% would have been released following an external examination. Based on this experience, strategies are being developed to reduce the incidence of these failures, specifically by pursuing ongoing educational efforts in local hospitals to address reporting issues. A key finding is that healthcare providers require additional and more concrete guidelines for determining which deaths fall within ME/C jurisdiction.

Some interesting trends and surprising categories within the data set will be presented. Some case examples will illustrate these points. Quality improvement initiatives will be presented that can be implemented within any medicolegal death investigation system to reduce the incidence of jurisdictional lapses.

#### **Hospital Deaths, Medicolegal Jurisdiction, Fail to Report**

## **G121 Medical Problems and Medical Neglect in the Elderly**

*Lakshmanan Sathyavagiswaran, MD\*, and Christopher B. Rogers, MD, Los Angeles County, Medical Examiner's Office, 1104 N Mission Road, Los Angeles, CA 90033*

After attending this presentation, attendees will understand problems encountered in nursing homes, a variety of medical problems found in the elderly, and risk factors for elder abuse.

This presentation will impact the forensic science community by increasing awareness of special circumstances often found in the elderly. It will raise awareness among forensic professionals of abuse of elderly individuals.

Approximately 1.5 million Americans or 6% of the population over 65 live in one of the 18,000 nursing homes in the United States. Seventy-two percent of the nursing home population is over the age of 75. By the year 2040 it is estimated that there will be 5.2 million nursing home residents, of whom 88% will be over 75. Currently 33% of nursing home residents expire there. The median length of stay of the elderly in the nursing homes is about 582 days and about 26% of the residents stay less than six months. Half of nursing home residents are demented and other half are incontinent.

Problems in nursing homes include malnutrition, falls, excessive drug use, decubiti, infection, and psychological problems including depression, dementia, late life paranoia, and behavioral problems. Iatrogenic disorders include inappropriate drug use and use of too high a dose of a drug or too many drugs. The prevalence of mental illness in nursing home patients is approximately 75%. Nearly 40% of residents have occult depression and may benefit from a low dose of antidepressant drug. Behavioral problems include passive and active aggression, verbal aggression, and manipulative behavior. About 1.5 million infections occurred annually in nursing homes and 27% of all hospitalizations in nursing home patients are due to infections. Influenza in nursing home settings has a case fatality rate of approximately 10%. It has been found that failure of the protective effect from influenza vaccination is probably related to impaired responses from poor nutritional status in nursing home residents. Similar questions also exist regarding the efficacy of pneumococcal vaccination in the frail elderly. Tuberculosis epidemics can occur in nursing homes.

Malnutrition has been found to be present in just under 60% of nursing home patients and weight loss is a major problem in the nursing home. Zinc deficiency has been important in the pathogenesis of decubiti, immune dysfunction and anorexia in the nursing home residents. Somatomedin is also low in this setting. Growth hormone deficiency is associated with increased adipose tissue and reductions in muscle mass, bone mass, renal blood flow, and liver drug metabolism. Dehydration is another major problem. There is decreased thirst perception with advancing age. Many nursing home patients live in a water desert. Hyponatremia may be due to tube feedings, low salt diet, or syndrome of inappropriate antidiuretic hormone secretion. Elderly institutionalized patients, 10 – 25% will have a major fall each year. Usually the falls are associated with use of antidepressants, sedatives, hypnotics, vasodilators, osteoarthritis, and depression. The risk of falls is increased after a meal.

It is estimated that half a million elders living with younger family members are abused. Some old people become ill, demanding, less productive and more difficult to care for as they age. They become stubborn, quarrelsome, and untidy, refusing to eat and losing high order psychological defense mechanisms acquired during developmental phases. They may even become aggressive and combative, so they provoke aggression and punishment from the caretaker. With the demise of an extended family, many working children find it difficult to care for aged parents both physically and financially. In modern society such care is expensive and exhausting.

The temptation to punish an elder who acts like a mischievous child may be severe. It has been found that if a child has been abused, he may later become an abusive caretaker. New conflicts arise when aged parents move into a child's household leading to violence if they are not resolved. Differences in life style, values, and religious practices may be some of the unresolved family conflicts which may be reactivated. The caretaker becomes depressed, despairing at the hopelessness of the situation.

Neglect by paid caretakers arises because of poor pay of aides, poor working conditions, long working hours, and interference of red tape and paperwork with efficient care. Staff members may become depressed and pessimistic because they believe that their patients are dying and there is no hope of successful outcome no matter what the efforts. In for-profit nursing homes, there may be proprietary efforts to cut cost, and show better profit to management and stock holders. This may result in neglect, for example, by providing less food, poorer quality of food, fewer personnel, and less maintenance of facilities.

Many elderly patients don't complain because of fear of retaliation, including possible expulsion to homes that are worse or further away from families, physical abuse, or ignorance of their rights, and legal recourse.

They may become apathetic and feel defeated, depressed, and helpless. Impending death enhances these feelings. Case examples of medical neglect involving dehydration, severe fecal impaction, and vitamin deficiency will be presented.

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**Nursing Home, Elder Abuse, Geriatrics**

## **G122 Pressure Sores In England: Comparing the Investigation of Two Cases**

*Stuart J. Hamilton\*, 9 Troon Close, Consett, Co Durham DH8 5XF, UNITED KINGDOM*

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After attending this presentation, attendees will be better informed about how potential neglect cases are investigated in England and the interactions between coroners, pathologists and law enforcement in such cases, as well as the way British pathologists interpret pressure damage.

This presentation will impact the forensic science community by stimulating discussion over the responsibilities of carers for frail and vulnerable people and the differences in how the law views the development of pressure ulceration in different jurisdictions.

With the general increase in the number of elderly people in modern society, the care and support given to vulnerable and unwell older people is becoming an increasingly significant social issue. This is particularly true in the setting of care homes, where companies often receive large sums of money from both government agencies and private funds. The development of pressure ulceration in immobile individuals who are in a care setting will almost always lead to an investigation by various authorities, and communication between the various bodies may occasionally be limited.

In England, such cases fall under the legal jurisdiction of Her Majesty's Coroner who, because of the possibility of neglect, will usually instruct a pathologist to perform an autopsy. A subjective opinion will be formed as to whether the case is one where criminal charges may arise or not, and this will influence the extent and nature of the subsequent investigations, and different coroners may approach the cases in very different ways.

Here, are presented the very different investigations of two cases of vulnerable elderly people with significant pressure sores and some of the complexities arising from the distinction between "suspicious" and "non-suspicious" deaths are explored.

The first case is of an elderly female who was in poor health who lived in a residential home but who, like many similar patients, spent prolonged periods in different hospitals. After her ultimate death, concerns were expressed about the fact that she had developed pressure sores, but it became difficult to identify exactly when, and therefore where these had begun. The coronial investigation focused on who had been responsible for their development and where they had occurred, as well as whether they had contributed to the patient's ultimate demise.

The second case also concerns a frail elderly person, but in contrast she was being cared for by a relative and had not been seen outside the house in which she lived for a long time. She was taken to hospital by her daughter, but on her arrival the medical staff found her to be already deceased. An initial examination revealed very severe pressure damage (certainly the worst this pathologist has ever seen) and she was referred for autopsy. As the relative was the sole caregiver, she was interviewed by the police and an investigation into the possibility of this case representing one of gross negligent manslaughter was begun and the death treated as a suspicious one. The relative was found to be of sound mind, and her defense was that the deceased "didn't like hospitals" and that the carer preferred to treat the pressure sores by "natural means."

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**Pressure Sores, Elderly Patients, Neglect**

## **G123 Extreme Shortness of Umbilical Cord Associated to Hematoma During Labor in Uncomplicated Pregnancy: A Fatal Case**

*Marco Di Paolo, MD\*, Via Roma 55, Pisa, ITALY; Laura Roas, MD, Via roma 55, Pisa, ITALY*

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After attending this presentation, attendees will receive exhaustive information about an uncommon case of fatal hematoma at labor in a short umbilical cord fetus following an uncomplicated pregnancy. A complete forensic examination was performed by autopsy including histological examinations. The cause of death was established as acute asphyxia caused by acute traumatic vascular rupture of umbilical arteries as a consequence of the protracted cord traction during labor.

This presentation will impact the forensic science community by expanding awareness of with a fatal complication related to alterations of umbilical cord. In particular, the present case report will inform attendees about the risks associated with a short funiculus in an uncomplicated pregnancy and the significance of an accurate evaluation of umbilical cord morphology during the gestational period.

**Case Presentation:** A 26-year-old woman with an uncomplicated antenatal course, at 40 weeks of gestation + 2 days, was admitted to hospital for a scheduled induce labor. The contraction spontaneously started in the evening of the same day. The next day, at 2:00 a.m., the cervical dilation was 2cm and the fetal heartbeat resulted regular. At 3:20 a.m., the dilatation was 6cm and a significant deceleration of fetal heart tracing was recorded: at this point, the head of the fetus was fully engaged in the pelvis. On the bases of this risky circumstance, at 4:20 a.m. the dilation was complete. Oxytocin (10 IU, i.v. infusion) was administered and Kristeller procedure as well as episiotomy were performed. At 5:18 a.m., the woman delivered a dead female newborn (body weight: 3.610kg; body length: 55cm).

No significant findings at external examination of the fetus were found. The autopsy showed intense polyvisceral stasis. Funiculus disclosed a reduced length (27cm vs normal value of 35-80 m) and diameter (1cm vs normal value of 1.6cm); it showed the presence of multiple hematoma. Placenta, that was normally inserted, didn't reveal any significant alterations.

Histological examination of lung samples revealed the presence of fetal squames and debris in pulmonary vessels, acute emphysema, and pulmonary hemorrhages. The histopathological examination of the placenta didn't show anything of interest. Sections of umbilical cord demonstrated significant periarteriolar hemorrhage ascribable to the rupture of the umbilical vessels. The wall of arteries was characterized by manifest signs of traumatic dissection. Weigert's elastic stain and PAS were negative.

The cause of the death was indeed attributed to acute asphyxia dependent by the intrapartum traumatic rupture of arterial funiculus vessels. Strong traction of the short umbilical cord had a basic role in the pathogenesis of the fatal complication.

The present case report provides the evidence about the absence of alterations in the physiological development of the fetus as well as of complications for the pregnant in the antenatal period, despite the presence of a short umbilical cord. However, this morphological alteration resulted significantly involved in the newborn death, since the traction of short funiculus during the labor, caused the occurrence of a diffused hematoma through umbilical vessels tearing. These data suggest the opportunity of an accurate evaluation of the umbilical cord morphology during the gestational period using virtual reality system measurement also in pregnant without apparent complications.

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**Shortness and Hematoma of Umbilical Cord, Fetal Death, Autopsy**

## G124 Disseminated Varicella as a Cause of Sudden or Unexpected Death: A Case Report and Literature Review

*Dawn B. Holmes, MD\*, Adrienne E. Segovia, MD, and James A. Filkins, MD, JD, PhD, Cook County Medical Examiner's Office, 2121 West Harrison Street, Chicago, IL 60612*

After attending this presentation, attendees will have a better awareness of the characteristics of disseminated varicella (chickenpox) infection as a rare cause of sudden or unexpected death.

This presentation will impact the forensic science community by presenting the autopsy findings and circumstances surrounding the death of a 28-year-old female who succumbed to disseminated varicella infection and by comparing her case with others reported in the literature.

**Introduction:** Varicella zoster virus (VZV) is an infection that commonly occurs in childhood and usually has a relatively benign course in immunocompetent individuals. Mortality from VZV infection in immunocompetent individuals is exceedingly rare. In immunocompromised individuals; however, VZV infection often produces severe manifestations, leading to significantly increased mortality.

**Case Report:** A 28-year-old African American female with a history of immune thrombocytopenic purpura and sarcoidosis presented to the emergency room with an acute onset of a diffuse vesicular rash throughout her body. Her medications included prednisone, and she reported a history of chickenpox as a child. She was subsequently diagnosed with chickenpox and was treated in the hospital with benadryl and calamine lotion for five days. No antiviral medications were administered. The night of her discharge, she complained of shortness of breath. The following morning she was found unresponsive in her bed at home. At autopsy, she was noted to have numerous macular and papular vesicular lesions in various stages throughout the body, predominantly involving the head/face, neck, torso, back, upper extremities, and proximal lower extremities. The lesions also involved the mucosa of the inner surfaces of the upper and lower lips. Several of the vesicular lesions were crusted. On opening the body, numerous erythematous vesicular lesions were noted on the surface of the lungs, liver, and the mucosal surface of the esophagus, trachea, and epiglottis. Multiple enlarged peribronchial, periaortic, and mesenteric lymph nodes were identified; the largest of these measured 3.0 cm in greatest dimensions, consistent with the patient's known history of sarcoidosis. Histologic investigation uncovered multinucleated giant cells in the enlarged lymph nodes with associated hyalinization. There were also patchy hyaline membranes in the lungs with hemosiderin-laden macrophages, scattered multinucleated giant cells, and pulmonary edema. Mucosal lesions revealed ulceration with marked chronic inflammation extending to the submucosal tissue. The Centers for Disease Control and Prevention confirmed via immunohistochemistry the presence of VZV in various tissues. Toxicologic analyses were negative for ethanol, opiates, and cocaine.

**Discussion:** Mortality from VZV infection is exceedingly rare. The annual varicella death rate in the United States is 0.4 deaths per million. Previous cases of disseminated varicella mortality have involved unvaccinated individuals who had a primary VZV exposure. These individuals were usually immunocompromised to various degrees, although mortality has been reported in otherwise healthy individuals. Consistent with the majority of the cases reported in the literature, this case report involves an immunocompromised subject; interestingly, however, this was not a case of primary VZV exposure.

Reported VZV complications include pneumonia, disseminated intravascular coagulation, septicemia, encephalitis, acute respiratory distress syndrome, nephritis, myocarditis, and myelopathy. VZV pneumonia is the most common complication with the highest mortality, seen in immunocompromised individuals. Case reports in the literature typically describe an insidious onset of pneumonia with dyspnea, usually developing 1-6 days following the appearance of a vesicular rash. A similar course of events was present in this case report.

This case is reported to demonstrate the clinical course and autopsy findings of disseminated varicella infection. Although an extremely rare entity, it remains a cause of sudden or unexpected death that one may encounter in a forensic setting.

### **Varicella, Chickenpox, Disseminated**

## G125 Lethal Short Falls Can be Accidental Injuries

*M.G.F. Gilliland, MD\*, and Karen L. Kelly, MD, East Carolina University, Brody School of Medicine, Forensic Pathology Division, Department of Lab Medical Pathology, Greenville, NC 27834*

After attending this presentation, attendees will learn that accidental lethal short fall child injuries can be identified and learn the investigative efforts necessary to reach such conclusions.

This presentation will impact the forensic science community by showing how accidental lethal injuries in children will provide reliable scientific knowledge to allow practitioners to competently certify cause and manner of death in children thus preventing inaccurate diagnoses and incarceration of innocent parents.

**Background:** For many years child advocates have regarded short falls as unbelievable explanations for injuries in young children. They relied on the common experience of parents and caregivers as well as literature describing survivability of short falls (Helfer 1977, Chadwick 1991, Williams 1991, Lyons 1993) to identify lethal injuries with a short fall history as non-accidental.

Report of a series of death investigations (Reiber 1993) identified accidental mechanisms in two of nineteen deaths with history of short falls. Fourteen others were considered homicides and three were undetermined. A contrasting report (Plunkett 2001) used the Consumer Products Safety database described 18 deaths from accidental injuries but having limited description of injury and investigations. The latter report has been thought-provoking but less reliable. Individual case reports continue to provide anecdotal evidence of the existence of lethal accidental short falls.

**Methods:** Two institutions' experiences were reviewed and identified three cases of child deaths attributed to accidental short falls. First was a recent case from the Eastern Regional Medical Examiner's Office where a 10-month-old male was reported to have fallen backwards at day care onto a concrete floor on the 17<sup>th</sup> of February but seemed unhurt. He began vomiting on February 22 and was taken to a local medical center, treated for flu and released. He continued to vomit and was taken March 1 and again on March 2, treated and released each time. The child was less active and slept most of March 4<sup>th</sup>. Put on the kitchen floor to play, he fell forward and hit his head. He was unresponsive when his mother picked him up. Rushed to the hospital, he was found to have a large subdural hemorrhage, retinal hemorrhages, and brain edema. He progressed to brain death on March 6<sup>th</sup>. The impending death was referred as a non-accidental injury death. Autopsy revealed a healing skull fracture consistent with the history of injury 17 days earlier. Organization was seen in the subdural membrane. Review of medical records confirmed the parents' description of the sequence of events and supported the conclusion of accidental injury.

The other two cases were taken from the prospective study of 169 child death investigations in Dallas, Texas. One was a 40-month-old female who had an unwitnessed fall from concrete stairs to a conglomerate-surfaced apartment complex patio. After a brief lucid interval, sleeping for two hours, and vomiting she was recognized to be unresponsive; medical attention was unsuccessful. Autopsy revealed a large subdural hemorrhage, brain edema, and retinal hemorrhages. Patterned injuries on her forehead and elbows were consistent with the patio. The second child was a 12-month-old male with a witnessed fall from his standing height on a washing machine to a tile-over-concrete floor. He seemed uninjured for approximately a day but fell from the bed to the floor the next day and was taken to hospital poorly responsive the following day. The child advocate team gave an affidavit describing the injuries as probably due to a fall. The child had bilateral subdural hemorrhage, brain edema, and bilateral posterior retinal hemorrhages.

Subsequent autopsy and investigations confirmed the subdural hemorrhage duration and accidental nature of the mechanism of injury.

**Conclusion:** Three accidental lethal short falls were found in review of medical examiner deaths at two institutions. Thorough law enforcement and autopsy investigation led to correct certification of these deaths, two of which were initially regarded as suspicious.

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### **Infant/Child Death Evaluation, Short Falls, Accidental Injuries in Children**

## **G126 Challenges in Evaluating Cause and Manner of Death in Palliative and End-of-Life Care Patients**

*Elizabeth A. Laposata, MD, and Cristina M. Padilla, MS\*, Biomedical Forensic Sciences, Boston University School of Medicine, 72 East Concord Street, Boston, MA 02116*

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After attending this presentation attendees will be educated on the ethical, legal, and medical issues in suspicious deaths of terminally ill patients and provide the forensic pathologist guidelines for complete review and interpretation of evidence.

This presentation will impact the forensic science community by providing guidelines for the forensic pathologist to investigate cases of suspicious deaths of terminally ill patients.

Medicolegal death investigations of death in the end-of-life and palliative care setting are challenging and will become more numerous as the population ages. More than 90% of the 500,000 deaths in critical care units in 2007 were preceded by withdrawing or withholding life support treatments. Further, in 2008 more than 40% of all decedents in the United States received hospice care. And increasingly, end-of-life care is provided in non-institutional settings.

The forensic pathologist who is called upon to investigate such deaths must have the ability to differentiate compassionate care from malpractice and even murder in these complex cases. Further, competent and comprehensive death investigation may also be a cornerstone for assessing of quality of care in end-of-life care setting where such traditional indicators of quality of health care as death and recovery rates are not appropriate.

The intentions of clinicians who manage end-of-life-care are often scrutinized because of the role they play in shifting care from curative to palliative measures. Frustration or misperception fueled by powerlessness on the part of even a single family member or ancillary health care provider can give rise to allegation of euthanasia or murder. Moreover, hospice providers point out that families often believe that the death of their loved one was premature.

No comprehensive methodology exists to guide the forensic pathologist in evaluating suspicious deaths of terminal patients. On reviewing the standard practices in the palliative care community and examining controversial cases in the literature of physicians accused of euthanasia, we propose a set of guidelines to assist the forensic pathologist in answering the question: What information should be studied in order to derive a complete evaluation and interpretation of the evidence available?

Determining cause of death in these patients is difficult since the death may occur from the disease process, a complication of the disease process, a withdrawal of life-sustaining therapies, the medications administered, or any combination thereof. One difficulty in enforcing adherence to proper end-of-life-care policy and standards is having the data necessary to establish the intent or motivation of the healthcare provider (i.e. intent to kill, to shorten the dying process, to relieve pain, etc.). This is a multi-layered and complex task. It involves a careful review of medical records, autopsy findings, toxicology results and witness accounts. The medical records establish the foundation and context for the investigation. The autopsy provides information about the physical manifestations of the disease and eliminates other potential causes. Postmortem toxicology must be interpreted with caution because of potential drug interactions, drug tolerance and disease-

altered drug clearance unique to each case. Inappropriately low levels of pain medication should raise the question of drug diversion by caretakers or family. Witness accounts given by family and friends who followed the care of the decedent can help determine whether the standard of care was met. The forensic pathologist must understand the symptom manifestations and pathophysiology of the dying process. Rarely does a single piece of evidence reveal the intent of the healthcare provider.

Understanding the bioethical aspects involved in treatment, such as the rule of double-effect, and using clear definitions of terms such as terminal sedation, active euthanasia, passive euthanasia and physician assisted suicide are essential are essential for proper determination of manner of death.

The goal of this study, is that the forensic pathologist will have the knowledge and structured format to investigate deaths in the end-of-life care setting. A competent death investigation will protect the vulnerable, dying patient population; protect practicing physicians from the distress of false accusation; and prevent terminal health care being used as a cover for euthanasia.

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### **Palliative Care, Terminal Sedation, End-of-Life Care**

## **G127 Using $\beta$ -Amyloid Precursor Protein Staining to Study Patterns of Axonal Injury in Young Children With Hypoxic Ischemic Encephalopathy**

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After attending this presentation, attendees will be able to appreciate whether the pattern of axonal injury in abusive head trauma in young children is caused by hypoxia or trauma.

This presentation will impact the forensic science community by presenting the findings in a group of infants and young children with hypoxic ischemic encephalopathy from causes other than abusive head trauma (AHT) using beta amyloid precursor protein (BAPP) immunohistochemistry reactivity. The study will detail the pattern/patterns of axonal injury caused by pure hypoxic/ischemic insults in young children. The hypothesis is that infants and young children with AHT have overlapping patterns of traumatic (tDAI) and hypoxic/ischemic axonal injury (VAI) and that these two patterns need to be able to be distinguished to determine whether traumatic axonal injury exists in the AHT group. In known fatal human cases of tDAI, the gross brains demonstrate numerous streak and punctuate hemorrhages in association with the axonal damage. These small hemorrhages are not seen in young children with tDAI. It has been questioned whether the vessels of young children do not tear because they are very elastic. Another possibility is that there is vascular injury without disruption of the vessels and that vascular damage causes failure to perfuse leading to hypoxia.

BAPP immunohistochemical reactivity is a very sensitive method of detecting axonal injury. Young children with AHT have been studied in several series to determine whether tDAI or VAI occurs in these children. Many of these children obviously have VAI as they have suffered respiratory arrest or distress early on in their course. Some of these children have evidence of both types of axonal injury present. Typically, tDAI produces a pattern of scattered small groups of axons reactive for BAPP in hemispheric white matter, corpus callosum, internal capsule, and brainstem. VAI appears to produce a pattern of reactive axons described as broad geographic areas often related to vessels. One possibility is that although young children with AHT frequently show the VAI pattern of damage, the more subtle pattern of tDAI is being obscured under the more pervasive damage caused by the VAI. Another possibility is that the pattern of VAI in young children with AHT is changed by the damage to small blood vessels and differs from VAI seen in non-AHT cases.

The first cases of intrauterine causes of hypoxic ischemic encephalopathy studied have demonstrated no or very minimal VAI BAPP

reactivity. These cases have survived several days with severe hypoxia which may have also existed for some period intrauterine. All cases demonstrate typical ischemic changes on H & E staining.

Because the axonal injury in cases of AHT may be present in a mixed pattern, it is important to study a non head injured group to discern true VAI patterns. An issue of great interest in AHT in children is the timing of the injury. If tDAI is the basis of AHT in young children, certain predictions can be made about the timing of the injury. Because tDAI is a type of immediate impact injury, the injury or disruption of the axonal processes occurs at the moment of injury and not subsequently as a result of hypoxia or increasing intracranial pressure. Damage of axonal processes at the level of the thalamus and deep gray matter has been demonstrated in experimental primate models of DAI as well as in the CT examination of brains of known human cases of tDAI to be lesions which produce immediate onset of unconsciousness.

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**Abusive Head Trauma, Axonal Injury, Hypoxic Encephalopathy**

## **G128 Correlation of Antemortem and Postmortem Retinal Hemorrhages in Children**

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After attending this presentation, attendees will gain information about retinal hemorrhages with regards to length of survival, cardiopulmonary resuscitation, level of intracranial pressure, coagulation parameters and presence of trauma.

This presentation will impact the forensic science community by providing a better understanding of the evolution of retinal hemorrhages in the pediatric population.

Retinal hemorrhages have been recognized as an important indicator of abusive head trauma for many decades. The ability to document the presence of intraocular injuries has greatly improved through the use of wide-field digital retinal imaging. Accurately describing the number, location, and morphology of retinal hemorrhages in children is extremely important for diagnostic as well as legal purposes.

Peripheral retinal hemorrhages are often asymptomatic and may be discovered incidentally during an ophthalmologic examination. Many systemic and ocular disorders are known to be associated with a mild nonspecific hemorrhagic retinopathy, such as hypertension, blood cell disorders (i.e., leukemia, anemia, idiopathic thrombocytopenia), sepsis, vasculitis, cerebral aneurysms, vitamin deficiencies (thiamine, vitamin C), retinal infections, increased intracranial pressure, prematurity and alterations in sodium, oxygen and glutaric acid levels. Also included in the differential for a few posterior pole retinal hemorrhages is abusive trauma. In most of these situations; however, the historical, systemic, or ocular findings allow for the correct diagnosis.

Birth related retinal hemorrhages have also been well documented in the literature, with an occurrence rate ranging from 3 to 50 percent. Vacuum-assisted vaginal delivery is a known risk factor for birth related retinal hemorrhages while delivery by cesarean section has a low rate of retinal hemorrhages. It is important to realize; however, that infants delivered by cesarean section may have failed prior vaginal delivery attempts. The retinal hemorrhages associated with birth may occur in all retinal zones, similar to that seen in suspected abusive head trauma. Over 90 percent of birth related intraretinal hemorrhages resolve within two weeks and none were detectable after four weeks in one study. Researcher and clinicians tend to agree that extensive confluent retinal hemorrhages identified in a child over the age of one month are not birth related.

Proper identification and description of retinal hemorrhages is crucial for developing an appropriate differential diagnosis. Traditionally, the gold standard for retinal examinations required the use of an indirect ophthalmoscope through neurologically or pharmacologically dilated pupils. Images could be taken through the ophthalmoscope; however, many times the hemorrhages seen by the ophthalmologist were crudely drawn in the

patients chart. Wide-field digital retinal imaging with a RetCam is being increasingly utilized over the indirect ophthalmoscope, particularly in pediatric patients. Images can be stored as part of the electronic medical record and therefore are available for evaluation at later dates.

Different techniques have been employed at the time of autopsy to visualize and document retinal hemorrhages. The "gold standard" and most commonly employed procedure is removal of the eyes and adjacent periorbital tissues. The eyes are subsequently fixed in formalin for a minimum of two weeks, sectioned into superior, inferior and middle segments, photographed and embedded into paraffin blocks which are made into glass slides. This process allows for the distribution, location and number of retinal hemorrhages to be assessed. Additionally, hemosiderin deposition, an indicator of prior hemorrhage, into the retina, around the optic nerve or in the periorbital tissues can be assessed. Postmortem monocular indirect ophthalmoscopy has been used by some as an inexpensive adjunct to enucleation; however it can be a challenging technique to master and is not widely used.

A large part of the problem in determining the significance of retinal hemorrhages is that their pathophysiology is not well understood. Several hypotheses exist; including raised intraocular venous pressure due to a sudden rise in intracranial and central venous pressures, hypoxia, single blunt impact, and increased intrathoracic pressure. Vitreoretinal traction due to cycles of rapid acceleration and deceleration is thought to be the causative process by some researchers.

In this presentation, antemortem documentation of retinal imaging (photographs and/or chart diagrams) will be compared to the postmortem retinal findings of children whose deaths fell under the jurisdiction of the Harris County Institute of Forensic Sciences from 2005 - 2011. Only cases with both antemortem and postmortem data will be analyzed. Progression or non-progression of hemorrhages will be described with regards to age, survival period, cardiopulmonary resuscitation attempts, intracranial pressure (when available), presence or absence of intracranial trauma, presence or absence of extracranial trauma, optic nerve sheath hemorrhage, coagulation parameters and cause of death. As intracranial pressure increases and patients become coagulopathic, it seems logical that the number of hemorrhages would increase; however this type of correlation in a large medical examiner's office has not been reported in the literature.

Ophthalmologists are often consulted to examine the retinas of children who present to the hospital with evidence of trauma and/or hypoxic encephalopathy. The time frame for the examination varies – depending on the degree of suspicion for abuse by the treating physician. Should these results indicate progression of retinal hemorrhages with increasing time and intracranial pressure, clinicians may opt for a "sooner-rather-than-later" approach to ophthalmologic examinations in order to properly interpret the results.

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**Retinal Hemorrhages, Pediatrics, RetCam Images**

## **G129 Spontaneous Non-Traumatic Subarachnoid Hemorrhage With Retinal and Optic Nerve Sheath Hemorrhages Associated With Segmental Cerebrovascular Fibromuscular Dysplasia**

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After attending this presentation, attendees will learn that retinal hemorrhages and optic nerve sheath hemorrhages can occur in young children who have a spontaneous non-traumatic subarachnoid hemorrhage.

This presentation will impact the forensic science community by emphasizing the need for routine postmortem ocular examinations in young children dying suddenly and unexpectedly.

Spontaneous non-traumatic subarachnoid hemorrhage (SAH) in children is usually attributed to arteriovenous malformations, aneurysms, neoplasm, infection, leukemia, hemophilia, or sickle cell disease. Aneurysms may arise with conditions such as polycystic kidney disease, Ehlers-Danlos syndrome, fibromuscular dysplasia (FMD) and other conditions. A case of a child with bilateral retinal hemorrhages (RHs) and optic nerve sheath hemorrhages (ONSHs) associated with a spontaneous SAH arising from a ruptured right vertebral artery aneurysm due to segmental FMD is reported.

A previously healthy 4-year-old boy had a witnessed collapse at his daycare facility. Paramedics found the child in asystole at the scene where he was intubated and received cardiopulmonary resuscitation along with epinephrine and atropine. By the time he reached the medical center he was in sinus tachycardia and had no neurological response. His cranial computed tomography revealed diffuse subarachnoid hemorrhage with marked cerebral edema causing cisternal effacement and downward displacement of the cerebellar tonsils. No parenchymal lesion was visible to account for the hemorrhage. Due to the grim prognosis, organ procurement was contacted and viable organs were recovered for transplantation after declaration of clinical brain death.

No clinical fundal examination was recorded in the medical record; however, postmortem monocular indirect ophthalmoscopy revealed bilateral RHs over the posterior poles. His autopsy revealed diffuse cerebral and spinal cord subarachnoid hemorrhage due to a ruptured right vertebral artery aneurysm arising within segmental fibromuscular dysplasia. Additionally, there was intraventricular hemorrhage, cerebral edema with cerebellar tonsillar herniation and hypoxic ischemic brain injury, pituitary necrosis and patchy acute spinal cord infarcts. He had bilateral optic nerve sheath hemorrhages, most pronounced in the optic nerve-globe junction. The bilateral ONSHs were located not just in the subarachnoid space but were also subdural and intra-dural with extension in the adjacent periorbital soft tissue. Blood extended along the pial septa into the interior of the left optic nerve. The left globe contained multiple RHs involving the nerve fiber layer with focal pre-retinal extension. No FMD was in the globes or orbital soft tissues.

Vitreous bleeding associated with SAH was first described by Moritz Litten in 1881; however, the association of vitreous hemorrhage concurrent with intracranial bleeding has been attributed to Albert Terson and later broadened by some authors to include any intra-ocular hemorrhage. It was initially believed that blood from a SAH could track along the optic nerve sheath and penetrate the lamina cribosa to appear in the vitreous space, but this theory has been refuted because a connection has not been demonstrated on electron microscopy. Another theory suggests that rapid increase in intracranial pressure transmitted through the optic nerve sheath and impairs venous drainage to the cavernous sinus, resulting in venous congestion and rupture of retinal vessels.

A noninflammatory, nonatherosclerotic disorder, FMD is characterized by abnormal cell growth in the walls of medium and large arteries leading to stenosis. The etiology is not clear but believed to relate to hormonal and mechanical factors. It is familial in about 10% of cases and affects the renal arteries about 60-75% of the time. The extra-cranial cerebrovascular arteries are involved in 30-60% of cases.

Extra-renal FMD can cause critical stenosis, aneurysm formation and rupture or cerebral thromboembolism.

Many authors consider that RHs in young children are uncommon in the absence of abusive head trauma. This case highlights the lack of clinical fundal examinations in young children when abuse is not suspected and the importance of routine postmortem ocular examinations in young children who die suddenly and unexpectedly. Although the brain and spinal cord had only subarachnoid hemorrhage, the presence of subdural hemorrhage in the optic nerve sheaths suggests that a sudden rise in intra-cranial pressure preceded the ONSHs and RHs.

**Forensic Science, Retinal Hemorrhage, Non-Traumatic Subarachnoid Hemorrhage**

## **G130 Complicated Suicide Versus Autoeroticism? A Case Involving Multiple Drugs and a Porta-Potty**

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After attending this presentation, attendees will appreciate a bizarre circumstance of suicide that initially masks itself as a homicide. Moreover, attendees will learn to recognize alternative behavioral practices associated with paraphilias and transvestism that may be present at the scene of a death.

This presentation will impact the forensic science community by presenting a unique and bizarre case of a suicide in a Porta-potty. The necessity of thorough investigation including autopsy is emphasized whenever scene investigation suggests the possibility of homicide. Careful correlation of autopsy findings and death scene investigation are essential in determining how a death may have occurred. Moreover, toxicology findings can provide abundant evidence regarding not only the cause of death but also the manner of death. In the current case, the extremely high level of tramadol in combination with amphetamine provides a tremendous amount of evidence for suicidal overdose.

Suicides can mimic homicide until further investigation and pathology are reviewed. In this report, a unique case of suicide by drug overdose with possible contributing factors of positional asphyxia, hyperthermia, and exposure to the chemical contents of a "porta-potty" is described.

A 36-year-old male was found dead in the waste receptacle of a porta-potty in a park on a late afternoon of a hot August day. Police were notified of an abandoned vehicle in the parking lot adjacent to a local park. The interior and exterior of the vehicle were covered with hand-drawn graphic images and writings alluding to the male genitalia and homosexual acts. The vehicle contained several articles of clothing and an open gym bag. The bag contained personal lubricant amongst other items. The driver's seat contained several tubes of lipstick that matched the color of writing on the car. The decedent was found in the tank of the portable toilet at the edge of the parking lot. The decedent's leather jacket, vehicle keys, and additional tubes of lipstick were found inside the porta-potty.

Scene investigation revealed an obese man lying in the fetal position within the tank of a porta-potty. The decedent was noted to be shirtless, wearing only women's stockings. To allow for further investigation and autopsy, the body was extricated from the basin of the toilet by cutting the top portion of the tank off.

At autopsy, the approximately 100kg decedent was noted to have early signs of decomposition, including skin discoloration, slippage, and bloating. He was wearing women's makeup, a pair of nylon leggings, and a left nylon sock. The body exhibited blue debris and had a chemical smell. The flanks, back, and arms showed confluent linear abrasions. Multiple apparent chemical burns overlapped the abrasions. The lesions were dry, thickened, and firm.

On internal examination, the abdominal organs showed signs of decompositional change but few other abnormalities. The stomach contained approximately 150ml of brown, viscous fluid which contained at least three capsules exuding a viscous material. A battery-operated vibrating device was present within the rectum.

Urine, serum, bile, and vitreous fluid samples were submitted for toxicological testing. The urine drug screen was positive for amphetamine, tramadol, methylphenidate, nicotine, cotinine, and caffeine. Serum drug testing revealed the presence of ethanol (50mg/dL), formic acid (45mg/L), tramadol (140mg/L), O-desmethyltramadol, methylphenidate, ritalinic acid, and-amphetamine (13.5mg/L).

Subsequent police investigation failed to reveal any evidence that suggested someone other than the decedent was involved in this death.

Family members denied knowledge of homosexual tendencies, as well as suicidal ideation. Nevertheless, DNA analysis was run on several samples collected from the obtained lipstick, swabs of the porta-potty, and body swabs; the results proved to match the DNA of the decedent.

Based on the autopsy findings, as well as the historical and investigative information surrounding the case, the cause of death was listed as the combined toxic effects of formaldehyde, amphetamine, and tramadol with possible contributing factors of positional asphyxia and hyperthermia. The manner of death was ruled "suicide."

Cases of suicides can mimic homicide upon initial investigation.<sup>1</sup> Nevertheless, the unique presentation of this case is of importance. An exhaustive literature/media search was unable to obtain a similar case. Cases of individuals getting trapped in the basins of chemical toilets have been reported in the media, but none resulted in fatality.<sup>2,3</sup> Provided with the immediate evidence, homicide must be suspected. Furthermore, it is possible to conceive that a "hate-crime" against an individual who practices alternative sexual lifestyles occurred.

The results of police investigation, autopsy, and toxicology indicated otherwise. The pattern of the flank and other abrasions was isolated to the area of central obesity, indicating a methodic approach to minimize injury when entering the relatively small opening within the toilet seat. No other evidence of trauma was present at autopsy. Ultimately, the habitus of the man would likely have prevented an assailant from forcing the body through the opening without excessive trauma being incurred (or the assailant simply giving-up because of the difficult task at-hand).

It is important to note the bizarre circumstances surrounding the decedent's death. Paraphilias are occasionally described in the forensic literature and may be evident at scenes of suicide.<sup>4</sup> Although the family reported no known history of cross-dressing, it is likely that the decedent had a history of partaking in practices of transvestitism, as he was found with an anal vibrator while wearing women's clothing and make-up, indicating a level of sexual arousal associated with the act.

The primary component of many chemical toilets, including the one in this case, is formaldehyde.<sup>5</sup> At autopsy, firm, chemical burns were noted over several parts of the body. The skin hardening was essentially due to the fixing of the tissue. The primary metabolite of formaldehyde, formic acid, provides an additional mechanism of injury in cases of formaldehyde exposure. Upon absorption into the blood, formaldehyde is rapidly metabolized to formic acid. Normal formic acid levels range between 0 to 12mg/L. The decedent's formic acid level was 45mg/L, well above the normal range, indicating systemic absorption of formaldehyde had occurred.

Fatal outcomes attributed solely to amphetamine have been observed in a wide range of blood amphetamine concentrations. In a review of 17 fatalities attributed solely to amphetamine, the peripheral blood concentration ranged from 1.1 to 7.4mg/L.<sup>6</sup> Therefore, the high concentration of amphetamine (13.5mg/L) reported herein is remarkable.

Tramadol is a synthetic analog of codeine that possesses opiate-like properties; however, tramadol is not derived from an opiate. Although fatal intoxication with tramadol is rare, several cases have been previously reported.<sup>7,8</sup> The highest reported blood tramadol level for cases of Tramadol intoxication alone was reported to be 15.1mg/L.<sup>7</sup> In a separate case of multiple drug intoxication, the highest reported blood tramadol level was 38.3mg/L,<sup>9</sup> at least 100 times the therapeutic range of 0.1 to 0.3mg/L.<sup>7,10,11</sup> The decedent reported in this case had a tramadol level of 140mg/L, which is approximately 470 times the therapeutic range. In no other case could such a level of tramadol be found in the literature.

With no anatomic explanation for death but a sufficient toxicological explanation, it would be reasonable to rule this death as simply being due to the combined toxic effects of the several drugs and toxins identified. However, because of the unique body position and the history of an extremely hot outdoor environmental temperature, positional asphyxia and hyperthermia are potential contributing factors in this death.

In a "complex suicide," more than one mechanism is applied resulting in death.<sup>12</sup> The case presented exhibits multiple factors contributing to the decedent's suicide; however, the secondary insult resulting from the constraints of the toilet basin, heat, and chemical contents of the toilet were

possibly unintentional mechanisms contributing to death. Therefore, the suicide more likely represents a "complicated suicide" – the initial insult resulting in suicide (the substances ingested) allows for a secondary insult that was not originally planned as part of the suicide.<sup>12</sup>

The current case is of interest to the forensic community for a variety of reasons. The case emphasizes the importance for forensic investigators and police to be aware of unorthodox practices and paraphilias. The bizarre practices of the decedent in this case produced a scene that suggested the possibility of a homicide. Whenever scene investigation suggests the possibility of a homicide, it is imperative that a thorough investigation be performed, including the performance of an autopsy. Careful correlation of autopsy findings and death scene investigation are essential in determining how a death may have occurred. Accurate toxicology findings can provide abundant evidence regarding not only the cause of death but also the manner of death. In the current case, the extremely high level of tramadol in combination with amphetamine provides a tremendous amount of evidence for suicidal overdose.

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#### Suicide, Tramadol, Porta-Potty

## G131 Anaphylaxis After the Injection of Buprenorphine

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After attending this presentation, attendees will become aware of the possibility of an anaphylactic reaction due to the intravenous use of buprenorphine, and attendees should consider analysis for tryptase in cases of its misuse.

This presentation will impact the forensic science community by increasing the awareness of the possibility of an anaphylactic reaction following the injection of abused prescription medications.

Buprenorphine is a partial opioid agonist that was developed for pain management in the 1970s and in 2002 became available for opioid substitution therapy (OST) in the United States. The formulations designed for OST are sublingual and include Subutex<sup>®</sup>, a tablet that contains only buprenorphine, and Suboxone<sup>®</sup>, a formulation of buprenorphine and naloxone in a 4:1 ratio designed to reduce the diversion and misuse of buprenorphine by causing withdrawal symptoms when administered intravenously. Despite the addition of naloxone to buprenorphine formulations, the drug is still diverted and misused. Buprenorphine is relatively safe when used appropriately, however multiple deaths have been reported due to buprenorphine use and misuse both with and without concomitant use of other drugs. In several of the cases reported, the levels of buprenorphine were not considered to be toxic; however, in many of these cases, since there was no other identifiable cause of death, buprenorphine toxicity was still listed as the cause of death. Two cases of anaphylaxis in decedents who had injected buprenorphine formulations just prior to death and suggest that anaphylaxis should be considered in individuals who misuse buprenorphine via intravenous administration regardless of the postmortem buprenorphine concentration are presented.

In 2009 and 2010, autopsies were performed on two individuals who were witnessed to die suddenly after the injection of buprenorphine. Initial thoughts were that the deaths were due to buprenorphine intoxication, however toxicologic analysis showed elevated tryptase. The first case was a 29-year-old woman with a history of asthma, drug abuse, and an allergic reaction in the past. Investigation showed that she and her boyfriend had bought 20 tablets of alleged 0.2mg buprenorphine via the internet from a Philippine based pharmacy. They soaked the tablets in water, filtered them and prepared two syringes each with a 1mg dose. After purging the air from the syringe, she injected the solution and was witnessed to immediately gasp for air and collapse. Her boyfriend administered two puffs of her albuterol inhaler without benefit. She received multiple doses of naloxone and epinephrine from EMS and in the ER. Analysis of the contents of the syringes revealed no buprenorphine. Autopsy showed hyperinflated lungs with mucous plugging of the airways, peribronchial smooth muscle hypertrophy and eosinophilic infiltration. Postmortem toxicology screen was positive for naloxone (22 ng/mL) and elevated serum tryptase concentration of 179ng/mL. The cause of death was Anaphylactic reaction complicating asthma and the manner was Accident. The second case was a 30-year-old woman who was three months postpartum with a history of heroin abuse. Investigation showed that her boyfriend witnessed her complain of not feeling well after injecting herself with Suboxone<sup>®</sup> from a 8mg/2mg strip that she had purchased on the street. She then went to her bedroom was found to be unresponsive five minutes later. Autopsy showed rare polarizable foreign material in the pulmonary macrophages, scattered multinucleated giant cells in the lungs and focal interstitial hemorrhage and edema of the larynx; however, no mast cells were identified. Postmortem toxicology screen was positive for buprenorphine (17ng/mL), norbuprenorphine (7.6ng/mL), and naloxone (96ng/mL). Postmortem serum tryptase concentration was elevated at >200.0ng/mL. The cause of death was Anaphylactic reaction due to intravascular injection of Suboxone<sup>®</sup>, and the manner was accident.

Anaphylaxis is an acute immunologic systemic reaction an allergen via IgE receptor activation on mast cells and basophils with the release of inflammatory mediators including histamine, tryptase, prostaglandins, and leukotrienes. Anaphylactic reactions can be elicited by many substances; therefore it is possible that any constituent in either formulation abused in these cases elicited the anaphylactic reactions. The postmortem diagnosis of anaphylaxis depends upon a complete investigative history with focus on the details of the reaction and the event surrounding it as there is individual variation in symptomatology. Investigation should include questions on how the decedent reacted immediately prior to death, how the person has reacted before from exposure to the substance, and a complete medical history including history of asthma or allergies. Autopsy should include gross and

microscopic evaluation of the airway and toxicologic analysis for serum for tryptase. Postmortem tryptase concentration has generally been considered elevated when greater than 10ng/ml. It has also been reported that postmortem tryptase can be elevated in the absence of anaphylaxis but usually not to the degree seen in these cases.

Given the increasing use of buprenorphine in opioid replacement therapy and the recent increase in the quantities of diverted buprenorphine being seized by law enforcement, the possibility of an anaphylactic death should be considered when the drug is misused. Serum tryptase concentration should be assessed prior to assigning the cause of death as buprenorphine intoxication in these types of cases. Elevation of tryptase is particularly useful in confirming the diagnosis of anaphylaxis triggered by injection of a medication or agent that is not normally used intravenously.

#### **Anaphylaxis, Buprenorphine, Injection**

### **G132 Increasing Efficiency in the Autopsy Suite: Rapid Drug Screening on Pericardial Fluid**

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After attending this presentation, attendees will understand usage of pericardial fluid for toxicology testing, and how to increase efficiency in the autopsy suite by utilizing rapid drug screens.

This presentation will impact the forensic science community by increasing efficiency during autopsies, reducing unnecessary laboratory work, and showing other offices how to reduce costs while maintaining high quality forensic work.

Suspected illegal and prescription drug abuse compose a large subset of autopsies that are performed. However, there are also many cases in which the death can be attributed to a natural cause, if drug abuse can be eliminated as contributing factor. In order to reduce the amount of toxicology work to be done by the laboratory, the medical examiners employ rapid urine testing at the time of autopsy in such cases. The TLC based test gives qualitative results for presence or absence of amphetamine, methamphetamine, cocaine, and PCP, the four most common illegal drugs associated with sudden death. If no drugs are detected, a cause and manner can sometimes be assigned at the end of the autopsy, expediting closure for the family. However, many of these cases yield toxicology laboratory work anyway, due to the lack of urine or unsuitable samples of urine in the body.

Several bodily fluids are available at autopsy. There is no doubt that fluid specimens are much more convenient to be handled for drug and chemical analyses than solid tissue specimens. The qualitative usefulness of urine and bile for analysis of drugs is well known, but there is little information on the usefulness of pericardial fluid in spite of usually sufficient amounts available at autopsy. Pericardial fluid is an ultra-filtrate similar to urine. Many of the substances that are detected qualitatively in urine are also detectable in pericardial fluid. By utilizing the same TLC based assay that is originally designed for rapid urine drug testing, 59 cases at the office were evaluated simultaneously via quick pericardial fluid testing, quick urine testing, and normal toxicology assays on routine toxicology specimens (routine toxicology assays include HPLC, gas chromatography, mass spectrometry and combinations thereof). Of the 59 cases, four were homicides, 15 were accidents, 10 were suicides, and 30 were classified as naturals. No decedents under the age of 12 were included, nor were cases in which urine or pericardial fluid was not present for testing.

The results of the quick toxicology tests were photographed and evaluated, and then compared to the final toxicology results. The sensitivity and specificity of the quick urine screen was 100% and 73%, and for the quick pericardial screen was 95% and 84%, respectively.

By utilizing pericardial fluid as a substitute for when urine is not available for rapid drug screening at the autopsy table, excess laboratory work can be eliminated, as well as expediting issuing of a cause and manner

of death. This has a three-fold effect of lowering laboratory costs, decreasing death certificate turnaround time, and also allows for families and loved ones to reach closure sooner. Further research includes expanding the pericardial drug screen from illicit stimulants to include major prescription drugs of abuse (i.e., – opiates, benzodiazepines, etc.).

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**Pericardial Fluid, Drug Screening, Efficiency**

### **G133 Two Unique Cases of Volatile Substance Abuse Death: Huffing Halogenated Hydrocarbons With Plastic Bag Over Head**

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The goal of this presentation is to elucidate the mechanism by which two individuals died from inhalation of a halogenated hydrocarbon, assisted by securing a plastic bag around their necks.

This presentation will impact the forensic science community by better understanding of the mechanism of death in volatile substance abuse cases.

The inhalation of volatile substances for their intoxicating effect, an act popularly referred to as “huffing,” is a relatively new form of recreational drug abuse in the United States. These volatile substances are commonly found in consumer aerosol sprays, such as keyboard cleaners and air fresheners. Easy access and low costs make these products particularly attractive to adolescents.<sup>1</sup> Presented in this study are two unique cases in which individuals died from inhaling an aerosol spray (containing halogenated hydrocarbons) within the confines of a plastic bag secured around the neck. The plastic bag serves to potentiate the intoxicating effects of the aerosol, by increasing the concentration of chemicals in the user’s breathing space. Given the presence of the plastic bags, the cause of death in both cases was certified as asphyxiation by placement of plastic bag over the head while huffing. In both cases, the volatile substances of abuse were halogenated hydrocarbons: difluoroethane and chloroethane. The purpose of this study is to investigate the mechanism(s) of death, i.e. determine its toxic effect. There are a number of possible mechanisms that may have contributed to death in these cases: anoxia, aspiration, vagal inhibition, respiratory depression, suffocation after loss of consciousness, and cardiac arrhythmia (due to sensitization of the myocardium by the chemical agent and/or from carbon dioxide in the user’s environment).<sup>2</sup> In addition, volatile substance abuse puts the user at risk for “sudden sniffing death syndrome,” a fatal cardiac arrhythmia caused directly by the sensitization of the myocardium to endogenous epinephrine. This later mechanism is supported by animal experiments which show that numerous halogenated hydrocarbons are capable of inducing cardiac arrhythmias. In the forensic setting, the postmortem diagnosis of a cardiac arrhythmia is often a diagnosis of exclusion due to the lack of pathognomonic changes within the heart.<sup>3</sup> Arrhythmias are reported in the literature as being the most likely cause of death in volatile substance abuse cases, but this is usually speculation and cannot be proven without more specific postmortem evidence of cardiac arrhythmia.<sup>4</sup> In the two huffing cases another significant variable was the placement of plastic bags secured over the head. It is proposed that hypoxia also plays a role in the abuser’s death possibly by contributing to a fatal cardiac arrhythmia. Hypoxia has direct effects on the cardiovascular system and may heighten the cardiac response to epinephrine. For example, animal experiments suggest that cardiac sensitization is amplified by both volatile substances and the presence of hypoxia. It may be that the combination of a volatile halogenated inhalant, elevated epinephrine blood levels and hypoxia has the most cardiotoxic effect.<sup>2</sup> These two cases seem to demonstrate this triad of causation; alternatively, depression of central nervous system function and subsequent asphyxiation by plastic bag remains another possible explanation. Further studies are required for a more exact diagnosis.

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**Huffing, Difluoroethane, Chloroethane**

### **G134 The Short-Term Effect of a Prescription Drug Monitoring Program on Prescription Drug Overdose Deaths Investigated at the Minnesota Regional Medical Examiner’s Office**

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After attending this presentation, attendees will be made aware of the impact of the Prescription Drug Monitoring Program of Minnesota (PDMP) on the drug overdose deaths investigated at the Minnesota Regional Medical Examiner’s Office.

This presentation will impact the forensic science community by attendees’ participation in a discussion of the epidemic of drug overdose deaths observed in the United States and the evaluation of the Prescription Drug Monitoring Program, a possible solution to this phenomenon.

The increase of drug overdose fatalities in the United States is a major public health concern. This phenomenon was related to an increase in the number of prescriptions and use of opioid medications. In January 2010, the State of Minnesota adopted an operational PDMP similarly to 34 other states in the United States. The PDMP was initiated in an attempt to improve the control of the opioid prescriptions and subsequently to reduce drug overdose deaths. This studies goal is to evaluate the short-term impact of the Minnesota’s PDMP on prescription drug overdose deaths investigated at MRMEO from 2006 to 2010.

This observational and retrospective study was using data from MRMEO. The study population was all residents of MRMEO counties who died of prescription drugs from 2006 to 2010. The death certificates, scene investigation, autopsy and toxicology reports of 154 prescription drug overdose deaths were systematically reviewed.

The mean age of the decedents was 43.3 years (10-73 years) and 51% were female. The manners of death were in 48.7% of the cases undetermined, in 33% an accident and in 17.6% a suicide. The number of mixed drug cases was similar to the number of cases with a single drug. Opioid analgesics were the most prevalent drugs, leading with 132 deaths (85.7%). Psychotherapeutic drugs were involved in 69 deaths (44.8%). Two third of the deaths (67.5%) were due to a drug prescribed to the decedent. There were more psychotherapeutic drugs prescribed to the decedent than opioid analgesic drugs (75.4% and 64.4%, respectively). Prevalence of diversion was higher in male decedent cases (70.6%) and in younger decedent cases (mean age 36 years). Of all 154 cases, 34 (22.1%) had an association with alcohol. Methadone and oxycodone were the two most common opioid analgesics identified. Fentanyl was responsible for the highest percentage of single drug deaths in this study (14 (58.3%) of 24 deaths). Methadone and morphine were the two most frequently diverted drugs. Indeed, these two drugs were prescribed to the decedents in less than

half of the cases (49% and 44.5%, respectively). The benzodiazepines detected in 51 cases (33.1%) were mainly found in association with the other classes of drugs (94.1%). Over the first four years studied, prescription drug fatalities increased annually. In the fifth year of the study (2010), the PDMP was implemented in Minnesota, and we observed a trend of a decrease in the number of the prescription drug overdose cases, opioid analgesic overdose cases, and prescribed opioid analgesic overdose fatalities.

In conclusion, it was observed as a short-term effect a decrease in the number of drug overdose deaths investigated at MRMEO. Therefore, this result confirmed our initial hypothesis that PDMP in Minnesota is effective in prevention among our study populations, as the majority of drugs involved in overdose deaths investigated at MRMEO were prescribed to the decedents. However, the trend has to be confirmed by analyzing the data from MRMEO in the coming years, possibly in conjunction with other jurisdictions with different population demographics.

**Prescription Drug Overdose, Prescription Drug Monitoring Program, Minnesota**



## H1 Effect of Obesity on the Accuracy of Age-at-Death Indicators of the Pelvis

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After attending this presentation, attendees will learn how obesity, measured using the body mass index, affects the accuracy of skeletal age-at-death estimations based on the pubic symphysis and auricular surface.

This presentation will impact the forensic anthropology community by demonstrating that caution should be used when estimating age in obese individuals using the auricular surface, and that age-at-death estimations based on the pubic symphysis are preferred for obese individuals.

Age-progressive stages or macromorphological changes of the auricular surface of the ilium and symphyseal face of the pubic bone are commonly examined by forensic anthropologists to estimate adult age-at-death from skeletal remains. However, individual rates of progression through these stages can vary considerably depending on life history events such as diet, disease, physical activity, and body mass. Since the sacroiliac and pubic symphysis are weight-bearing joints, it is likely that the rate of progression through age-related stages is influenced by body mass, especially obesity. To date, no study has examined the effects of body mass on the progression of age-related morphological changes in the pubic symphysis or auricular surface. Since 1990, adult obesity rates have been dramatically increasing. As the number of obese individuals increase, so will the representation of obesity in forensic cases. Therefore, it is vital that forensic anthropologists know whether obesity affects the rate of progression through the different age stages in the pubic symphysis and auricular surface. This study investigates if obesity affects the age-related progression of morphology in the auricular surfaces and pubic symphyseal faces, and how these modifications affect the accuracy and precision of age-at-death estimates. In addition, sexual dimorphism in age-related changes was examined. It is hypothesized that obesity causes acceleration in degenerative age-related changes in these two anatomical regions, especially the auricular surface, and therefore, the inaccuracy of age-at-death estimations will be greater in obese individuals compared to those with a clinically normal body weight for stature.

The hip bones of 245 adults (23-90 years of age) of known age, sex, stature, and body weight from the William M. Bass Donated Collection were used in the study. Specimens with gross pathological anomalies of the pelvis or lower limb were not used. BMI was calculated for each individual by dividing recorded body weight in kilograms by stature in meters squared. Age-related stages were scored on the hip bones of 119 adults of clinically normal body mass (BMI between 19 and 25) and 126 obese (BMI  $\geq 30$ ) adults using the Suchey-Brooks method for the pubic symphysis and the Buckberry-Chamberlain method for the auricular surface. In some analyses the obese sample was subdivided into obese (BMI 30 to 39) and morbidly obese (BMI  $\geq 40$ ). In addition to the overall progression through the age-related stages, specific traits (transverse organization, surface texture, microporosity, macroporosity, and apical lipping) were scored for each auricular surface to reveal which of these features, if any, are affected by obesity. The accuracy of each method was calculated by subtracting the actual age from the mean and median age of the stage for the pubic symphysis and auricular surface, respectively. The correlation between actual age and the estimated age was calculated for both obese and normal body mass individuals using Pearson's correlation coefficient. Bias was calculated to determine the under- and over-aging results for the different

age groups of each population, while inaccuracy is the average absolute error of age without reference to under- or under- age prediction.

As expected, the degree of bias and inaccuracy generally increases with age using both methods regardless of BMI. However, young adult obese individuals exhibit greater inaccuracy in age-at-death estimations using the auricular surface, but not the pubic symphysis compared to individuals with a normal BMI. This is probably due to the greater weight-bearing function of the sacroiliac joint and postural changes during locomotion in obese individuals. In addition, age was estimated with less precision in obese individuals compared to clinically normal weight individuals for both methods. Obese males show greater inaccuracy than obese females. There was also a greater tendency to over-age morbidly obese compared to obese individuals. Morbidly obese individuals also exhibited greater inaccuracy than either clinically normal or obese BMI individuals. The specific characteristics all follow the same general pattern of onset regardless of BMI.

This study helps to elucidate how obesity affects the rate of age-related skeletal change of the human pelvis. The results indicate that the pubic symphysis method is preferred when estimating age in obese individuals, especially males. However, the results also indicate that forensic anthropologists should use caution when assessing age-at-death from the skeletons of obese individuals using either auricular surface or pubic symphysis methods.

### Obesity, Age-at-Death, Auricular Surface

## H2 A Test of the Mann Maxillary Suture Aging Method

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The goal of this presentation is to inform attendees about a test of the Mann et al. (1987; 1991) maxillary suture aging method performed on the Hamann-Todd Collection.<sup>1,2</sup>

This presentation will impact the forensic science community by demonstrating the accuracy of the Mann maxillary suture aging method on a large sample of known remains.

Accurate estimation of age-at-death from the human skeleton can be especially difficult in cases of fragmentary or incomplete remains. When presented with an isolated cranium, estimating the age-at-death is often limited to dental development, cranial, or maxillary suture closure. A previous test of the Mann method by Gruspier and Mullen found the variation in age was too great for recommendation of widespread usage in forensic cases, while another test suggested the method was useful when combined with other age indicators.<sup>3,4</sup> However, Gruspier and Mullen's sample was limited to White males predominantly over 40 years old, and the Ginter sample was heavily biased towards individuals above 60 years of age.<sup>3,4</sup> The goal of this project is to test the Mann method on a large, diverse sample, and to present a recommendation for forensic applications.

The present sample consists of 200 male and female individuals of European and African American ancestry from the Hamann-Todd Collection, with ages ranging from 10-82 years. Efforts were made to avoid any individuals expressing bony pathologies of the cranium as well as to create a balanced sample.<sup>5</sup> This large and diverse sample will allow for investigations into possible differences between sex and ancestry. Following Overbury et al, the side with greater fusion was scored as present when confronted by asymmetrical palatal fusion. Palatal suture closure was

scored using the criteria described in Mann et al. and Meindl and Lovejoy for cranial suture closure in order to readily compare the two scoring systems and determine if one system performed better than the other for estimating age from palatal suture closure.<sup>2,6</sup>

Preliminary results of this study support those findings of Gruspier and Mullen, although the findings of Ginter are not disputed.<sup>3,4</sup> Other than the reliably early fusion of the incisive palatine suture and the delayed or minimal fusion of the anterior median palatine suture into adulthood, too much variation exists in the fusion of the other sutures to recommend widespread use of Mann et al. in forensic contexts.<sup>2</sup> That does not mean the method has no value, as it is best used in conjunction with other methods in a supporting role. In addition, the Meindl and Lovejoy and Mann scoring methods each resulted in Spearman's correlations of  $r=0.377$  and  $r=0.380$  with age (in years), respectively.<sup>6,1</sup> While the Mann method has a larger correlation to age than Meindl and Lovejoy, this may not be significant in terms of scoring palatal fusion.<sup>1,6</sup>

Based on results of this study, the Mann method is most useful in separating younger from older individuals. Smith and Tondury, and more recently Kroman and Thompspon, demonstrated that cranial suture closure appears to be more closely related to individual brain and connective tissue (dura) development and somatic dysfunction than advancing chronological age.<sup>7,8</sup> In light of this finding, it is unclear if maxillary suture closure will follow this trend.

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#### Mann Method, Age-At-Death Estimation, Palatal Suture Fusion

### H3 Comparison of Vulture Scavenging Rates at the Texas State Forensic Anthropology Research Facility Versus Off-Site, Non-Forensic Locations

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After attending this presentation, attendees will gain an enhanced understanding of vulture scavenging occurring at the Texas State Forensic Anthropology Research Facility (FARF), and the impacts vulture scavenging may have on future forensic investigations involving decomposition rates obtained at this site.

This presentation will impact the forensic science community by presenting an overview of vulture scavenging at FARF in comparison to vulture scavenging at off-site, non-forensic locations. Furthermore, the results from this study illustrate the need to incorporate avian scavengers into models pertaining to scavenger succession to help insure an accurate interpretation of taphonomic events during forensic anthropology investigations.

FARF is an outdoor laboratory dedicated to studying the rates of human decomposition and has an abundance of turkey vultures and black vultures residing nearby. Both vulture species are obligate scavengers and common visitors to the FARF site, thereby providing an excellent opportunity to study how soon after death vultures will arrive to feed on either carrion or a human cadaver. However, vultures are intelligent birds capable of recalling their prior successful scavenging locations, which raises the question, "Have the vultures residing near FARF learned that this site provides a reliable source of food, and if so, are the vulture scavenging rates at FARF the same as scavenging rates at off-site, non forensic locations?"

Substituting pigs for human models, two series of decomposition trials were conducted at FARF and in surrounding locations on the Freeman Ranch, which is a 4,204 acre working cattle ranch, to determine at what point during the postmortem interval vultures will arrive to scavenge and if these arrival times are the same at FARF in comparison to off-site locations.<sup>1</sup> Trial one began on July 01, 2011 and Trial two began two weeks later. Each trial involved three fetal pigs weighing between 1.38kg and 2.06kg and three separate field placement sites. The first pig was placed at FARF, the second pig was placed at an off-site ranch location that remained constant during both trials, and the third pig was placed in an off-site location that differed between trials. The sites shared similar vegetation and geographical features, but all sites were at least 1.5km apart. The three pigs used during Trial one were placed at their corresponding sites on same day, and all pigs were left to decompose without the protection of a cage. Trial two involved identical methods, with the only difference being the start date. Furthermore, each site was equipped with a motion activated infrared wild life camera and a weather station programmed to record climatic variables using one-minute sampling intervals. The cameras and the weather stations were in operation 24-hours a day throughout the duration of the study.

Results indicate that vultures are the primary scavengers at FARF and surrounding Freeman Ranch locations. Vultures arrived at five of the six pigs during the first 17 hours following pig placement, and at two of the pigs, a Crested caracara (Mexican Eagle) fed alongside turkey vultures and black vultures. A canine living on the ranch property scavenged the sole pig not scavenged by vultures. The failure of vultures to scavenge this pig is attributed to the pig's placement occurring late in the day and close to the time that vultures were preparing to return to their roosts. The canine took the pig in the middle of the night approximately eight hours following field placement.

For each of the five pigs scavenged by vultures, the vultures arrived, skeletonized the pig, and departed the site in less than three hours. This rapid scavenging reduces the probability that forensic investigators will observe vultures feeding at a crime scene, but systematic field searches conducted during this research revealed that the presence of down feathers in the surrounding vegetation and an intact vertebral column can be reliable indicators of recent vulture scavenging. Lastly, no differences were detected between the vultures' arrival times and scavenging behavior at FARF compared to the off-site locations, indicating that vultures are currently not impeding on the applicability of uncaged decomposition rates obtained at this facility.

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#### Vulture Scavenging, Postmortem Interval, Taphonomy

## H4 Validation of Two Age Estimation Methods Based on the First and Fourth Ribs in the Colombian Population

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The goal of this presentation is to show the application of two aging methods, one with the first rib and one with the fourth rib in the Colombian population.

This presentation will impact the forensic science community by showing how the aging process of the first rib on the costal facet and the face of the tubercle, as well as the morphological changes of the costal facet of the fourth rib and the general aspects of each method and their application to Colombian populations.

This study was carried out with the skeletal collection curated by the National Institute of Legal Medicine and Forensic Sciences in Bogotá, Colombia. The collection consists of 135 adult individuals, both male and female, with ages ranging from 19 to 93 years at the time of death.

The method used for the first rib was developed by DiGangi et al.<sup>1</sup> In this method, the geometric features of the costal facet are observed together with the texture of the surface of the tubercle. Each one is assigned a score according to the tables developed for this method. Changes were observed and recorded, excluding structures that showed diseases, where the area of interest was concealed by remnants of dry soft tissue, were incomplete or deteriorated.

The Loth & Iscan method was used for the fourth rib.<sup>2</sup> This method observes the changes produced by the aging of the costal face; morphological changes of this area were recorded, taking into account the features present on the articular surface, the borders, the walls, the depth and shape of the pit, and the porosity and quality of the bone in general. Both studies took into account both the right and left ribs, regardless of sex.

Results indicate that current methodologies utilizing both the first rib and fourth rib capture age-related change more accurately in young and middle-aged adults. For the first rib, the correlation coefficient between the best point estimate and real age was 0.623 ( $p$ -value < 0.001). Not surprisingly, adults aged 60 years and older had the most amount of error associated with their age estimates. In the case of the first rib method, these individuals were most likely to fall outside of both the 50% and 95% posterior density regions and in the case of the fourth rib method, these individuals often fell outside of the mean and standard deviation. Overall, the first and fourth rib methods do appear to contribute to age estimates and should continue to be tested.

These efforts to validate the methods according to the characteristics of the Colombian population provide more scientific rigor to the forensic work in the country. This contributes to the standardization process in the research of the biological profile of modern populations. Additionally, estimates will be more precise and they will strengthen the practice of forensic anthropology in Colombia and its role in the resolution of criminal cases.

The most important aspect of these projects is that they provide support to the families of the victims of the Colombian armed conflict. They are part of the resolution of forensic cases and facilitate identification. Consequently, they contribute to the return of individuals to their families.

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### Age Estimation, First Rib, Fourth Rib

## H5 Human Identification from CT and MRI Scans: Novel Approaches to an Old Problem

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After attending this presentation, attendees will learn about the various ways in which computerized tomography (CT) and magnetic resonance imaging (MRI) scans can be used for comparison with radiographs, scans and models of unknown decedents to establish personal identity.

This presentation will impact the forensic science community by demonstrating novel approaches to human identification based on CT and MRI medical records. These approaches allow multiple avenues for antemortem/postmortem radiographic comparison and address the issue of pattern recognition.

Prior to the advent and widespread use of computerized tomography (CT) and magnetic resonance imaging (MRI) techniques in clinical medicine, patients who had sustained traumatic injury to the head and face were evaluated using plain film radiography. Anthropologists used these skull series and various facial radiographic projections to compare with postmortem radiographs of unknown decedents to establish the decedent's personal identity using frontal sinuses, grooves for the middle meningeal arteries, the *sella turcica*, and other structures thought to be morphologically unique in each individual. Over the past decade, plain film radiology has been abandoned as a diagnostic tool for most head and face injuries. Instead, the more detailed and clinically useful CT and/or MRI are used. This shift in diagnostic radiology has resulted in a dearth of available plain film radiographs from which to identify putative decedents. Investigators are, instead, presenting CT and MRI studies to anthropologists, while anthropologists are generally limited to plain film technology for radiographic imaging of skeletal remains. CT and MRI scans may be available as a film series, digital imaging and communications in medicine (DICOM) images on a digital recording medium, or scan volume data.

An obvious solution is to perform a CT scan on the unidentified cranium, matching the transverse or axial images between the antemortem and postmortem scans. Volume scan data may be used to recreate, in three dimensions, a virtual model of the skull that provides further information, such as frontal sinus volumes, upon which identity may be established. However, this route towards identification is costly and dependent on the availability of CT and MRI scanners (which are usually running at capacity in clinical areas), and one must deal with the probability that the two scan formats may not be precisely aligned. Another solution is to use the antero-posterior and/or lateral "scout films," taken as part of the CT or MRI protocols, as a proxy for the antemortem "plain film" which can then be compared to postmortem images. However, scout films are small and generally become pixilated when expanded to a useable size.

More creative uses of CT and MRI scans are:

- Using axial scans of the head to capture the dental pattern of the putative decedent for comparison with a postmortem odontogram. The investigator may then use Adams et al. OdontoSearch 2.0 program to determine the relative frequency of the matching pattern, and then assign probative value of the match based on that information.<sup>1</sup>
- Using scan volume data to produce a three-dimensional model of the putative decedent's skull, this can be compared with the unknown specimen both metrically and via digital radiography. This method requires access to a three-dimensional model printer. Once the model is printed it can be radiographed. Comparison of this "antemortem" film with the radiographs of the unknown skull is only limited by the resolution of the printed models. In most cases; however, the model represents a faithful reproduction of most of the anatomical variants used by anthropologists to establish identity. Additionally, one may compare metrics obtained from the

model, the unidentified skull, and the population from which the individual is derived.

- Evaluating scans for idiosyncratic variation, pathology or trauma that might correspond with findings based on examination of an unknown skeleton.

This presentation will use case reports to explore the various ways in which CT scans may be used to establish personal identity in forensic contexts.

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- <sup>1</sup> Adams BJ, Shigeta CK, Drogosch AC, Schumann RW. OdontoSearch version 2.0. Joint POW/MIA Accounting Command, Central Identification Laboratory, 2007.

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### Forensic Anthropology, Human Identification, Radiographic Comparison

## H6 Taphonomy of Infant and Child Sized Remains in Western North Carolina

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After attending this presentation, attendees will have a better understanding of the arrival sequence of scavengers, their impact on the decomposition, scattering, and recoverability of remains deposited both on the surface and buried in western North Carolina.

This presentation will impact the forensic science community by highlighting the impact avian and mammalian species have on the scavenging and scattering of infant and child sized remains placed within a wooded area of western North Carolina.

There have been limited studies of the scavenging and scattering of infant and child sized remains. This research focuses on the relationship that scavengers play in the decomposition process and the associated pseudotrauma that they can cause to remains, and examines the recoverability of scavenged remains. These data are of importance to personnel tasked with the search and recovery of such remains, as well as the pathologist and the physical anthropologist.

The first phase of this research was conducted from February to July, 2011. Six sets of pig remains weighing less than 10 pounds were placed in a wooded environment both on the surface and buried. Three areas were chosen to place remains. Each area contained a shallow burial and a surface deposit. In Area 1, the burial and surface deposits were both covered with small piles of brush. In Area 2, both sets of remains were wrapped in common shopping bags from the local market. In Area 3, the remains were deposited with no additional alteration. Motion activated game cameras were utilized to track scavenger activity and field visits were conducted to track changes in the remains.

The second phase was conducted from March to June, 2011 and involved the deposition of one 30 pound pig on the surface in a wooded environment. Multiple motion activated game cameras were utilized to track scavenger activity and field visits were conducted to track changes in the remains.

The two phases of this research project resulted in varying observations of animal behavior in response to the remains. In the first phase no buried set of remains was disturbed, whereas two sets of surface remains were completely removed from the area. The set of remains placed on the surface in a bag was finally fully scavenged but only after an extended period of time. In the second phase arrival and contribution of the scavengers was not as expected from previous studies. In particular, the role of canines seemed to be highly diminished in comparison to avian involvement with scavenging and scattering.

This research shows the relationship that scavengers in western North Carolina have to the destruction of infant and child sized remains. It is

recommended that additional studies be conducted throughout the United States. By becoming more familiar with the results professionals involved with the search and recovery of infant and child sized remains will be better prepared to develop effective search strategies in cases where scavenging and scattering of remains have occurred.

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### Taphonomy, Scavenging, Forensic Archaeology

## H7 I'd Give My Eye Teeth for Cementum Increment Analysis

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After attending this presentation, attendees will gain an understanding of how dental cementum is deposited and how it is used to determine age and season at death in humans. Specific to this talk will be a discussion of which tooth to choose for dental cementum increment analysis for season at death.

This presentation will impact the forensic science community by marking one step in preparing a new method for use throughout anthropological and odontological forensics.

Zooarchaeologists have long used dental cementum increment analysis to estimate the season at death in mammals, yet testing the validity of using this method to determine season at death in human teeth is very limited and still on-going.<sup>1,2</sup> Cementum is the tissue that binds the tooth to the periodontal ligament, and in the interest of teeth being retained throughout adulthood, cementum is deposited throughout the course of life. From seminal zooarchaeological pieces, it was learned that cementum is secreted in pairs of bands each year. Pilot work by Wedel demonstrated that the seasonal transitions of the outermost cementum band in humans change from translucent to opaque in teeth extracted in fall/winter (October - March), and from opaque to translucent in teeth extracted in spring/summer (April - September).<sup>3</sup> Counting these pairs of bands and adding that number to the age at which the tooth erupts derives an age at death estimate. This study focuses on distinguishing the outermost increment in transverse thin sections of the middle third of the root by using transmitted polarized light microscopy.

Wedel<sup>3</sup> took Lieberman<sup>4</sup> and Wittwer-Backofen<sup>5</sup> at their word, namely that "any tooth will work." However, neither paper demonstrated the veracity of this statement by supporting it with data; they simply used all four types of teeth (incisor, canine, premolar, molar). All adult teeth that are erupted and in occlusion do exhibit increments (Wedel)<sup>3</sup>, but here is no consensus on which adult tooth derives the most repeatable and reliable results. Pinichi et al. tested the different types of teeth in predicting age and showed that premolars and third molars have the highest prediction efficiency.<sup>6</sup> Kagerer and Grupe's study supported the use of impacted third molars having very reliable precision in age determination.<sup>7</sup> On the other hand, they showed that premolars have one of the highest standards of deviation when cementum increment analysis was used to determine chronological age. Moreover, very few studies are testing the use of the outermost band of cementum to determine the season-at-death in humans. Recent studies using this method have focused on animals. Wall-Scheffler and Foley tested the use of dental cementum on first molars of sheep without justifying their research subject choice.<sup>8</sup>

This study seeks to test the hypothesis that the cementum bands transition from light to dark on multiple teeth from the same patient at the same time, and while examining 10 teeth from any one individual, determine whether one tooth type or another is most useful for determining season at death in humans. Two sample pools containing a total of 300 samples were included in the study. Sample pool #1 contains teeth donated by the patients from a school of dentistry. Ten individuals who each had 10 teeth extracted in the normal course of dental treatment were embedded,

sectioned, ground and polished, and examined under 10X magnification. Of these 100 teeth, 94 valid specimens resulted. The most precise results, where season observed matched actual season at extraction (our proxy for season-at-death) were obtained from the canines (eye teeth, cuspids). Sample pool #2 contains teeth donated by patients from a Santa Cruz doctor's office and are being evaluated at present. The data will be presented side by side.

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#### Dental Cementum Increments, Season at Death, Anthropology

## H8 Validation of the Kindschuh et al. (2010) Method for Determining Sex from the Hyoid Body

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After attending this presentation, attendees will understand: (1) the sex estimation method presented by Kindschuh et al.; (2) the significance of analyzing comparable datasets for metric studies; and, (3) the importance of utilizing modern skeletal collections for forensic studies.

This presentation will impact the forensic science community by illustrating the importance of utilizing comparable datasets for metric studies of sex determination. In addition, this study demonstrates that the accuracy of the Kindschuh et al. method may be difficult to judge when subtle differences in data collection are utilized.<sup>1</sup>

The hyoid bone is widely used in forensic contexts as an indicator of neck trauma; however, other studies such as the work of Kindschuh et al. have suggested that the hyoid bone can be used to estimate sex.<sup>1</sup> In their study, 398 hyoid bones were utilized from the Terry Collection to generate six discriminant function equations with classification accuracies that ranged from 82% to 85%.

Two of the equations developed by Kindschuh et al. exclusively utilized unfused hyoid bodies to estimate sex and the current study was designed to test the accuracy of those discriminant functions.<sup>1</sup> This study made use of original data collected by Devlin on a large sample of unfused hyoid bodies drawn from the William F. McCormick Collection (n=1,033).<sup>2</sup> Both males and females of European and African ancestry were utilized and age-at-death of the sample ranged from 20-79 years. Two measurements of the hyoid body were employed: body height and body length. In both studies, Kindschuh et al. and Devlin define these measurements identically; therefore, data from the McCormick Collection were applied to the Kindschuh et al. formula.<sup>1,2</sup>

While the original study performed with a rate of 82% accuracy, a rate of only 40% was achieved here and in the majority of cases, males were classified as females. This discrepancy is explained by decreased means for both body length and body height between populations. For example, in the Terry European-American sample, mean body length was 25.11mm for males and 21.04mm for females while in the McCormick European-American sample, mean lengths were 20.89mm and 17.67mm, respectively. Mean body heights follow a similar decrease in size between samples. For example, mean body height for Terry European-American males was 12.25mm and 10.46 mm for females, while in the McCormick sample mean heights were 11.04mm and 9.20mm, respectively. Overall, mean body length decreased by 17.01% in males and 16.01% in females and mean body height decreased by 9.88% in males and 12.04% in females.

While metric differences between the Terry Collection and contemporary samples like the McCormick Collection have been attributed to secular change in other studies, it is possible that the discrepancy observed here is due to variation in measurement technique.<sup>3,4</sup> Recent contributions have documented the importance of demonstrating low inter-observer error when multiple datasets are compared, though no studies have systematically compared techniques utilized for measuring the hyoid bone.<sup>5,6</sup> Additional data collection and direct comparison between the Terry and McCormick samples are necessary to elucidate a clear pattern.

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#### Sex Estimation, Hyoid Body, Metric Measurement Techniques

## H9 Pubic Aging Indicator Symmetry

*Kevin Hufnagel, MA\*, University of Tennessee, 250 South Stadium Hall, Knoxville, TN 37996; and Donna McCarthy, PhD, 1502 Northshore Woods Drive, Knoxville, TN 37919*

After attending this presentation, attendees will understand that age estimation from the pubic symphysis is not as straightforward as it appears and that assumptions about the methods employed may not always hold true.

anthropologists should consider more carefully how they create age estimates from pubic symphyses. Forensic anthropologists routinely determine age estimates from established and commonly-used methods as part of the biological profile. However, whether age is estimated by a student anthropologist or seasoned professional, precision of the estimation may be affected by as minor a factor as which side of a bone is chosen for analysis. In forensic cases where only partial remains are recovered, this factor becomes even more significant in age estimation.

If aging indicators are truly linked to biological age then it is logical to presume that, barring gross pathological exceptions, the left side indicators should mirror the right side, and vice versa. In this study, the degree of symmetry between the right and left sides is measured on various age indicators of the pubic symphysis. One hundred seventy-six pairs of pubic symphyses from the William M. Bass Donated Collection housed at the University of Tennessee, Knoxville were scored according to the definitions of 35 different aging indicators compiled from eight aging methods. Both

component and phase methods were used. Five of the variables are newly created variables based on variations or alternative interpretations of variable definitions from the literature. The two sides were observed independently by the same observer to maintain consistency.

The data were analyzed using simple statistical calculations on the differences of the observations between the sides. Calculations included the range of the differences and the magnitude of the asymmetry. The percentage of observations that demonstrated a difference between sides was calculated, as well as the average magnitude of asymmetry for those observations scoring differently on the two sides.

There is significant difference between the right and left pubic symphysis. Generally, the differences between the two sides are no more than one stage removed from one another. Thus, an aging indicator having only three or four possible states would be highly unlikely to score a difference measure of three or even two between the two sides. Likewise, methods with a large number of different states can more easily achieve a greater magnitude of asymmetry. Therefore, the percentage of observations that exhibited a difference bilaterally is more meaningful than the magnitude of the difference. None of the 35 variables tested exhibited 100% concordance between the two sides, although a few were relatively close. Eleven of the 35 variables had more than 30% of the observations differ between sides, with a maximum range of 47%.

These data illustrate that anthropologists cannot assume the right pubic symphysis will be aged the same as the left. This factor should also be taken into account when establishing definitions for new aging indicators.

#### **Aging, Pubic Symphysis, Symmetry**

## **H10 Decomposition of Dismembered Pig Carcasses in Insect Repellent and Conventional Waste Disposal Bags**

*Kassra L. Scholl, BSc\*, and Tal Simmons, PhD, School of Forensic & Investigative Sciences, University of Central Lancashire, Preston, Lancashire PR1 2HE, UNITED KINGDOM*

After attending this presentation, attendees will gain an understanding about how decomposition of heads, limbs, and torsos in separate, sealed plastic waste disposal bags proceeds; and, if insect activity and the decomposition process can differ between regular waste disposal bags and insect repellent waste disposal bags.

This presentation will impact the forensic science community by providing results from a controlled experiment in an area with very little previous research. This presentation will add to research being carried out in forensic taphonomy by broadening the understanding of how dismembered remains decompose in particular settings, enabling a better appreciation of these processes in human decomposition.

Dismemberment of remains to complicate identification and for ease of transportation may be encountered in homicide cases. Heads and limbs, especially hands, of victims are often found severed from the trunk and disposed of separately for this reason. Furthermore, perpetrators may strive to complicate forensic analysis of remains by trying to distort indicators that aid postmortem interval estimation. Since insects are commonly known to be of forensic importance, disposal of remains in insect repellent plastic bags could be an additional way to complicate a time-since-death estimate. It has been shown previously that wrapped remains pose a greater barrier for insects. Research conducted into the effects of various coverings on decomposition by Dautartas observed that insects seemed to appear at a later stage on wrapped remains compared to exposed remains.<sup>1</sup> Furthermore, an examination of Louisiana cases by Manhein showed that remains buried and covered in plastic exhibited a markedly delayed decomposition.<sup>2</sup> It is therefore assumed that the rate of decomposition is distorted in wrapped remains as a result of reduced or delayed insect activity as well as other factors. Previous research and case studies have also found that products such as insecticide, patchouli perfume, HCl, and

gas can result in delayed insect activity, and therefore may have a masking effect on decomposition of cadavers, which possibly leads to an underestimation of the postmortem interval.<sup>3,4</sup>

In this experiment, 24 *Sus scrofa domestica* carcasses were used as research subjects. The experiment was conducted at the Taphonomic Research in Anthropology Centre for Experimental Study (TRACES), Northwest England. The 24 carcasses were dismembered into heads, limbs, and torsos and distributed separately into 72 waste disposal bags (36 insect repellent experimental and 36 regular control bags). The 72 bags were subdivided into four sets; three of which were opened alternatingly at intervals of approximately 50 Accumulated Degree Days (ADD) for data collection. The fourth set was held back to act as a disturbance control. A scoring scale based on the degree of surface area covered to quantify insect activity was developed for the purpose of this research, as well as a scale based on Heaton et al. for scoring decomposition stages of heads, limbs, and torsos in separate, sealed plastic bags.<sup>5</sup>

Insect activity was first observed at 98.5 ADD in both the experimental as well as the control group. Preliminary statistical testing found there to be no significant difference in insect activity over time between the experimental and control groups ( $F_{1,250} = 2.165$ ;  $p$ -value = 0.143). Analysis of insect data did show a significant difference in insect activity over time between heads, limbs and torsos respectively ( $F_{2,250} = 8.477$ ;  $p$ -value = 0.000). Trends for insect scores show that while torso bags have a continuously higher insect activity compared to limb bags they progress at the same rate, whereas heads show relatively little insect activity in the earlier stages, but then exhibit a markedly faster progression. Trends for decomposition scores of both the experimental and control groups indicate that torsos progress at the fastest rate, followed by heads and ultimately limbs with a distinctly slower rate. These observations support earlier findings that heads and limbs decompose at a slower rate than whole bodies, likely due to limited bacterial action caused by the absence of gastrointestinal organs and hence gastrointestinal bacteria, which drive most of the putrefaction process.<sup>6,7</sup>

In conclusion, this study provides evidence that insect repellent bags neither deter insect activity nor distort the decomposition of remains when compared to regular bags, and that decomposition rate and insect activity differs between different elements in separate bags. Furthermore, data gained from this research may be used to generate a formula for PMI calculation of heads, limbs, and torsos in separate, sealed plastic bags.

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#### **Taphonomy, Dismemberment, Plastic Bags**

## H11 An Evaluation of the Use of Modern Medical Imaging Techniques for the Determination of Biological Sex From Craniometric Measurements

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After attending this presentation, attendees will be aware of the range of imaging techniques that may be used as alternatives to direct physical craniometric measurements. They will understand the relative advantages and disadvantages and levels of accuracy of Computed Tomography (CT) Scanning and Computed Radiography (CR) and their suitability for deployment.

This presentation will impact the forensic science community by increasing awareness of alternative non-invasive methods of taking measurements from the cranium for the determination of biological sex in human subjects.

The acquisition of direct anthropological measurements from human remains can sometimes involve the removal of flesh. This practice raises many ethical, cultural, and religious issues, and, in the United Kingdom, is in conflict with the recommendations of the Clarke enquiry. The process is also time-consuming and involves the manual handling of biological material. Radiography has long been an alternative, non-invasive method for obtaining measurements from fleshed remains, but has traditionally been a very time-consuming process requiring correction for magnification. However, the advent of modern digital imaging techniques appears to offer more efficient methods of gathering anthropological data non-invasively. This paper will present the findings of a study that aimed to evaluate the viability of two modern imaging methods; Multi-Detector Computed Tomography (MDCT) Scanning and Computed Radiography (CR) for determining cranial measurements to aid human sex identification.

Twenty skulls from a museum collection were examined using both MDCT and CR and five standard measurements were taken from each skull using the image data from both techniques. These measurements were compared with direct physical measurements taken using callipers, mandibulometer, and osteometric board.

The results showed that measurements taken from CT scan images were as accurate as direct osteometric measurements, and measurements taken from CR images were affected by magnification proportional to the distance of the body part from the image receptor. The results from this study suggest that the effect of magnification on measurement data from digital radiography is significant enough to alter any resulting assessment of biological sex and should be corrected for. However, the process of examination and measurement from Computed Radiography is rapid and the technology is far more widely available to investigators than CT scanning and can be deployed easily in field situations. It is recommended that an accurate and reproducible magnification correction method for use at various object-to-film distances should be developed for CR technologies.

It is recommended that CT should be used as the method of choice for taking craniometric measurements from fleshed remains. However, where this is not possible digital radiography is an acceptable alternative, provided that the magnification can be accurately corrected for.

### **Craniometrics, Radiography, CT Scanning**

## H12 Cranial Sexual Dimorphism and Anthropological Standards: Preliminary Investigations in a Western Australian Population

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After attending this presentation, attendees will gain awareness of: (1) the relationship between contemporary population specific standards and the expected accuracy of sex estimation in adults; and, (2) the value of 3D medical databases as potential sources of contemporary morphometric data for creating forensic anthropological standards.

This presentation will impact the forensic science community by quantifying population specific standards in forensic anthropology. Further, it will demonstrate how 3D medical databases provide abundant sources of contemporary research data that are complementary to traditional approaches based on direct examination of physical remains.

The formulation of a biological profile (osteobiography) is a crucial tool when unidentified skeletal remains are investigated. Accurate sex estimation ensures that the most appropriate and accurate statistics and standards for analyzing the remaining aspects of the biological profile are applied, e.g., sex-specific age, ancestry, and stature. Also, the most accurate biological profile is achieved using contemporary population-specific standards.

Australian forensic anthropology is constrained by a paucity of population specific standards as repositories of documented skeletons, traditionally the main source of population-specific data, do not exist. Today, if a morphometric approach is required for sex estimation, then a standard formulated from a non-Australian reference sample is the only option. The general effect of applying non-population standards creates an accuracy reduction in classification, the magnitude of which is proportionately related to the degree of dissimilarity (increasing biological distance) between the original reference sample and the individual to which those standards are being applied.

This research provides preliminary results of sexual dimorphism in Western Australian crania. The primary purpose is to formulate a series of morphometric standards for the estimation of sex and to demonstrate the effect on classification accuracy when non-population specific vs. population-specific standards are applied. The sample comprises cranial multi-slice computed tomography (MSCT) scans at a maximum thickness of one millimeter on 100 adult females (mean age of 36 years) and 100 adult males (mean age of 40.33 years). The 3D coordinates, using 3D volume rendering of 48 landmarks were acquired (by AF) using *OsiriX*<sup>®</sup> (v.3.9). A total of 55 linear measurements were calculated using *Morph Db* (an in-house developed database application). Measurements were analyzed using basic descriptive statistics and discriminant function analyzes using *SPSS 19.0*. The degree of correct sex classification in the Western Australian (WA) sample was also explored using the Giles and Elliot and Steyn and Iscan standards.<sup>1,2</sup>

Results reveal strong dimorphism in the WA population with bizygomatic breadth, mastoid height and maximum cranial length contributing significantly to sex discrimination. Maximum cross-validated classification accuracy using a step-wise analysis of five variables is 93% with a 4% sex bias. When classifying the WA sample using Giles and Elliot (Function #16 – American Caucasians) and Steyn and Iscan (Function #1 – South African Caucasians), sex was estimated correctly in 83% and 80% of cases respectively. However, the sex-bias is 31% and 36% respectively. Using the same variables required by the aforementioned foreign functions to formulate specific WA standards, results had an expected classification accuracy of 89% (sex bias 1%) for Giles and Elliot and 88% (sex bias 2%)

for Steyn and Iscan. While the overall sex classification accuracy is still relatively high, using foreign standards for the WA sample results in an unacceptably large bias. These non-Western Australian standards will thus frequently misclassify females. These results highlight the importance and necessity for developing population-specific standards for Western Australians.

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1. Giles E, Elliot O. Sex determination by discriminant function analysis. *Am. J. Phys. Anthropol* 1963;21:53-68.
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#### Sex Estimation, Cranium, Western Australia

### H13 Fetal Sexual Dimorphism of the Ilium: A 3D Geometric Morphometric Approach

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After attending this presentation, attendees will be familiarized with the use of 3D geometric morphometric measurements for the study of sexual fetal dimorphism.

This presentation will impact the forensic science community by demonstrating feasibility of outline analysis for immature forms.

This study aims to look for a sexual fetal dimorphism, using 3D geometric morphometric measurements, by applying Elliptic Fourier analysis (EFA) based on the study of 93 European fetal iliac bones aged between 21 weeks and 40 weeks from amenorrhea (WA), recorded by multi-slice computed tomography (MSCT), and 3D reconstructions. This study demonstrates the feasibility of outline analysis, especially for immature forms; however, there was no sexual dimorphism established by this study.

The evidence of sexual dimorphism in human adults has been established and studied for decades. Therefore, the information for fetuses is not well known, with few studies and contradictory results. Ninety-three French fetal iliac bones aged between 21 weeks and 40 weeks from amenorrhea(WA), coming from the anatomic collection of Marseille Hospital, were recorded by multi-slice computed tomography (MSCT), and 3D reconstructions. The 3D geometric morphometric analysis was based on outline analysis. EFA was used because of the immature morphology and the difficulty of landmark positioning. Reconstructions allow shape descriptions for each harmonic. Principal Component analysis (PCA) was performed separately for each age group (cut off at 30 WA).

According to the literature, the collection was divided into two age groups, with a limit at 30 WA. Both groups are well balanced (sex ratio respectively about 0.86 and 0.94). The first ten Principal Components axes represent 96% of the global variability, with harmonics or with amplitudes study. To allow best choice about the most discriminant principal component axes, PCA axes were selected using the most important eigenvalues (PC1- PC2), then by Wilks' Lambda analysis, and finally by applying Jolliffe eigenvalue threshold of 0.7 on the most discriminant PC axes with Wilk's test. In order to describe the best methodology in the Elliptic Fourier outline analysis, PCA was applied on amplitudes and harmonic coefficients derived from the Fourier series.

The accuracy of the reconstructions increased with the number of harmonics. The simultaneous analysis of the step-by-step reconstructions and the harmonics ellipses demonstrates that harmonics presenting the greatest amplitudes (magnitudes) and greatest axis ratios have the greatest morphologic contributions, which can be related to anatomic features. The convergence between the reconstructed outlines and the original outline by the use of an increasing number of harmonics can be easily appreciated visually, and can be quantified by the fit index, which is the sum of the squared distances between the reconstructed points and the original outline. The major morphologic characteristics were mainly described by the first seven harmonics presenting the greatest amplitudes. Finer features were described by harmonics of higher order. The iliac crest was the first to be described in step by step reconstruction from the second harmonic. The ischial tuberosity appeared as a positive relief around the fourth harmonic and its asymmetry around the fifth. The greater sciatic notch is described by the fifth harmonic. Its real depth and its asymmetric aspect appeared with the seventh harmonic. A perfect description is acquired around the seventh. After the eighth harmonic, there are no visible significant differences between shapes described by each one.

The best graphical representations in terms of sexual dimorphism were different between the age groups. But, these representations were not so easy to interpret, because of the absence of clear discrimination between female and male groups, regardless of the PCA selected. The overlap between age groups was too large to allow a sexual discrimination.

For fetuses between 21 and 29 WA there was no clear discrimination between age groups, regardless of which method was used (PC1-PC2, PC1-PC21 and PC1-PC11). The study for oldest fetuses leads to the same findings: big overlap and no clear discrimination between sex groups.

While various pelvic indicators showed marked sexual dimorphism in adults, there are no comparable levels of dimorphism in subadults and especially in fetuses. Several studies tried to answer questions asked by anthropologists and forensic physicians. Even if a significant sexual dimorphism in 2D elliptical Fourier analysis of the iliac outline was revealed, it was not possible to localize the source of this dimorphism. Computer-assisted image analysis allowed an automatic quantification of the outlines shape. It presents many advantages like greater objectivity and reproducibility, greater rapidity, and facilitation of measurements traditionally impossible to determine directly. Unlike the adult hip bone, where landmarks are relatively simple to define, the fetal ilium is a smooth continuous form with few or no landmarks. These characteristics make a classic Procrustes analysis unreliable. Summary forms need another type of morphometric study, which overcomes reference points. For cases where landmarks are difficult to obtain, outline analysis is the preferred option for analyzing shape. Elliptic Fourier analysis (EFA) used in this study is Fourier series for closed outlines. EFA is a Fourier method that interpolates the outline to get a large number of points. Many shapes can be described by the amplitudes of only few first harmonics. This method has been tested extensively in the literature. It has its applications in many fields, like biology, paleobiology, palaeontology, zoology, and biological anthropology, with different methods such as harmonic coefficients or amplitudes. Data agree that there are no significant differences between Fourier analysis and analyzes using landmarks for simple shapes, or shapes with reproducible point references. This study is the first to apply outline analysis using Elliptic Fourier analysis on shapes derived from immature remains. 3D was used because of its better accuracy in outline description, with homogenous age and sex groups. Even if a description was possible for the different anatomic regions of the ilium by the step-by-step reconstructions, there was no difference between sex groups demonstrated by these methods on the population. The intraobserver variability has been tested and was less than 5%, highlighting good reproducibility of the outline delimitation.

The question of the fetal sexual dimorphism is quite difficult to resolve. In immature bones, the relationship with age and the difficulty to use reproductive homologous points are factors of limitation of the geometric morphometric studies like Procrustes or eigenshape analysis. Furthermore, the small size of age and sex group and the possible poorly

marked dimorphism need more investigation, with larger samples in order to assess the absence of a sexual dimorphism.

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**Outline Analysis, Sexual Dimorphism, Ilium**

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## **H14 Morphometric Comparison of Nasal Aperture Shapes Among Modern South Africans**

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After attending this presentation, attendees will learn of the variation in mid-facial characteristics of African, European and Colored South Africans and to understand the statistical framework used to describe similarities and/or differences within and among these groups.

This presentation will impact the forensic science community by contributing to the knowledge base of human variation within a modern South African population, in providing a more scientific evaluation of this variation, and in presenting a mathematical approach to the classification of ancestral groups.

With more than 49 million people of various social identities, languages and belief systems, South Africa is an ideal country to evaluate human variation and the statistical relationship between social identity and biological characteristics. Because patterns of variation within and between populations are shaped by culture, language, geography, and secular change; it is necessary to define the effect these parameters may have on the reliability and accuracy of the commonly used methods for estimating ancestry as well as sex, stature and age-at-death. With a large database of population groups, FORDISC 3 has addressed problems regarding osteometric differences among populations. Mid-face and nasal bone morphology has been shown to be the most accurate region of the cranium to sort population groups in North American (Hefner) and South African samples (L'Abbé et al.).<sup>1,2</sup>

The purpose of this study was to assess variation in nasal bone structure, interorbital breadth and nasal shape, among African, European and Colored South Africans using elliptical Fourier analysis (EFA), geometric morphometrics (GM) and traditional linear measures through discriminant function analysis (DFA). Colored refers to a heterogeneous group of people in South Africa who are defined socially and geographically (Adhikari).<sup>3</sup>

A total of 310 crania of African, European, and Colored South Africans (165 males; 145 females) from the Pretoria Bone, Raymond A. Dart and Kirsten skeletal research collections in South Africa were used. All crania were photographed in the Frankfort plane, at a distance of 46 cm, using an Olympus 305 digital camera. Standard landmarks, which include subspinale, inferior point of nasal borders, alare, nasale inferius, dacryon, nasal superius, nasion and glabella, along with three nasal arcs were digitized using a MicroScribe G2. Inter- and intra-observer error was evaluated.

Geometric Morphometric (GM) analyzes including Procrustes fit (generating Procrustes Coordinates) and Elliptical Fourier analysis (EFA) were used to obtain shape variables. These variables as well as linear measures were imported into FORDISC 3.1 for linear discriminant function analysis (DFA). Statistical significance was assessed within and between ancestral groups. Each group was tested for normality and each was proven to be normally distributed. Outliers were identified through boxplots.

For all linear measurements, statistically significant differences between the sexes were observed in each ancestral group. But, these size differences did not affect classification of these groups. Mahalanobis distance was used to test the statistical significance between each ancestral mean for all variables. DFA with linear measures demonstrated a statistical significance among all groups, except for blacks and Coloreds which were

metrically indistinct ( $p$ -value=0.062). However when size was removed, nasal aperture shapes were statistically different between these two groups. For osteometric and shape analyzes of the nasal aperture, European and African South Africans as well as European and Colored South Africans were significantly different ( $p$ -value < 0.01). Inter- and intra-observer agreement was high (0.7 and 0.8, respectively).

The differences observed between these groups may be used as a tool for estimating ancestry, especially with separating European and other South African groups. Colored groups were more likely to misclassify as Africans than Europeans, which may reflect the heterogeneous nature of the group and the history of the country. In evaluating and defining the nasal aperture, shape does provide more ancestral information than size.

To approach the evaluation of ancestry from unknown skeletal remains, the relationship between social and biological race has to be examined, understood and continually evaluated on modern groups. Large databases are needed and an understanding of the cultural history of the population is crucial for the interpretation of these differences.

**References:**

- <sup>1</sup> Hefner JT. Nonmetric cranial traits: new approaches for the determination of ancestry. *J Forensic Sci* 2009;54(5):985-95.
- <sup>2</sup> L'Abbe EN, van Rooyen C, Nawrocki SP, Becker PJ. An evaluation of non-metric cranial traits used to estimate ancestry in a South African sample. *Forensic Sci Int* 2011;209(1-3):195.e1-7.
- <sup>3</sup> Adhikari M. Not white enough, not black enough: racial identity in the South African coloured community. Athens, OH: Center for International Studies, Ohio University Press, 2005.

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**Ancestry Estimation, Geometric Morphometrics, Discriminant Function Analysis**

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## **H15 Estimation of the Postmortem Interval of Human Remains in a Subtropical Humid Environment Using Accumulated Degree-Days and Total Body Scoring**

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After attending this presentation, attendees will understand the utilization of the total body score (TBS) and accumulated degree days (ADD) to quantitatively estimate the postmortem interval (PMI) in a subtropical, humid environment. This presentation will show the accuracy of the TBS-ADD method to estimate PMI from experimental field studies using human subjects of known date of death and recovery and known postmortem interval.

This presentation will impact the forensic science community by revealing the variability in decomposition scoring on four subjects in two environmentally different settings. The presentation will also impact the forensic science community by showing the accuracy of the TBS-ADD method for estimating PMI based on data from experimental field studies using a controlled sample of human cadavers of a known postmortem interval. It will also show that the method is region-specific and equations must be tailored to each particular environment.

Most forensic taphonomy studies involving postmortem interval data are derived from research conducted in temperate and arid climates and has been qualitative in nature. More limited research has been conducted in the cold weather climate of Edmonton, Alberta (Komar, Weitzel).<sup>1,2</sup> This postmortem interval data has been applied to interpret the rate of decomposition in climates in the US and abroad that are environmentally distinct.

Although qualitative studies have been very useful, there are more recent studies that utilize quantitative data (Megyesi, et al., Adlam and Simmons).<sup>3,4</sup> The Megyesi and colleagues' pilot study claims "80% of the observed variation in human decomposition could be accounted for by the combination of elapsed time and temperature as it is reflected in accumulated degree-days (ADD)" (Megyesi et al.), therefore a more accurate assessment of PMI can be obtained quantitatively.<sup>3</sup> However, Megyesi et al.'s study was based on assessment of PMI from case photos and not from a known sample.

The Southeast Texas Applied Forensic Science Facility (STAFS) at Sam Houston State University is a human decomposition facility located in a subtropical, humid climate zone similar to climates of Louisiana, Mississippi, Alabama, Georgia, most of Florida, South Carolina, and portions of North Carolina.

Human cadavers were used in experimental field studies using TBS during the human decomposition process and ADD to estimate postmortem interval in a subtropical, humid climate.

Four unclothed male subjects of similar weight and age were placed in the outdoor research facility in the summer of 2011. All individuals were placed in a supine position and cages made of a wood frame and galvanized mesh hardware cloth were placed over them. Two individuals were placed in direct sunlight and two were placed in a shaded area. Photographs of each body region were taken daily as well as gross observation descriptors of the human decomposition process. The descriptions previously established for the various stages of the human decomposition process in temperate (Bass) and arid climates (Galloway) were revised to coincide to the process seen in the subtropical, humid environment of southeast Texas.<sup>5,6</sup> Variability in decomposition scoring was also noted. Daily recording of temperatures were also recorded.

Initial results show that there are deviations from and similarities to the human decomposition process recorded for temperate, arid, and cold weather environments.

Human decomposition descriptions were adjusted to correspond to gross observations seen in the subtropical humid environment. For example, desiccation of tissue was moved to early decomposition. Also, as a result of desiccation throughout the body tissue, including the abdominal tissue, bloating is prolonged well into advanced decomposition and therefore the descriptors for advanced decomposition in determining TBS were adjusted.

Subjects placed in the sun reached bloat (TBS 9-11) approximately two to three days after placement, which corresponded to an ADD of 57-88 (C). Subjects placed in the shade reached bloat (TBS 9-11) approximately four to six days after placement, which corresponded to an ADD of 124-189 (C). Megyesi, et al. record bone exposure of the face occurring at a TBS between 14-26.<sup>3</sup> In the present study, no subjects with a TBS between 14-26 had bone exposure of the face.

With these and other distinct differences, the equation designed by Megyesi et al. was adjusted to align with the subtropical humid environment of southeast Texas.<sup>3</sup> This study confirms that the use of the TBS-ADD equation designed by Megyesi et al. to determine postmortem interval must be tailored to fit various types of environments.<sup>3</sup>

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3. Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci* 2005;50(3):618-26.
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and experimental field studies. *J Forensic Sci* 1990;35:103-111.

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#### Postmortem Interval, Human Decomposition, Total Body Score

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## H16 Decomposition Pattern and Rate in Hanging Pigs

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After attending this presentation, attendees will understand the major differences in the decomposition patterns and rates between hanging pigs and those decomposing on the ground.

The presentation will impact the forensic science community by adding to the knowledge concerning decomposition in hanging bodies carried out under controlled conditions and by providing a scale analogous with Megyesi, et al. for scoring hanging bodies which, combined with accumulated degree days (ADD), will allow for the calculation of time-since-death.<sup>1</sup>

Establishing the postmortem interval (PMI) is an essential part of any death investigation. Critical to establishing PMI is an understanding of the process of decomposition. Using decomposition scoring and ADD, the PMI can be calculated for bodies. The scoring tables currently used have been prepared from bodies lying in contact with a surface. There is little data for the decomposition pattern in hanging bodies<sup>2</sup> and previously there has been no scale for scoring hanging bodies that compares with the total body score (TBS) scale introduced by Megyesi, et al.<sup>1</sup>

The findings of a decomposition study carried out under controlled conditions at TRACES (Taphonomic Research in Anthropology: Centre for Experimental Study), University of Central Lancashire, United Kingdom will be presented. Twenty freshly killed pigs (*Sus scrofa*) of the same age and weighing between 19.5kg and 57.0kg were used as human analogues. Ten pigs were hung by the neck using nylon rope attached to hooks hung from an A-frame of scaffolding poles. The animals were between 60 and 90cm distance apart with their hind feet approximately 100cm off the ground at the start of the experiment. To protect the pigs from vertebrate and avian scavengers, the A-frame was surrounded at the bottom with chicken wire to a height of 60cm above ground, and bird netting was stretched over the whole frame. A further ten control pigs were placed on the ground and covered with chicken wire cages to protect them from scavengers.

The pigs were observed and the pattern of decomposition recorded and photographed for head and neck, torso, and limbs at approximately 50 ADD intervals until 932 ADD (the end of the study period). Continuously recording data loggers, set to take temperature readings at six-hourly intervals, were used to measure the ambient and internal temperatures. Total Body Score (TBS) score was assigned to the control pigs at each visit using the existing Megyesi, et al. scale.<sup>1</sup> The hanging pigs were weighed at seven and eight day intervals throughout the experiment to compare the percentage body weight loss between the males and females.

As the TBS system was constructed using bodies in contact with the ground and not hanging, a new scale for hanging bodies analogous with Megyesi et al.'s was constructed using the observations and photographs from the study. This enabled the assigning of a Total Hanging Body Score (THBS) to each of the hanging pigs, and allowed a direct comparison to be made with the control pigs when looking at rates of decomposition as a function of ADD.

In the control animals the pattern of decomposition showed no differences between the sexes. For the hanging pigs; however, the post

bloat body shape was markedly different for males and females. Females retained an equally swollen rectangular shape throughout the length of the torso with an evenly swollen profile. Swelling in the males centered around the mid-torso being at its greatest width around the umbilicus and penis.

As decomposition progressed, the hanging males and females displayed differences in the expanded anal opening. Initially in both sexes the anus opened to a diameter of approximately 10cm. In the males the back of the scrotal sack subsequently “gave way” and merged with the perforated anus producing a large clear hole directly below the hanging pig from which the bones dropped. The females remained swollen for longer with a pouch of stretched skin forming below and to the front of the anal opening.

While there initially appeared to be differences in the rates of decomposition between the sexes in the hanging pigs these are not statistically significant.

The new scale for hanging bodies will be presented together with photographs of the differing decomposition patterns.

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#### Hanging, Decomposition, Score

### H17 An Evaluation of the Use of Modern Medical Imaging Techniques for the Estimation of Human Stature

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After attending this presentation, attendees will be aware of the range of imaging techniques that may be used as alternatives to direct physical measurement of skeletal elements. They will understand the relative advantages and disadvantages and levels of accuracy of computed tomography (CT) Scanning and Computed Radiography (CR) and their suitability for deployment.

This presentation will impact the forensic science community by increasing awareness of alternative non-invasive methods of measuring skeletal elements for the estimation of human stature.

The acquisition of direct anthropological measurements from human remains can sometimes involve the removal of flesh. This practice raises many ethical, cultural, and religious issues, and, in the United Kingdom, is in conflict with the recommendations of the Clarke enquiry. The process is also time-consuming and involves the manual handling of biological material. Radiography has long been an alternative, non-invasive method of obtaining measurements from fleshed remains, but has traditionally been a very time consuming process requiring correction for magnification. However, the advent of modern digital imaging techniques appears to offer more efficient methods of gathering anthropological data non-invasively.

This paper will present the findings of a study that aimed to evaluate the viability of two modern imaging methods; Multi-Detector Computed Tomography (MDCT) Scanning and Computed Radiography (CR) for the measurement of long bones to aid human identification.

Twenty hind pig legs were examined using both MDCT and CR and three measurements (length, breadth, and diameter) were taken from the

femora, tibiae and fibulae of each leg. Following de-fleshing by dissection and maceration, each measurement was repeated using an osteometric board.

The results showed that measurements taken from CT scan images were as accurate as direct osteometric measurements, and measurements taken from CR images were affected by magnification proportional to the distance of the body part from the image receptor.

The results from this study suggest that the effect of magnification on measurement data from digital radiography is significant enough to alter any resulting stature estimates and should be corrected for. However, the process of examination and measurement from Computed Radiography is rapid and the technology is far more widely available to investigators than CT scanning and can be deployed easily in field situations. It is recommended that an accurate and reproducible magnification correction method for use at various object to film distances should be developed for CR technologies.

It is recommended that CT should be used as the method of choice for taking osteometric measurements from fleshed remains. However, where this is not possible digital radiography is an acceptable alternative, provided that the magnification can be accurately corrected for.

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#### Stature Estimation, Radiography, CT Scanning

### H18 Determination of Sex from 2nd to 4th Digit Ratios of the Hand

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After attending this presentation, attendees will be familiar with the developmental basis for differences in 2<sup>nd</sup> and 4<sup>th</sup> digit ratios of the hand between males and females and the practical application of this knowledge to the determination of sex from skeletal remains.

This presentation will impact the forensic science community by demonstrating that, despite its initial promise, the 2<sup>nd</sup> to 4<sup>th</sup> digit ratio does not appear to be an accurate method for determining sex from hand bones. The results of this discriminant function analysis suggest that total length of the 2<sup>nd</sup> and 4<sup>th</sup> digits or a combination of phalanx lengths provide a more accurate means for determining sex.

The ratio between the length of the 2<sup>nd</sup> digit (index finger) and 4<sup>th</sup> digit (ring finger) is strongly influenced by fetal testosterone in utero and has been shown to vary significantly between males and females (Manning et al.; Manning).<sup>1,2</sup> In general, males are expected to exhibit a lower 2D:4D ratio (ring finger longer than index finger) while females exhibit a ratio closer to 1.00 (ring finger and index finger of identical length). Garn has shown that this ratio is fixed by the 14<sup>th</sup> gestational week, suggesting that 2D:4D may be appropriate as a factor to differentiate males and females using skeletal remains.<sup>3</sup>

For this study the utility of finger length, phalanx lengths, and 2D:4D ratios for discriminating between males and females in a population of 342 adult individuals (171 Females, 171 Males) from the Terry collection are tested. All individuals were classified as “White.” Metacarpals and phalanges were measured using a mini-osteometric board from Paleo-Tech Concepts to the closest hundredth of a millimeter. Statistical tests were made using PASW Statistics (SPSS) version 18 for the Windows 7 operating system. Fingers missing bones were dropped from the analysis, as were outliers that failed a Grubb’s Test. A D’Agostino-Pearson K<sup>2</sup> omnibus test was used to evaluate whether measurement distributions were normally distributed.

Significant differences existed between 2D:4D ratios of males and females for the right and left hands (One-Way ANOVA: right:  $F=8.767$ ,  $p\text{-value}=0.003$ ; left:  $F=18.424$ ,  $p\text{-value}=0.000$ ). Three Discriminant Functions were used to evaluate the ability of measures to differentiate

males and females. The sum of phalanx lengths for the 2<sup>nd</sup> and 4<sup>th</sup> digits yielded a correct classification rate of 81.2% for the right hand and 85.3% for the left hand. A step-wise discriminant function analysis of phalanx length was also conducted for right and left elements independently. Length of the distal 2<sup>nd</sup> phalanx and medial and distal 4<sup>th</sup> phalanges yielded a correct classification rate of 84.3% for the right hand. For the left hand the medial and distal 4<sup>th</sup> phalanges correctly classified 82.8% of individuals. Contrary to the initial expectations, 2D:4D ratio correctly classified only 58.1% of individuals for the right hand and only 58.8% for the left hand.

These results show that despite the ability of the finger bones to differentiate males and females with a reasonable degree of accuracy (> 80%), digit ratio, at least as measured from hard tissue, does not. This may be due to several factors. The racial category "White" used by the Terry collection may obscure important differences between sub-populations from the European continent. In addition, the historic nature of the Terry collection may mean that its individuals exhibit more "feminized" digit ratios (males and females differed by only about 1%) due to developmental, health, and nutritional dissimilarities with current populations. Further work is needed to determine if these conclusions are applicable to other population groups.

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#### Sex Determination, Digit Ratios, Discriminant Function Analysis

## H19 Sex Determination: A Study of Sexual Dimorphism in Complete and Fragmentary Cuneiform Bones

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After attending this presentation, attendees will understand how to use the cuneiform bones to aid in the estimation of sex in unknown individuals.

This presentation will impact the forensic science community by showing new measurements which will aid in the identification of sex in fragmented remains.

Accurate sex estimation is a necessary step in the identification process and sexing techniques that can be applied to all bones is advantageous. Since the 1970's, metric sexing techniques have been applied to the talus and calcaneus more than any other tarsal bone. Steele was one of the first researchers to examine sexual dimorphism of the talus and calcaneus.<sup>1</sup>

Other researchers including Barrett et al, Bidmos and Asala, Bidmos and Dayal, Gualdi-Russo, Murphy, and Wilbur repeated Steele's research and confirmed that the talus and calcaneus are useful in determining sex with accuracies as high as 96%.<sup>2-10</sup> These researchers not only verify that the talus and calcaneus are sexually dimorphic, but that the accuracy rates are repeatable and the techniques can be applied to different populations from the past and the present. Other than the talus and calcaneus, little research has investigated the utility of other tarsal bones, particularly the smaller bones of the foot, for their potential in estimating sex. In addition, most of these studies require the tarsal bones to be in good condition and that most, if not all, of the bone is present. Kidd and Oxnard added the navicular and the cuboid to their study of the talus and calcaneus, but failed

to include the three cuneiforms.<sup>11</sup> Sheena Harris, using The William M. Bass Skeletal Collection and a mini-osteometric board, measured the maximum length, width, and height of all seven tarsal bones.<sup>12</sup> However, her measurements required the bones to be complete.

The present study examined cuneiform bones from 100 adult individuals (50 male and 50 female) including both Blacks and Whites from the William M. Bass Skeletal Collection for their potential in sex determination. This study not only examines the typical "maximum" measurements from complete cuneiform bones, but also devises new measurements that divides the bone into smaller segments (e.g., measurements involving articular surfaces and tubercles). Using a digital sliding caliper, this study examines eight new measurements each from the medial cuneiform, the intermediate cuneiform, and the lateral cuneiform. Previous researchers (Wilbur; Barrett et al, Bidmos and Asala), have shown that there is no significant difference between the right and left foot and therefore, only tarsals from the left side are used in this study.<sup>10,2-4</sup>

To test the accuracy of the new measurements, the data in this study was analyzed by importing all measurements into FORDISC 3.0 (Ousley and Jantz) and applying discriminant function analysis.<sup>13</sup> The results were positive and showed that not only were Harris' maximum measurements repeatable, but including smaller dimensions of the bones can be equally useful in determining sex. When all measurements are taken into account, the medial cuneiform was shown to exhibit the most dimorphism of the three with an accuracy rate of 94.2%, followed by the lateral cuneiform at 86.7%, and then the intermediate cuneiform at 84.7%. While some accuracies of the measurements fell below 75.0% when taken individually, the accuracy rate raised above 75.0% if they were combined with at least one other measurement. Three measurements (one from the medial cuneiform and two from the intermediate cuneiform) were excluded due to the fact that they were not useful in sex determination. When these measurements were excluded, the overall accuracy for the intermediate cuneiform increased from 84.7% to 88.3% while the medial cuneiform's accuracy decreased by 0.1%. This study has shown that while the cuneiforms are smaller and more difficult to side than other bones, they can be equally useful in the determination of sex. In addition, the use of new measurements developed for this study allows incomplete or fragmentary cuneiform bones to be used to determine sex.

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**Sexual Dimorphism, Tarsals, Sex Determination**

**H20 Analysis of Recovery Korean War Remains**

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After attending this presentation, attendees will learn how the Korean War, an international conflict that occurred between 1950 and 1953, has important implications for the development of forensic sciences in South Korea. It is estimated that approximately 137,000 Korean soldiers were killed during the Korean War. MAKRI takes a leading role in the identification of Korean soldiers. Here we present our preliminary results of the analysis of 2,470 Korean KIA remains recovered between 2009 and 2010 are presented.

This presentation will impact the forensic science community by combining all the above, the Korean War KIA were mostly 17 – 25 year olds and 160 – 170 cm tall. The “Asian” characteristic of shovel-shaped teeth, cranial ossicles, and linear enamel hypoplasia were the most observed non-metrical and pathological characteristics observed. The majority of observed trauma included gunshot and projectile wounds. Recovered Korean War remains sustained 60 years of taphonomic processes causing poor preservation, meaning that many of the specimens were unable to go through the forensic anthropological identification process. Eighteen cases (0.73%; n=2,470) were identified through material evidence, testimonies, and DNA. Approximately 3,000 KIA remains are stored since they are not identified due to a lack of personal (antemortem) information. Further international collaborative research is necessary to help expand and further elaborate on these initial findings.

Forensic science is a relatively new discipline in Korea that is currently being influenced by the more established research organizations found in the Western Europe and North America. In 2007, with government support, the Republic of Korea Ministry of National Defense (ROK MND) established the MND Agency for Killed in Action Recovery and Identification (MAKRI) research organization. MAKRI is a forensic sciences research organization whose main goal is the identification of recovered killed-in-action (KIA) Korean soldiers using standard theoretical and methodological research approaches in the international forensic sciences, particularly forensic anthropology.

Of the remains available, those in a good state of preservation were selected for creating a biological profile, including data on sex, age, stature, trauma, and non-metric traits. It was possible to estimate stature based on the long bones using 19.1% (471) of cases; 38.4% ranged between 160.1 – 165.0 cm and 38.2% ranged between 165.1 – 170.0cm. Age could be estimated in 44.6% of cases and the majority of ages ranged from 17- 25 years (86%). Sex could be accurately determined in 55.6 % of cases with 49.1% being male, and 50.8% containing masculine characteristics. Non-metric traits were documented from whole crania (15% of cases, 371 skulls). Ten percent had cranial suture ossicles, and 9.2% had a metopic suture. Approximately 10% of cases had intact teeth and 89.0% had shovel-shaped incisors. Enamel hypoplasia was highest in specimens with pathology (80%). Five percent of the subjects had observable trauma, of which 26.6% exhibited gunshot wounds and 72.4% projectile wounds. Approximately 63% of the gunshot wounds were observed in the cranium and 36.4 % were observed in the extremities.

**MAKRI, Korean War, Recovery Remains**

**H21 Anthropology and the AAFS: A Significant Impact in Its First 40 Years**

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After attending this presentation, the attendees will understand the origin of the Physical Anthropology (PA) Section of the AAFS, its significant growth, and patterns of its demographic evolution. Attendees will also learn of the impact that the Section has had in the Academy’s annual programs.

This presentation will impact the forensic science community by showing the significant growth of the field and the increase of forensic anthropologists available to pursue identification of primarily skeletal remains.

Excellent accounts of the origins of the Physical Anthropology Section are available in Snow, Ubelaker and Scammell and Rhine.<sup>1-3</sup> Origins generally reflect the state of osteological identification at the time. Of the original 14 members, there were 11 PhDs and 3 MAs; 11 males and 3 females; the East was most heavily represented, followed by the Rocky Mountain region. More importantly, they were bright, broad based but focused, moderately assertive, and enjoyed the fruits of research and the dissemination of knowledge. All but two were, or would work in, academics and all but two continued as productive members of the Section. The section experienced a rapid growth: at the end of its first decade it numbered 66; by 1992 it had climbed to 159; the following decade to 245; and currently there are 406 individuals spread over eight membership categories. It is believed that the Physical Anthropology Section has grown faster than any other section during the past 40 years. Based on the AAFS Membership Matrix presented at the Chicago meeting, the PA Section is currently composed of 45 Applicants, 80 Associate Members, 90 Fellows, 47 Members, 9 Retired Fellows, 1 Retired Member, 121 Student Affiliates, and 13 Trainee Affiliates for a total of 406 individuals. The sex ratio is 33% males and 67% females.

More importantly, the PA section has been a leader in the presentation of scientific papers. The Connective Tissue has chronicled the PA section relative to other AAFS sections seven times since 1994. The most recent Academy profile (Table 1) presents the data from the Chicago meeting in 2011 and is generally indicative of earlier years.

	N	PP	MM	R <sub>1</sub>	PP <sub>2</sub>	R <sub>2</sub>	%MP	R/M
Criminalistics	2845	(207)	41.2	1	27.3	1	7.3	10
Path/Bio	935	(136)	13.5	2	17.9	2	14.5	5
General	847	(54)	12.3	3	7.1	4	6.4	11
Toxicology	526	(52)	7.6	4	6.9	5	9.9	9
Odontology	446	(50)	6.5	5	6.6	6	11.2	8
Phys. Anth	406	(102)	6.7	6	13.5	3	25.1	1
Qu. Doc	212	(27)	3.1	7	3.4	9	12.7	7
Jurisprudence	210	(47)	3.0	8	3.6	8	22.4	3
Engineering	174	(24)	2.4	9	3.2	10	13.8	6
Psychiatry	152	(36)	2.2	10	4.8	7	23.7	2
DMS	106	(21)	1.5	11	2.8	11	19.8	4
<b>Total</b>	<b>6919</b>	<b>(758)</b>	<b>100.00</b>					

Table 1. Summary statistics of interest to our section, 2011.

N = section size in rank order;  
PP = number of papers presented by members of that section;  
MM = percent of members within Academy;  
R<sub>1</sub> = rank order of membership in the Academy;  
PP<sub>2</sub> = percent of total presented papers from that section;  
R<sub>2</sub> = rank order of percent of total papers from that section;  
%MP = % of members presenting papers from each section;  
R/M = rank order of members presenting papers by Academy section

For being 6th in size, the PA Section has shown to be the most productive in scholarly presentation at any of the recent Annual Meetings! To put this in perspective, the members of the Criminalistics Section presented 27.3% of all papers at the Chicago Meeting, but they comprise 41.2% of the Academy membership. The Pathology/Biology Section presented 17.9% of all papers at the Chicago Meeting and they comprise 13.5% of the Academy Membership; a rather balanced presentation production. The Physical Anthropology Section, presented 13.5% of the

\* Presenting Author

papers presented at the Chicago Meeting while holding only 6.7% of the Academy membership. As in the past few years, the members of the Criminalistics Section presented a lower relative percentage of papers; the members of the Pathology/Biology Section presented papers commensurate with their size in the Academy, and members of the Physical Anthropology Section appear to be more industrious in presenting their scholarly work at the annual meetings. There are excellent reasons for this distribution of data: the criminalists have a large number of individuals doing the applied work and may have less time for research or case presentation, while the anthropologists, mostly university faculty and graduate students, are expected to have a commitment to research and the dissemination of knowledge which are necessary for professional promotion and salary increases. The Central Identification Laboratory also maintains a high level of research and scholarly activity despite a significant level of deployment for Search and Recovery missions and subsequent identifications.

Student, Trainee Affiliate, and Members have been very competitive in applying for and receiving considerable, significant grant monies from within the section, within the AAFS and external sources. As well, numerous student presenters have received the J. Lawrence Angel Award, and 28 members or colleagues have received the T. Dale Stewart Section Award, three members have been named as Distinguished Fellows, and one has been the recipient of the International Adelaide Award.

It will be very interesting to watch the continuing evolution of the Physical Anthropology Section over the next ten years. Changes that currently reside in the Anthropology Scientific Working Group and the Executive and Legislative branches of our government will provide continued excitement for both the near and long range future of the Academy's Physical Anthropology Section.

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#### Section History, Growth, Impact on AAFS Program

## H22 Further Femmes Fatales: Do Women Dominate Forensic Anthropology Professional Practice in the United States, Canada, and the United Kingdom?

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After attending this presentation, attendees will gain awareness of the phenomenon that women outnumber men in forensic anthropology professional practice. It is anticipated that many attendees will have seen last year's presentation on this trend in the United Kingdom, and therefore will obtain more in-depth appreciation into whether this trend is reflected in the United States and Canada. Attendees will be able to compare gender distribution, motivations, career pathways and career progression in North America versus the United Kingdom.

This presentation will impact the forensic science community by presenting additional research that extends the investigation into professional practice and competence, allowing an overview of the uneven gender distribution that exists today. The composition of the forensic anthropology community has implications for deployment opportunities around the world, career progression, and acceptance of the discipline by male-dominated institutions such as police and law enforcement agencies.

This research extends that presented at the 2011 AAFS conference (Williams),<sup>1</sup> which examined the fact that more consistently women than men enroll in forensic anthropology university courses in the United Kingdom. The present study aims to determine whether this trend in education is reflected in professional practice and membership of professional forensic anthropology-related associations in the United States, Canada, and the United Kingdom, and to elucidate reasons for this trend.

Gender distribution statistics for forensic anthropology professionals were gathered via two mechanisms. Member details from the main professional Forensic Anthropology associations in the United States, Canada, and the United Kingdom for the last decade were collated, including the Physical Anthropology Section of the AAFS, the Forensic Anthropology section of the International Association for Identification (IAI), the British Association of Forensic Anthropologists (BAFA) and others. Questionnaires were also circulated to Forensic Anthropologists employed at leading universities, forensic laboratories, museums, and professional institutions, in order to determine the gender distribution of professionals, and to record their motivations for entering the discipline, career ascent and pathways, and persistence in career.

Preliminary results suggest that greater numbers of women than ever before are applying for forensic anthropology professional positions and membership to professional associations. Female membership of the AAFS Physical Anthropology section has risen by 11% over the last 10 years. However, the apparent saturation of women at lower levels is not translated vertically, and the distribution of women in the higher echelons of the discipline has not yet reached equilibrium with men. The number of female Diplomates of the ABFA has risen to 35%, and has remained relatively constant over the last 10 years, a trend which is projected to continue, but this is not representative of the distribution of women throughout the educational system. This research aims to raise questions as well as answer them, and to determine why there is this saturation at lower levels, without equality of progression. Is it simply a matter of time? Are female forensic anthropologists reluctant to apply for more senior positions, or are men better at securing the more senior positions? Is the discipline still dominated by men from the early growth years of the discipline? Are the competency tests giving equal opportunity to both genders?

This research is the first trans-Atlantic study to examine the apparently global profusion of women in forensic anthropology, and to elucidate reasons for the lack of gender parity at all levels of the discipline. This has important implications for the future of professional practice, membership of professional organizations, and higher education in the discipline.

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#### Forensic Anthropology, Professional Practice, Gender

## H23 Forensic Anthropology Fellowship Training Model

*Deborah C. Pinto, PhD\*, 208 Hollyberry Trail, Toronto, ON M2H 2P4, CANADA; and Jennifer C. Love, PhD, Sharon M. Derrick, PhD, and Jason M. Wiersema, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will learn a model for a forensic anthropology fellowship training program and to discuss potential funding sources.

This presentation will impact the forensic science community by raising awareness of the insufficiency of the current on-the-job training model practiced in the field of forensic anthropology and the advantages of a formal training program modeled after the American College of Graduate Medical Education Forensic Pathology Fellowship Program.

Few opportunities for formal post-graduate training in forensic anthropology exist. Although doctoral programs in physical/forensic anthropology are well suited for teaching the fundamentals of the field, necessary analytical skills are best obtained through casework. Medical examiner offices with in-house Diplomats of the American Board of Forensic Anthropology (D-ABFA) should develop formal training programs for emerging forensic anthropologists that involve them in diverse casework.

The American College of Graduate Medical Education (ACGME) Forensic Pathology Fellowship Program provides an excellent training model for the field of forensic anthropology. The program is a minimum of 12 months and requires the fellow to perform 200 - 300 autopsies under the supervision of a practicing forensic pathologist. The program also encourages the fellow to participate in crime scene investigation and court proceedings. The fellow must keep a log of his experiences, including: autopsies, external examinations, crime scene visits, and opportunities to observe or provide court testimony. The program requires objective assessment of fellow competence by multiple evaluators and to provide each fellow with documented semiannual evaluation of performance with feedback. ACGME requires the faculty to include a board certified forensic pathologist.

The Harris County Institute of Forensic Sciences (HCIFS) Forensic Anthropology Division created a Forensic Anthropology Fellowship Program following the ACGME model. A recent doctoral graduate from a physical anthropology program was hired. The fellow was assigned a diverse caseload and was responsible for the processing, analyzing and report writing of each case. Each case was supervised by a forensic anthropologist and each report was co-signed by the fellow and supervising anthropologist. In addition to the casework, the fellow was required to conduct a research project and to present the findings at a national scientific meeting. The program was funded through the National Institution of Justice Paul Coverdell Forensic Science Improvement Grant Program. Funding was awarded for salary, including a standard benefit package, and registration fees and travel expenses for participation in the national scientific meeting. The salary rate was set following the National Institute of Health Salary Guidelines for Postdoctoral Scholars.

During the program, the HCIFS Forensic Anthropology Fellow completed nine biological profile analyzes, 83 trauma analyses, 23 scene recoveries, and 41 autopsy consultations. The fellow conducted research on the trauma pattern associated with the use of an automated cardiopulmonary resuscitation device and presented the results at the American Academy of Forensic Sciences Annual Scientific Meeting. The fellow also participated in pre-trial meetings with prosecutors and defense attorneys and observed court testimony presented by a forensic anthropologist and pathologist. She attended continuing education courses that included ethical training, advancements in decedent identification, pediatric trauma seminars and medicolegal death investigation. The fellow completed rotations in the investigation division and crime laboratory.

The current on-the-job training model used by the field of forensic anthropology should be replaced with a formal training model as described here. The American Board of Forensic Anthropology requires an applicant to have three years of experience after the receipt of a doctoral degree and to submit three case reports for review. With a formal training program, the three years of experience should be replaced with a year-long fellowship program. The proposed requirement ensures that the applicant is receiving training from a D-ABFA as opposed to possibly practicing without supervision until eligible for board certification.

#### **Forensic Anthropology, Training, Fellowship Program**

## **H24 Recent Activities of the Scientific Working Group for Forensic Anthropology (SWGANTH)**

*Thomas D. Holland, PhD\*, Department of Defense JPAC, Central ID Laboratory, 310 Worcester Avenue, Hickam AFB, HI 96853; and Angi M. Christensen, PhD, Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA 22135*

After attending this presentation, attendees will become familiar with the recent activities of the Scientific Working Group for Forensic Anthropology (SWGANTH).

This presentation will impact the forensic science community by raising awareness of the SWGANTH's work to establish, identify, and publish "Best Practices" within the forensic anthropology discipline.

In late 2007, the U.S. Department of Defense Central Identification Laboratory (DOD CIL) and the Federal Bureau of Investigation (FBI) Laboratory cosponsored the creation of the Scientific Working Group for Forensic Anthropology, or SWGANTH. With the formation, success, and continued effort of SWGANTH, Forensic Anthropology has demonstrated its dedication to the advancement of discipline practices, improving communication, and building consensus among forensic anthropology professionals and with forensic community partners.

SWGANTH's initial purposes were to identify best practice guidelines for the Forensic Anthropology discipline and to disseminate guidelines, studies, and other findings that may be of benefit to the forensic anthropological community. To achieve this goal, the group's 20-member Board, comprised of professionals representing a broad cross-section of expertise and jurisdictional involvement, created committees to address specific topics relevant to the practice of Forensic Anthropology. Each committee, populated by forensic anthropologists from the United States and around the world, is charged with researching, capturing, and distilling the current best practices for the topic addressed by that committee. In addition to the development of Bylaws and a Code of Ethics and Conduct, topic areas addressed by the SWGANTH to date include:

- [Individual] Qualifications
- [Forensic Anthropology] Laboratory Management and Quality Assurance
- Determination of Medicolegal Significance
- Sex Assessment
- Identifying and Describing Pathological Conditions, Lesions and Anomalies
- Facial Approximation
- Age Estimation
- Skeletal Sampling and Preparation
- Personal Identification
- Resolving Commingled Remains
- Stature Estimation
- Trauma Analysis
- Statistical Methods
- Ancestry Estimation
- Postmortem Interval
- Documentation, Reporting and Testifying
- Detection and Recovery

Most of these guidelines have already been approved and published, and the remaining committees are close to issuing their final recommendations. In addition, the SWGANTH recently created committees charged with developing a proficiency testing program for forensic anthropology laboratories, identifying basic components of forensic anthropology educational programs, identifying research needs in the field of forensic anthropology, and creating an audit checklist that will aid forensic anthropologists in evaluating their performance relative to the larger community. The SWGANTH has also arranged for these documents and recommendations to be translated into Spanish, French, Arabic, and Russian. These guidelines, recommendations, and other materials of interest are published on the publicly accessible website: [www.swganth.org](http://www.swganth.org).

Several major developments that have occurred within the last year involve organizational changes to the structure and intent of the SWGANTH. First, the Board voted to expand the membership from 20 to

25 members. While logistical and administrative constraints argue for maintaining the Board's composition at or near 20 members, the group voted to add the International Committee of the Red Cross (ICRC) as a member of the SWGANTH, allowing the ICRC to participate as an organization and bring a broad international perspective to the group. Second, the group voted to enact term limits for Board members. Board members will now serve three-year terms beginning in 2011 and 2012. Third, the group voted to expand the purpose of SWGANTH to include the possibility of developing consensus Minimum Standards rather than being limited to identifying only Best Practices. Fourth, the group created a committee to examine the concept of Forensic Archaeology with an eye toward integrating this emerging sub-field into the larger Forensic Anthropology discipline.

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### Forensic Anthropology, SWGANTH, Best Practices

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## H25 Sexual Dimorphism in Crania and Humerus of Central Indian Population

*Nasir M. Ahmad, MSc\*, and Ruma Purkait, PhD, Sagar University, Department of Anthropology, Dr. H.S. Gour Central University, Sagar, Madhya Pradesh 470003, INDIA*

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After attending this presentation, attendees will understand new osteometric techniques devised for fragmentary bones for central Indian population for sexual dimorphism in crania and the humerus bone. Attendees will also gain knowledge regarding the parameters useful for sexual dimorphism in the fragmentary regions of crania and humeri.

This presentation will impact the forensic science community by devising measurements for fragmentary bones, which is the form bones generally arrive to forensic experts. India is a multi-ethnic country and area-specific equations should be applied. This is the first time that equations specific to the central Indian population are reported.

Each individual has a right to retain his or her identity, even after death. In cases of mass disaster or incidents of unnatural death where only skeletal remains of an individual are found, it is a tough task for the forensic anthropologist and the medicolegal personnel to complete identification. Therefore any data set or a statistical formula based on the particular population is vital to the investigator. One of the four main attributes of biological identity that a forensic anthropologist tries to establish is sex.

In India, skeletal remains are minimal, due to cultural practices related to disposal of dead bodies. The remains that require a medicolegal opinion are usually taken back either by relatives or by law enforcement personnel following the analysis. So the collections of known bones are very few. The remains used in this study are from the collection of the Department of Anatomy and Forensic Medicine of various medical colleges in central India. They belong to cases macerated in the medical college or cases whose identity has been established by experts. All the cases are from central India. The age and sex of skeletons are documented 173 (100 male and 73 female) crania and long bones included in the study were free of any orthopedic and pathological disorder.

All the measurements included in the study were taken following the methods prescribed by Martin and Saller (1957).<sup>1</sup> Few measurement are devised by the authors, keeping in mind the practical need where the forensic anthropologist is frequently confronted with fragmentary bones as compared to complete bones in criminal and disaster cases.

Sixteen cranial measurements and ten humeral measurements were taken. The data were subjected to discriminant function analysis with SPSS 16. The level of accuracy achieved for this population with a single variable ranged from 67.7% to 90.3% for the cranium and 77.5% to 98.4% for the humerus. The equations were applied on a test sample to check the efficacy of the parameters and gave nearly 90.0% and 95.0% sexing accuracy for the cranium and humerus, respectively.

The formulae generated from the present study can be used by the forensic anthropologist and the law enforcement agencies to diagnose sex of unknown bone of central Indian origin.

### Reference:

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### Forensic Anthropology, Osteometric Techniques, Mass Disaster

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## H26 Non-Metric Assessment of Ancestry through Cranial Macromorphoscopies: A Validation of the Hefner Method

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After attending this presentation, attendees will learn the reliability and the validity of the newly proposed Hefner (2009) method.

This presentation will impact the forensic science anthropology community by assessing the validity of this method for ancestry estimation in human crania and will allow attendees to evaluate the utility of the method when applied to forensic cases and assess the method's ability to meet the *Daubert* requirements.

Ancestry estimation is essential for the construction of biological profiles for unidentified individuals found in forensic contexts. The human skull has historically been considered the best indicator of ancestry and has been analyzed in a number of metric and non-metric studies. Arguably, metric assessment of population affinity is primarily done with FORDISC 3.0 (Ousley and Jantz); although, non-metric methods of ancestry estimation continue to be employed and taught, as they are the suggested methods presented in a number of introductory osteology and forensic anthropology textbooks (cf. Bass, Byers).<sup>1-3</sup> The benefits of non-metric methods are numerous including ease of use, no need for specialized equipment, and relatively quick data collection.

Hefner (2009) presented a new method of ancestry estimation by creating ordinal scores with descriptions and corresponding illustrations for a set of 11 macromorphoscopic traits commonly, or historically, applied to ancestry estimation in the crania. The frequency distribution of those traits was analyzed in four populations (African, American-Indian, European, and Asian). Ten of the eleven traits analyzed by Hefner were found to be significantly different between groups and, when analyzed within a statistical framework, can reliably predict ancestry with accuracy rates ranging from 84-93% depending on the method applied and variables used. Also in the original study, tests of observer error were found to be low, suggesting that this method can be accurately applied for ancestry estimation.

Since publication, the Hefner method has been applied and cited in a number of actual forensic cases and has been incorporated into Osteoware (Smithsonian Institution), a free program developed for data collection and storage. However, in order for the Hefner method to be reliably applied for the estimation of ancestry, independent tests of both the reliability and validity of the method must be conducted by alternative observers and in populations not previously studied for scientific rigor.

Two observers, with some previous familiarity with the Hefner method, scored a sample of 84 crania from the Hamann-Todd (HTH) Collection. Two ancestral groups were analyzed: American whites (20 female, 21 male) and American blacks (22 female, 21 male). All crania used in this study had no apparent pathological conditions and were complete enough to score at least 14 of the 16 traits (the 11 traits originally used by Hefner 2009 and five additional traits included in the Osteoware 2011 package).<sup>4,5</sup>

The data were analyzed through linear discriminant function to examine ancestral affiliations. The variables for each analysis were forward stepwise selected. Analysis of inter-observer error was also conducted using Cohen's Kappa (K). Initial results provided accuracy results considerably lower than those found by Hefner with correct cross validated, classifications of 52.4% for observer one and 54.8% for observer two.<sup>4</sup> The

Mahalanobis  $D^2$  from WM and WF means were not significantly different, while the  $D^2$  for BM and BF means were found to be significantly different in each of the observer's analyzes ( $p$ -value > 0.05). Intra-observer agreement was similarly lower than that found by Hefner.<sup>4</sup> Six of the traits had a moderate level of agreement ( $K=0.41-0.60$ ), six traits showed a fair level of agreement ( $K=0.21-0.40$ ), and four traits had only a slight level of agreement ( $K=0-0.20$ ) based on Landis and Koch.<sup>6</sup>

Lower classification accuracies than those found by Hefner may be the result of less experience with the method and trait scores.<sup>4</sup> Additionally, some of the trait representations found in the HTH collection lacked corresponding descriptions and illustrations and were forced into the most similar score. Results from this study suggest that caution should be used when applying this method to samples other than the one with which it was created and suggest that this method may require extensive practical experience with the traits, scores and illustrations before being used for ancestry estimation.

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3. Byers SN. Introduction to forensic anthropology: a textbook. 2nd ed. Boston: Allyn and Bacon, 2004.
4. Hefner JT. Nonmetric cranial traits: new approaches for the determination of ancestry. *J Forensic Sci* 2009;54(5):985-95.
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6. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33(1):159-74.

#### Ancestry Estimation, Cranial Non-Metric Validation, Discriminant Function Analysis

## H27 Using Metric Analysis to Investigate Ancestral Affinity of the Mandible

*Beatrix Dudzik, MA\*, 3909 Briargate Avenue, Knoxville, TN 37919; and David Echeverry, BA, 2334 Jefferson Avenue, Knoxville, TN 37917*

After attending this presentation, attendees will gain an appreciation of the morphometric variation of the mandible of seven distinct geographic regions. Numerous studies have examined metric landmarks of the human skull to investigate geographic differences among human populations. However, most of the research has focused on the cranium, and far fewer studies have utilized the mandible to assess variation within human populations. Previous research has established that morphological differences of the mandible exist between sexes and ancestral groups. Berg (2006, 2011) has shown that discriminant function analysis of mandibular morphology is an accurate tool for classification of individuals (including sex and ancestry).<sup>1,2</sup> This study utilizes discriminant function analysis, and calculation of Mahalanobis distances to further assess morphological patterns in distinct populations.

This presentation will impact the physical anthropological and forensic community by identifying the morphological differences of the mandible in distinct populations. The purpose of this study is to test the hypothesis that metric analysis of the mandible can be used to interpret ancestral relationships and thus aid in the classification of unidentified individuals.

This paper assessed metric differences of the mandible between 1,259 modern individuals. The individuals represented seven geographic groups which consisted of United States blacks (n=151), United States whites (n=166), North Eastern Asia (n=434), Eastern Asia (n=151), Southeastern Asia (n=115), Latin America (n=129) and West Africa (n=113).

The sample consisted of a combination of individual measurements compiled from the University of Tennessee's forensic anthropological

database and the Hanihara craniofacial data set. The combination of datasets has not been previously utilized to address the amount of mandibular morphological variation within differing geographic and ancestral modern human groups. The mandibular measurements utilized in this analysis consisted of seven measurements as defined by Martin (1928).<sup>3</sup> Bicondylar breadth (CDL), bigonial breadth (GOG), height of the mandibular symphysis (GNI), corpus mandibular width (TML), minimum anteroposterior width of the ramus (WRL), and maximum ramus height (XRL). As population differences and sexual dimorphism has been described by previous studies, discriminant function analyses were run on males and females separately to assess what is driving differences among population groups in regards to the combination of the effects of the mandibular measurements of CDL, GOG, GNI, TML, WRL, and XRL.

Results of the discriminant function analyses utilizing the measurements described on the male only sample indicated that the population groups were most clearly distinct in the combination of WRL, CDL, and TML, which accounted for 65.5 % of the variance observed, the combination of GNI, WRL and negatively correlated CDL (23.3%) and GNI (9.8 %).

Centroid plots from the discriminant function analysis of males only indicated that in regards to the first combination of measurements, the Northeast Asian sample exhibited the largest averages and were distinct from East Asian and Southeast Asians samples that plotted together in the middle of the axis. The North American blacks and West African samples plotted near each other below the Asian groups. The Latin American showed a large amount of variation but mostly plotted in the middle of the axis. The North American white sample exhibited the smallest averages and plotted at the negative end of the axis. The combination of GNI, WRL and negatively correlated CDL indicated a more linear relationship, with North American blacks and West Africans exhibiting the highest means and clustering together. The Asian samples plotted together with Latin American and North American whites, which exhibited the smallest average. Centroid plots from the discriminant function analysis of females only indicated the same patterns observed in the male analysis. Observation of Mahalanobis distances grouped the North American black and West African samples together with the North American white samples furthest from these groups. The Latin American and Asian samples grouped together and exhibited distances intermediate between the North American black and white groups.

Results of these analyses confirm conclusions of previous studies in that mandibular morphology can be used to infer ancestral affinity. This study shows that metric analysis of the mandible can be used to assess intracontinental variation as seen in comparison of Northeast and Southeast Asian samples, in addition to broad ancestral categories. Further studies are required to assess the degree in which morphological variability of the mandible is affected by environmental factors, and which traits (metric and non-metric) are most susceptible to plasticity.

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#### Mandible, Metric, Variation

## H28 Pubic Symphyseal Age Estimation from Three-Dimensional Reconstructions of Pelvic CT Scans of Live Individuals

Alexandra E. Wink, MS\*, 51 Elm Street, #1, Charlestown, MA 02129

After attending this presentation, attendees will understand the growing role of computed tomography (CT) and digital imaging technology in forensic anthropology, specifically how these techniques are applied to age estimation of both living and deceased individuals.

This presentation will impact the forensic science community by demonstrating how three-dimensional CT (3D-CT) technology can aid in anthropological assessment of live individuals as well as remains that are not completely skeletonized, thus expanding the purview of forensic anthropology.

Age estimation is a critical component of the biological profile in forensic anthropology. The pubic symphysis has been widely studied and is thought to be a reliable indicator of skeletal age; however, traditional pubic symphyseal aging techniques rely on access to the bony pelvis, which is not always feasible. Such is the case with fleshed or partially-decomposed remains as well as living, undocumented individuals. In recent years, 3D-CT technology has been developed to visualize the skeleton digitally. These “digital osteology” techniques are employed by forensic anthropologists to establish aspects of the biological profile. Previous studies have been performed on 3D reconstructions of CT scans of dry pubic bones. The goal of this study is to test the applicability of 3D-CT pubic symphyseal aging to CT scans of living individuals performed using clinical parameters.

A sample of 44 patient abdominal-pelvic CT scans from the Boston Medical Center was analyzed retrospectively for age at the time of the scan. The scans were de-identified in compliance with IRB guidelines, and only the sex of the patient and age of the patient at the time of the scan were recorded. Representation of males and females was approximately equal, and the subjects’ ages ranged from 19-89 years at the time of the scan. Three-dimensional images were created using the volume-rendering capabilities of OisriX software and analyzed for age using the Suchey-Brooks criteria for pubic symphyseal age estimation. The assigned Suchey-Brooks age ranges were compared to the age of the patient at the time of CT scan.<sup>1,2</sup> The images were analyzed randomly a second time to test for intra-observer reliability.

The features best visualized in the three-dimensional reconstructions were the symphyseal rim, the ossific nodule, and depression of the symphyseal face. The pubic symphyseal age estimates made based on the 3D images captured the true age of the subject 79.5% of the time, and intra-observer agreement was high (Krippendorff’s alpha coefficient of 0.65). In cases in which the true age of the patient was not captured, the errors primarily concerned Suchey-Brooks phases III and IV. All misclassifications were underestimations (i.e. the actual age of the patient was higher than the assigned age range). Idiosyncratic variation in pubic symphyseal morphology could very well account for the majority of the error in this study.

The results of this study demonstrate that three-dimensional reconstructions of clinical CT scans of living individuals are useful for visualizing the pubic symphysis for forensic anthropological age determination. As radiological techniques and imaging software capabilities improve, digital osteology may be utilized even more frequently to assess age and other aspects of the biological profile in fleshed remains and in living individuals.

### References:

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### Digital Osteology, Pubic Symphysis, Age Estimation

## H29 Reassessment of Cranial Trait Scores: Effects of Sex, Population, Age, and Body Size

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After attending this presentation, attendees will understand the effects of sex, population, age, and body size on cranial trait scores, and how these effects are relevant to sex determination methods.

This presentation will impact the forensic science community by providing knowledge regarding the reliability of cranial sex traits, the factors influencing cranial trait scores, and suggestions regarding which of these factors should be considered during sex determination methods.

Forensic anthropologists continue to rely on morphological cranial traits as key variables in sex determination methods. Typically, forensic anthropologists score the morphological expression of the glabella/supraorbital ridge, orbital margins, mastoid process, mental eminence, and nuchal crest using ordinal scales, such as those presented in Buikstra and Ubelaker (1994).<sup>1</sup> While numerous studies evaluate the diagnostic significance of these trait scores, investigations of the factors influencing these trait morphologies remain much more limited. These factors can be vital when applying sex determination methods using these traits. Traits may be highly variable in their expressions or patterns between populations, which would support the need for population-specific standards of assessment. For example, some populations may display relatively more “masculine” or “feminine” traits, and would therefore be more likely to be misclassified by a set of universal standards. The reliability of specific cranial traits may also vary between populations with differing levels of sexual dimorphism. Furthermore, if age-at-death or body size is highly correlated with cranial trait scores, these factors should be taken into consideration during sex determination methods to obtain accurate results.

In order to address these problems, cranial trait scores and postcranial size measurements were obtained from males and females across six different samples: (1) Bass Donated Collection European-Americans; (2) Terry Collection European-Americans; (3) Terry Collection African-Americans; (4) Hamann-Todd Collection African-Americans; (5) Kulubnarti medieval Nubians; and, (6) Arikara Native-Americans. Data were obtained from at least 30 males and 30 females of each sample (total  $n = 498$ ). Age-at-death and sex were known for the documented samples, and were estimated in the archaeological samples using traditional aging techniques and pelvic morphology. Pooled and sample-specific statistical analyses were conducted to evaluate how cranial trait scores vary with each other, as well as with sex, population, age, and postcranial size.

Across- and within-sample analyzes revealed significant sex differences in all five cranial traits ( $p$ -value  $< 0.05$ ). Discriminant Function Analysis (DFA) results revealed that glabella and the mastoid process were the best cranial trait sex indicators, whereas nuchal crest scores were the least reliable. Sample-specific correct DFA classification rates ranged between 86 and 94%. Kruskal-Wallis tests confirm significant sample differences in all cranial traits, including significant differences between Terry European-Americans and Bass European-Americans (e.g.,  $p$ -value  $< 0.01$  for nuchal crest, mastoid, and orbital margin differences in both sexes), and between Terry African-Americans and Hamann-Todd African-Americans (e.g.,  $p$ -value  $< 0.05$  for nuchal crest, orbital margin, and mental eminence differences in both sexes). These results suggest not only ancestral differences, but also more specific sub-population differences or possible secular trends. On average the Bass European-Americans exhibited greater (more “masculine”) scores in all traits, except the mental eminence. However, no other consistent patterns in sample differences could be discerned. For example, a greater average score in one trait did not coincide with greater scores in other traits. Furthermore, samples of similar ancestries did not necessarily display similar trait scores. Jonckheere-Terpste test results support an overall increase in trait scores

with femoral head diameter when males and females are pooled separately ( $p$ -value < 0.05 for all traits except mental eminence in males and orbital margin in females), but these relationships are mostly lost when samples are analyzed independently. Similarly, pooled sample results indicate a significant trend in nuchal and glabella scores with age ( $p$ -value = 0.000 for both traits in both sexes), but in many of the sample-specific analyzes these relationships do not reach statistical significance.

In summary, regardless of the sample, glabella and mastoid process were found to be the most reliable cranial sex indicators. Significant sample differences in trait expressions, even between samples of the same ancestral group, suggest that sample-specific standards would likely increase the accuracy of sex determination methods. When all samples were pooled, however, discriminant function analyzes were still capable of correctly sexing 86.5% of individuals, indicating that in the absence of sample-specific standards, a universal set of sex standards will still provide accurate results. Although the results of this study support a general increase in trait scores with body size and age, these relationships are weak ( $r^2 < 0.14$ ) and not likely of significance during sex determination methods.

#### Reference:

1. Buikstra JE, Ubelaker DH. Standards for data collection from human skeletal remains. Fayetteville: Arkansas Archeological Survey, 1994

#### Sex Determination, Cranial Traits, Population Variation

### H30 A Test of the Revised Auricular Surface Aging Method on a Modern European Population

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After attending this presentation, attendees will learn more about this revised aging method and see whether it is applicable on modern populations, as previous tests employed historical collections. The results of this test are more relevant to modern forensic practice.

The presentation will impact the forensic science community by helping current forensic practitioners decide whether this is a method that they should be using as a standard assessment technique for the examination of human skeletal remains. It may also prompt further testing on modern populations from other regions of the world.

Age estimation methods for human skeletal remains attracted considerable attention during the 1980s, with old techniques being revised and new ones being developed. The method for estimating age from the auricular surface of the ilium, as developed by Lovejoy and colleagues (1985), was recently revised by Buckberry and Chamberlain (2002). One of the reasons for this revision was to render the method easier to apply. Tests on historical populations were conducted for the revised method, with mixed results. The present study tests the Buckberry and Chamberlain method on the Athens Collection. This collection consists of documented skeletons of individuals who lived in the 20th Century. Sex, age, and ancestry information was derived from death certificates. A blind study of 120 remains was carried out by using the definitions outlined in the revised method. The auricular surfaces were scored in each individual component: transverse organization, texture of surface, microporosity, macroporosity, and apical changes. Statistical analysis was carried out using SPSS statistical software package.

An independent sample t-test was performed to test for significant differences between males and females for each composite score. It was found that there were no significant differences between ages for males and females ( $p$ -value > 0.05). In addition, Spearman's rank correlation statistics

were applied to identify relationships between each individual component as well as the derived composite score and documented ages. In all cases there was statistically significant positive correlation between features and the composite score with documented age ( $p$ -value < 0.01 in most cases, except for macroporosity where  $p$ -value < 0.05). A positive correlation means that the higher score assigned to a particular trait expression, the more frequently it was associated with older age. The same is true for the composite score (higher composite score means older age). Surface texture showed the strongest correlation among the features followed by transverse organization. As was expected, the composite score also presented a very strong correlation with age ( $r_s=0.756$ ,  $p$ -value < 0.01). Furthermore, partial correlation coefficients between features controlling for the effects of age were calculated. It was found that the partial correlations among the features are low and mostly non-significant, confirming that the features provide independent sources of information about age. A significant correlation was found only between transverse organization and surface texture, as well as surface texture and microporosity. Some of the results echoed those of Buckberry and Chamberlain. Finally, a Spearman correlation coefficient was calculated to test the correlation between documented and estimated age. It was found that there was a significant positive correlation between documented and estimated age ( $r_s=0.730$ ,  $DF=118$ ,  $p$ -value < 0.01).

The data generated from the present study suggest that the revised auricular surface method can be reliable for age estimation on a modern European population. There may be applications for the Buckberry and Chamberlain method in bioarchaeology as well.

#### Forensic Anthropology, Age Estimation, Auricular Surface

### H31 Estimation of Body Mass from Measurements of the Calcaneus and Talus

*Paul D. Emanovsky, PhD\*, Joint POW/MIA Accounting Command, Central Identification Laboratory, 310 Worcester Avenue, Hickam AFB, HI 96853*

The goal of this presentation is to introduce regression equations for the prediction of body mass from measurements of the calcaneus and talus.

This presentation will impact the forensic science community by presenting alternative skeletal elements for use in estimation of body mass, which typically relies on femoral head dimensions.

Estimation of body mass has been intermittently examined in the forensic anthropological context; however, body mass estimates are not a main component of the biological profile. In contexts outside the medicolegal field (e.g. paleoanthropological, bioarchaeological, ecological), estimation of "body size" from skeletal material is of interest. Allometric comparisons, for instance, are best made with reference to body mass.

The current study utilizes anthropometric data collected from white and black males and females from the Hamann-Todd Collection (HTH). The available anthropometric data (e.g., stature and weight) are combined with osteometric data from the foot in order to test proportionality and scaling consequences, as well as to generate predictive regression equations for body mass estimation. Measurement data for four variables, two from the calcaneus and two from the talus, were collected for a sample of 105 individuals (females:  $n=29$ ; males:  $n=76$ ) who exhibited clinically normal body mass indices (i.e.,  $BMI=18.5 - 25.0$ ). These four variables were entered into a Least Squares regression analysis using stepwise selection to choose the variable or combination of variables that best predict body mass. These elements both directly transmit the weight of the body during locomotion and are irregular bones comprised primarily of trabecular bone. This feature, as well as their functional relationship as articular joints, makes them ideal choices for estimation of body mass as they are not expected to vary in external dimensions with increasing or decreasing levels of activity, but are expected to correlate with overall size.

In a combined group analysis ( $n=105$ ) forward stepwise selection identifies the maximum length of the calcaneus (body mass = (CALMAX

\* 0.813) – 3.279;  $r = 0.643$ ; Adj.  $r^2 = 0.408$ ; SEE = 5.81 kg) as well as the combination of CALMAX and the maximum length of the talus (body mass = (CALMAX \* 0.534) + (TALMAXL \* 0.438) - 7.142;  $r = .0670$ ; Adj.  $r^2 = 0.438$ ; SEE = 5.66 kg) as the most useful predictors of body mass. These equations predict body mass in kg, though the bony variables are in mm. Tests of efficacy on the Least Squares regression equations were conducted using a separate HTH sample for which maximum lengths of the calcaneus and talus were made available. These tests of efficacy were also limited to individuals who fell in the range of a normal BMI ( $n=38$ ). Three measures were used to evaluate the performance of these predictive models – percent prediction errors (%PE= [(observed-predicted)/predicted] \*100), accuracy ( $\sum |\text{observed} - \text{predicted}| / n$ ) and bias ( $\sum \text{observed} - \text{predicted} / n$ ).

Using the CALMAX only equation, results in %PE ranges from -27.12kg to 15.44kg (mean = -4.60kg, SD = 10.11kg), accuracy is 5.63kg, and bias is -2.89kg. While using the model that incorporates both CALMAX and TALMAXL, results in %PE ranges of -26.21kg to 16.84kg (mean = -4.27kg, SD = 9.93kg), accuracy is 5.34kg, and bias is -2.69kg. The elements of the foot are on par with or superior to, existing methods for estimating body mass and may be considered superior for theoretical and empirical reasons. Based on the merits of the regression equation statistics and tests of efficacy, body mass estimation may not be as farfetched a goal as previously perceived in the forensic context.

#### **Body Mass, Linear Regression, Biological Profile**

### **H32 Estimating Sex from the Human Skeleton: A Validation Study on Recent Scapular Methodologies with Emphasis on Population Diversity**

*Ian C. Bell, BA\*, 5681 Rhuland Street, Apartment 903, Halifax, NS B3H 4J6, CANADA*

After attending this presentation, attendees will have a better understanding of population diversity as it relates to recent methodologies established for estimating sex from the human scapula.

The presentation will impact the forensic science community by introducing and validating previous methodologies established for estimating sex on different population groups.

The objectives of the study were to determine which methodologies by Dabbs and Moore-Jansen (2010) are more accurate and which are more reliable to the forensic investigator; to examine, through metric analysis, the sexual dimorphic traits of the human scapula; and to better understand the relationship between biological sex and population differences.<sup>1</sup>

One of the integral parts of developing the biological profile is the estimation of sex. Not only does it constitute a large part of the biological profile, but it provides for a better understanding of other elements of the profile. Methodologies for age-at-death and stature are generally sex specific and if there are no accurate methodologies to estimate sex, an entire individual may go unidentified. The human pelvis, skull and various long bones have been shown to be the best predictors of sex for an osteologist. Unfortunately, within a forensic and archaeological context, degradation of the skeletal material may render these bones unusable to the investigator. Therefore research to investigate new methods of estimating sex from other skeletal elements is crucial. Since 1887, the human scapula has shown potential for estimating sex through metric analysis.

In this study 298, contemporary white European individuals (168 males and 130 females) were used from two different skeletal collections: The William H. Bass Collection at the University of Tennessee, Knoxville and the Athens Bone Collection at the University of Athens, Greece. The methodologies follow those outlined in Dabbs and Moore-Jansen (2010) in which only left scapulae were used to measure six landmarks.<sup>1</sup> These six measurements were used in two previously established discriminate function equations to estimate sex of an individual (the “five-variable model” and “two-variable model”). Those measurements were: the

maximum length of the spine, maximum height of the scapula, maximum breadth of the scapula, height of the glenoid prominence, lateral curvature, and the thickness of the lateral border.

Using a two sample t-test to compare the Greek population with the North American population, the results indicate that four out of the six measurements were statistically different for males and one measurement was statistically different for females ( $p\text{-value} \leq 0.05$ ). Similar results were found when two sample t-tests were used to compare each population group with the results of Dabbs and Moore-Jansen (2010).<sup>1</sup> This indicates that, with regard to these six measurements, the two population groups are statistically different. Inter- and intra-observer error was performed on both population groups. However, the overall accuracies for correctly identifying the sex of the individual from the “five-variable model” for both the Greek and North American populations were: 91.89% in males, 85.18% in females and 96.80% in males, 94.73% in females, respectively. The overall accuracies of the “two-variable model” for both the Greek and North American populations were: 85.13% in males, 87.03% in females and 94.68% in males, 82.89% in females, respectively. To test for statistical similarities of these accuracies a chi-squared test was performed. The results indicate that the “two-variable model” for estimating sex in males is statistically different between the Greek and North American population groups ( $p\text{-value} \leq 0.05$ ). This could be a consequence of age. The “two-variable model” uses the maximum height and breadth of the scapula. As male individuals age, the ventral curvature of the scapula increases and the maximum height of the scapula decreases, which could have a direct impact on the “two-variable model” (Dabbs).<sup>2</sup> Further research could be done to confirm those findings.

Results show that although there are statistical differences in scapular measurements between the two population groups there is still a high overall accuracy of the two methodologies presented by Dabbs and Moore-Jansen.<sup>2</sup>

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#### **Sex Estimation, Population Diversity, Scapula**

### **H33 A New Method for Histological Age Estimation of the Femur**

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After attending this presentation, attendees will understand the current issues with histological age estimation and be introduced to a new method that uses the anterior midshaft of the femur. In addition, newly defined and tested histological variables will be presented and distributed.

This presentation will impact the forensic science community by providing a new method for histological age estimation. This will result in a higher quality of forensic practice and reduce errors in histological analysis.

Estimating adult age is problematic owing to biological variability in skeletal age indicators and their differential response to environmental factors over an individual’s life. Furthermore, when standard age indicators (e.g., pubic symphysis and sternal rib ends) are absent or altered by post-depositional taphonomic factors, anthropologists often resort to less accurate methods such as cranial suture closure. In order to improve age estimates, the use of multiple age indicators and various modalities of assessment should be considered. Histological methods are based on the continuous turnover of primary cortical bone with secondary cortical bone, which has been argued to occur at a more predictable rate than other degenerative changes. Despite this, a histological approach is typically not employed owing in part to inherent methodological issues ranging from

subjective definitions to difficulty reproducing microscopic field sizes. This research evaluates histological age estimation using the anterior femur and explores the biological limitations of bone turnover as an age indicator. The study builds upon previous histological methods, recognizing their importance and impact to our current understanding of bone turnover as an age indicator.

The sample includes femur cross-sections of known age individuals from three histological collections. The sample consists of 206 individuals (102 males, 104 females) from the Ericksen collection and 14 individuals (8 males, 6 females) from the Kerley collection, and 16 (10 males, 6 females) from a modern forensic collection of known age individuals.<sup>1,2</sup> Prior to this study, research was performed to redefine and validate histological variables. The variable definitions are available to attendees as a supplemental document provided by the authors. The following variables were collected:

- Surface Area (Sa.Ar.) per mm<sup>2</sup>
- Intact Secondary Osteons (N.On.):
- Fragmentary Secondary Osteons (N.Fg.On.)
- Intact Secondary Osteon Population Density (I-OPD) per mm<sup>2</sup>
- Fragmentary Osteon Population Density (F-OPD) per mm<sup>2</sup>
- Osteon Population Density (OPD): sum of I-OPD and F-OPD
- Mean Osteonal Cross-Sectional Area (On.Ar.) per mm<sup>2</sup>
- Mean Anterior Cortical Width (Ant.Ct.Wi.) per mm<sup>2</sup>

Histomorphometric data were collected using a transmitted light microscope and a firewire digital camera. The topographic sampling method was modeled after Iwaniec and colleagues and Stout and Paine.<sup>3,4</sup> The method evaluates ten columns from the periosteal to the endosteal cortex located at the anterior femur midshaft. Using a Merz counting reticule at 200x magnification (field area = 0.2304 mm<sup>2</sup>), 50% of the microscopic fields were evaluated in each column by alternating fields. This sampling strategy accounts for 95% of the remodeling variability within the anterior cross-section. Principles of stereology were followed, thus different magnifications or counting reticules may be employed to collect the histological variables. Osteon areas and cortical widths were calculated using imaging software.

Statistical analyzes were performed in SPSS 19 to examine the relationship between age and cortical bone histomorphometrics. Stepwise linear regression was used to develop the prediction equation and bootstrap methods were performed to assign measures of accuracy to sample estimates. Two variables (F-OPD and On.Ar.) required log transformation to meet normality requirements. Analysis of observer error was performed using Bland and Altman's procedure for testing the repeatability of methods.<sup>5</sup>

To examine the relationship between histomorphometrics and age, a general linear model was employed. Pearson correlations show moderate and strong relationships with age for all collected variables except I-OPD. Due to this finding it was determined that the constituent variables for OPD should remain separate in the regression model. A slight level of collinearity between predictor variables was recognized and influenced the selection of variables. A one-way ANOVA indicated that all variables, with the exclusion of I-OPD ( $p=0.296$ ), demonstrate significant sex differences at the 0.05 level. Stepwise regression analysis of the male dataset produced a model using F-OPD [log] and I-OPD as predictors, while the female model selected F-OPD [log] and Ant.Ct.Wi. as predictors. The standard error of the estimate is 12.87 years and 10.49 years, respectively. In the event that sex cannot be determined, a general equation was developed using F-OPD [log] and I-OPD, providing a standard error of 11.98. Observer error results indicate the method passed repeatability standards as described by Bland and Altman.<sup>5</sup>

Current histological methods demonstrate significant issues that affect their reliability and accuracy. The method developed from this research demonstrates several advantages over previous methods. The method is based on validated variable definitions, accounts for 95% of the spatial variation in osteons within the anterior cortex, and is not restricted to a specific field size or magnification. The age-related biological significance observed in the histological variables demonstrates that age-related accumulation of intact and fragmented osteons is not equivalent. While the

literature suggests combining I-OPD and F-OPD to reduce observer error, the results suggest that doing so may impact the ability to interpret age-related bone remodeling. Overall, the results of the study indicate that histological analysis of the anterior femur provides reliable age estimates. Considering the biological variation in both macroscopic and microscopic adult age indicators, the standard error in this study is similar to that of previous studies with large sample sizes. One of the most prevalent issues regarding adult age estimation is the inability to accurately age older adults. The described regression model is most accurate for individuals over 50 years of age. Bearing in mind that the elderly are a rapidly growing percentage of North American populations and that unidentified adults are a common occurrence in the forensic setting, this research will significantly increase the accuracy of estimating age for older adults.

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#### Histomorphometry, Age Estimation, Forensic Anthropology

### H34 The Unique Biodiversity of Avian and Mammalian Carrion Scavengers in Southern Illinois and Their Effect on Decomposition Rate and Pattern

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After attending this presentation, attendees will be aware of the unique biodiversity of the faunal assemblage in southern Illinois and how it affects the rate and pattern of soft tissue decomposition in the region. Those involved in human remains recovery and investigation will benefit greatly from the information and visuals presented.

This presentation will impact the forensic science community by highlighting the previously unknown differences between the faunal assemblages of southern Illinois and other regions housing forensic anthropology research facilities. Furthermore, this presentation will demonstrate how these unique assemblages can affect the rate and pattern of decomposition, further supporting arguments for multiple outdoor research facilities in various environments.

Scavengers can alter or destroy evidence pertinent to the cause and manner of death, destroy or scatter remains, and cause taphonomic effects that could potentially mimic trauma. Within specific microenvironments unique assemblages of mammalian and avian scavengers affect decomposition rates and patterns. Thus, it is imperative to study local fauna and the specific effects that they can have on both soft and hard tissues. Studies of scavenger behavior will aid in efforts to locate and recover dispersed remains, interpret the relative time the scavenging took place, and distinguish between faunal and other modifications on soft tissue and skeletal elements.

This study utilized a sample of 12 pigs (*Sus scrofa*) obtained from the Southern Illinois University-Carbondale (SIUC) Swine Center. Each pig

was humanely euthanized with a 15mm captive bolt gun (approved by the SIUC Institutional Animal Care and Use Committee) and all were scheduled to be euthanized due to illness or trauma. Once euthanized, the animals were immediately brought to the Complex for Forensic Anthropology Research for placement. Pig size ranged from approximately 1-64kg (nursery-15 weeks). Seven carcasses were buried (25-46cm). Five carcasses were placed on the surface in a mix of sun and shade (two frozen, three fresh). Subjects were placed at CFAR during two trials (October and December 2010) to assess seasonal differences. The five surface subjects were covered in 18-gauge wire mesh allowing scavengers access the subject from all angles, but preventing them from carrying off the entire carcass. Daily observations of the subjects were made by one of the authors and multiple measures of collection were used (Total Body Score, photographs, and written personal observations). Site-specific temperature data were recorded by iButton Link thermochrons (DS1921G). Motion-activated cameras were used to take still images and video of research subjects in the absence of the authors. All subjects were exposed for nine months before skeletal elements were cleaned and assessed for taphonomic damage.

Results show a multitude of scavengers feeding on the subjects beginning on the first day after placement for up to six months, with some evidence that feeding occurred even later. The most commonly observed species were the turkey vulture (*Cathartes aura*), Virginia opossum (*Didelphis virginiana*), and several species of rodent such as the eastern woodrat (*Neotoma floridana*). There is also moderate evidence of the common coyote (*Canis latrans*), bobcat (*Linx rufus*), and white-tailed deer (*Odocoileus virginianus*) being involved in the scavenging process.

Each species affected the decomposition rate and pattern of the subjects differently. This is clearly visible in the time of scavenger onset. Rodents and the opossum were present early, while the turkey vulture appeared after and seemed to be present only on the warmer days of the colder months. While rodents would gnaw on several small areas of the carcass, turkey vultures would rip apart the limbs of the subjects and abscond roughly 10m away to imbibe the flesh from the bones. The opossum was seen entering into the body of the carcass through the anal cavity to remove visceral tissue. Though the surface subjects were scavenged sooner, in some cases six months sooner, all subjects exhibited evidence of scavenging. The rate of decomposition was most affected by the turkey vulture and opossum, both accelerating the decomposition process by removing large quantities of flesh, thus exposing more carcass surface area to environmental and entomological elements. After skeletal elements were cleaned, characteristic light scores with flat floors were witnessed most prominently from rodent scavengers, while deep furrows and punctures point to possible carnivore or vulture damage. The bones collected exclusively from the vulture scatter pattern exhibit deep punctures, furrows, and multiple fractures.

It is clear that the southern Illinois region displays a unique biodiversity of scavengers that could affect the rate and pattern of decomposition, and possibly hinder a criminal investigation or positive identification. Further research is needed in this area and is currently ongoing at CFAR.

#### **Taphonomy, Scavenging, Forensic Anthropology**

### **H35 The Development of Forensic Anthropological Standards in Western Australia**

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The goal of this presentation is to increase awareness of the need to develop population-specific standards for identifying unknown human

skeletal remains in Western Australia and calls attention to the importance of a statistically-valid foundation for those standards.

This presentation will impact the forensic science community by demonstrating that, in the absence of demographically-sound skeletal collections, medical scans and measurements on living individuals offer an appropriate and reliable source of contemporary population-specific data from which skeletal standards for the estimation of age, sex, and stature can be developed. The presentation will highlight the importance of quantifying not only the degree of error associated with forensic standards, but also the accuracy and precision of the raw data (measurements) from which they are derived.

In Western Australia, there is an absence of population-specific standards for the estimation of sex, age and stature from skeletal remains and the living. Therefore, we apply skeletal standards from non-Australian populations. These skeletal standards are an inaccurate representation of our modern regional society both geographically and often temporally. In a global era of terrorism, crime, and natural disasters, the need for precise Western Australian standards, and novel approaches to identify unknown remains, are greatly overdue. To this end, our purpose is to fortify the capabilities of forensic scientists in Western Australia through the development and implementation of a 'Human Identification Package' (HIP): a software tool designed to provide statistically quantified estimations of standard biological features commonly utilized in the creation of an osteobiography e.g., sex, age, and stature. And, as they become available, additional modules capable of complementary analyzes will be incorporated (e.g. identifying human versus non-human remains).

In the age of *Daubert* and other relevant decisions, the statistical quantification of error and uncertainty in forensic science is vital. As the acquisition of morphometric data from clinical computed tomographic (CT) scans is still a relatively novel approach, our first level of analyzes are designed to validate raw data to formulate forensic standards. The two primary goals in our validation study include: (1) assessing precision in acquiring bone CT measurements, e.g. extent to which repeated measures provide the same value; and, (2) evaluating the accuracy of bone CT measurements, or the extent to which measurements depart from their true value.

Six dry human skulls from the Centre for Forensic Science (CFS) teaching collection were subjected to clinical multislice computed tomographs (MSCT) at Royal Perth Hospital using a *Philips Brilliance 64 Scanner*<sup>®</sup> with 0.9 millimeter slice thickness. Following 3D volume rendering, 90 bilateral landmarks were designed and acquired using *OsiriX*<sup>®</sup> (v.3.9); a total of 33 linear measurements were then calculated using *Morph Db* (an in-house developed database application). The same linear measurements were also acquired from the six dry skulls using traditional anthropometric instruments (sliding and spreading calipers – *GPM*<sup>®</sup>). Each CT scan and its corresponding dry skull were digitized and/or measured a total of six times, with a minimum of one day between re-measurement. The significance of difference between CT and dry bone ('true value') measurements are quantified using ANOVA; intra-observer error (precision) is assessed using standard anthropological statistics (e.g., TEM; rTEM; R).

No significant differences between the CT data and dry bone measurements were found. Intra-observer error was within accepted standards (rTEM < 5%; low TEM and high R values) for all measurements indicating high measurement repeatability for both data acquisition methods. The most "imprecise" measurements were expectedly those with Type III landmarks – points that have at least one deficient coordinate, e.g., mastoid height. Those landmarks are more accurately located by feeling for the tip of a rounded bump or bottom of a concavity, which is not possible in CT data. Irrespective, we demonstrate that the raw data underlying our forensic standards are valid and can be reliably acquired in CT and dry skulls.

#### **Osteobiography, Multislice CT, Measurement Error**

### H36 Multiplication Factor versus Regression Analysis in Stature Estimation from Hand and Foot Dimensions

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After attending this presentation, attendees will understand the usefulness of stature estimation methods in forensic examinations and will realize the variability in estimated stature and actual stature using multiplication factor and regression analysis methods as the literature on this aspect has been scanty.

This presentation will impact the forensic science community by confirming that stature estimation is more accurate and reliable with regression analysis method than that of multiplication factor method.

Estimation of stature is an important parameter in identification of human remains in forensic examinations. The objective of the present study is to compare the reliability and accuracy of stature estimation and to demonstrate the variability in estimated stature and actual stature using multiplication factor (MF) and regression analysis (RA) methods. The study is based on a sample of 246 subjects (123 males and 123 females) from North India aged between 17 to 20 years. Four anthropometric measurements; hand length, hand breadth, foot length and foot breadth taken on the left side in each subject were included in the study. Stature was measured using standard anthropometric techniques. Multiplication factors were calculated and linear regression models were derived for estimation of stature from hand and foot dimensions. Derived multiplication factors and regression formulae were applied to the hand and foot measurements in the study sample. The estimated stature from the multiplication factors and regression analysis was compared with the actual stature to find the error in estimated stature. Significant male-female differences were observed for stature, based on hand and foot measurements ( $p$ -value < 0.001). Significant sex differences were also observed for the MF derived in the study ( $p$ -value < 0.05) except for the MF derived for Hand Length that was almost similar in males and females ( $p$ -value = 0.712). Hand and foot measurements show a significant correlation with the stature in males and females ( $p$ -value < 0.001). Mean actual stature and stature derived from MF analysis and from regression analysis did not show any differences between them. However, it is evident that the range of stature estimated from MF analysis is broader and that from regression analysis is narrower than that of the actual stature. It is apparent that the MF analysis overestimates the maximum actual stature whereas the regression analysis underestimates it. The minimum actual stature is mostly underestimated in MF analysis and overestimated in regression analysis. However when error of estimate was calculated, it is observed that the minimum and maximum error in estimating stature in the study group is significantly larger in multiplication factor analysis than from the regression analysis. The study showed that the range of error in estimation of stature from the regression analysis method is less than the multiplication factor method; thus indicating that the regression analysis method is better than multiplication factor analysis in stature estimation.

**Forensic Anthropology, Personal Identification, Methods of Stature Estimation**

### H37 The Forensic Anthropology Center at Texas State University

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After attending this presentation, attendees will be familiar with the willed body donation program at the Forensic Anthropology Center at Texas State (FACTS).

This presentation will impact the forensic science community by highlighting the myriad research opportunities FACTS has to offer. FACTS aims to advance the field of forensic anthropology and other associated forensic science disciplines by creating a modern documented skeletal reference collection and providing access to both an osteological laboratory and outdoor human decomposition research facility for forensic scientists to conduct innovative interdisciplinary research in human identification studies.

The Forensic Anthropology Center at Texas State (FACTS) established a willed body donation program and an outdoor research facility in April 2008. Both the Forensic Anthropology Research Facility (FARF) and the Texas State Donated Skeletal Collection are available for research use by both national and international scholars. The mission of FACTS is to advance forensic science and anthropology through world-class education, research, and outreach. FACTS will: (1) provide the highest quality education and training for students and professional scientists in forensic anthropology; (2) provide training and certification for the medicolegal community; (3) assist national and international scholars from numerous forensic science disciplines in conducting quality scientific research that will benefit the medicolegal community; (4) facilitate interdisciplinary research and study, including providing state-of-the-art facilities and collections for research that advances forensic anthropology and other forensic sciences; (5) disseminate research in forensic anthropology through peer-reviewed conference proceedings and professional journals; (6) provide technical and scientific expertise and services to law enforcement, the medicolegal community, and the general public; and, (7) build local, national, and international partnerships with law enforcement agencies, laboratories, and research institutes to advance knowledge in forensic anthropology and other forensic sciences that deal with skeletal and decomposing bodies.

The Forensic Anthropology Research Facility is a large outdoor forensic decomposition facility, with 26 acres available for use on the Freeman Ranch. The decomposition facility provides opportunities for researchers to conduct studies that increase the understanding of variables affecting decomposition in climates similar to south central Texas. Longitudinal decomposition data for both human and non-human subjects are currently being collected. In addition, FARF is currently host to several national and international research projects covering the fields of anthropology, entomology, geography, molecular biology, and pathology. Accessibility to a weather station on the Freeman Ranch facilitates collection of weather and environmental data that assists researchers in outdoor studies. Approval for use of FARF requires submission of a research proposal and approval from the Director of FACTS.

The Texas State Donated Skeletal Collection is a growing modern skeletal collection consisting of permanently accessioned donated human remains from FARF research. Although a young center, FACTS has been quickly growing. Anatomical donations per year have risen from three donations in 2008 to 15 donations in 2010. As of July 2011, FACTS has already received 12 donors for the year. To date, there are 78 living donors on file that have willed their bodies to FACTS. The Texas State Donated Skeletal Collection currently has 37 accessioned donors, both males and females, ranging in age from 32-91 years, of African, European, and Hispanic ancestry available for research. Collaboration with other research institutions is an important aspect of FACTS; therefore FACTS has developed policies and data collection protocols that allow researchers to utilize the FACTS collection in conjunction with other similar research

facilities and standard osteological databases. The required paperwork for donation includes the basic biological profile, geohistory data, facial photographs, brief medical history, and life history. The data collected by FACTS prior to each donation are available to researchers and national databases. Approval for use of the Texas State Donated Skeletal Collection requires submission of a research proposal and approval from the Director of FACTS.

In addition to collaboration with other decomposition research facilities, FACTS also works with local law enforcement, FBI, and Texas Extension and Engineering Services (TEEX) offering educational lectures and workshops. The opening of a new multipurpose building located on Freeman Ranch will facilitate future workshops designed for the forensic education of graduate students and professionals.

The Forensic Anthropology Center at Texas State has immense research and outreach potential as a resource for the medicolegal and forensic science communities. It is important that researchers are aware of the resources available at FACTS and are encouraged to apply for use of the outdoor decomposition facility and/or the skeletal collection.

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#### **FACTS, Decomposition, Body Donation**

### **H38 Histological Aging of Neurocranial Bone**

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After attending this presentation, attendees will be introduced to the microstructure of several neurocranial bones and the utility of numerous features in formulating regression equations to estimate age-at-death.

This presentation will impact the forensic science community by expanding knowledge of bone histology to include neurocranial specimens as a solitary means of age estimation in biological profiles.

Successful skeletal age-at-death estimation employs a variety of traditional methods. One disadvantage is the necessity of near complete and proper preservation of target elements to reliably estimate age. Instances lacking traditional gross odonto-skeletal features force anthropologists to rely on bone or dental microscopy. Relevant histological research has focused on numerous bones, including the ribs, clavicles and mandible, but primarily the long bones. One limitation in some previous research is the insufficient attention to how biomechanical and metabolic factors affect the osteonal remodeling process in long bones and the accuracy of aging techniques. The influence of variation resulting from localized trauma, as well as the generalized effect of diet, disease, or excessive or minimal physical activity, is also important.<sup>1-2</sup>

Past histological research has focused on bones believed to be less vulnerable to environmental stressors.<sup>3-4</sup> This research examines the neurocranium, specifically the frontal, parietal, and temporal. Hypothetically, this region is less affected by biomechanical remodeling factors, given fewer muscle attachments, and has rarely been utilized in age-related histological research, with only Clarke and Cool performing histomorphometrics.<sup>1,3</sup> Their results indicate a low correlation of variables to age resulting from the irregular organization of features. However, these results may be due to a small sample size and a skewed older age distribution. Curtis focused on histomorphometrics of the frontal bone.<sup>4</sup> It was concluded that age-predictive equations should be controlled for sex when new variables were included. While promising, her large sample size is primarily reliable when estimating age on individuals over sixty years. Notwithstanding these issues, it is necessary to continue neurocranial histomorphometrics by expanding the younger to middle ages.

This research was performed at the University of Tennessee, Department of Anthropology's Mineralized Tissue Histology Laboratory, the Forensic Anthropology Laboratory at the University of Tennessee Medical Center, and the Pima County Office of the Medical Examiner in Tucson, Arizona. Sixty white male and female decedents of known age (20 through 82 years old), sex and ancestry from the University of Tennessee Medical Center were sampled during autopsy to remove three one-by-one

centimeter specimens from the sectioned margin of the left frontal, parietal and temporal bones. Complete medical histories were available; so retrospectively, if outliers demonstrate differential bone remodeling or atrophy, they could be excluded from analyses.

A research light microscope and computer imaging software were used to examine slides at 40, 100, and 200 magnifications; a photographic series of the entirety of each thin section was captured using a mounted digital camera attachment. The following histological features were examined: external table thickness, the number of secondary osteons, secondary osteon area, secondary osteon perimeter, secondary osteon maximum and minimum diameters, secondary osteon diameter ratios, secondary osteon Haversian canal area, secondary osteon Haversian canal perimeter, secondary osteon Haversian canal maximum and minimum diameters, secondary osteon Haversian canal diameter ratios, number of secondary osteon fragments, and osteon population density.

Pearson's correlation coefficients identified which of the above-mentioned variables (measurements from the frontal, parietal, and temporal bones) proved significant to estimate age. Also, stepwise selection determined which independent measurements were predictors. The significance level required for entry into the model was  $\alpha=0.15$ ; predictors with coefficients greater than a significance of  $\alpha=0.05$  were dropped and the model rerun. Statistical results demonstrate that the ratio of secondary osteon maximum to minimum diameter is a significant predictor. Also, the frontal bone has a stronger correlation to age than does the parietal or temporal bones. The results indicate the utility of neurocranial histomorphology in formulating age-at-death estimation regression equations.

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#### **Histomorphology, Neurocranium, Age Estimation**

### **H39 Sex Differences in Vertebral Centra Height From a Modern Autopsy Sample of Adolescents and Young Adults**

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After attending this presentation, attendees will be introduced to preliminary findings of sex differences in vertebral centra height for purposes of better understanding normal human variability in this region of the skeleton.

This presentation will impact the forensic science community by showing the ways in which vertebral centra height was found to vary in females and males, which may lead to the future development of a sex determination method for use in human identification.

The goal of this study was to examine sex differences in the maximum height of vertebral centra in a sample collected at autopsy for purposes of exploring normal human variability. The sample comprised 42 sets of as many as all 12 thoracic (T1-T12) and the first two lumbar vertebral centra (L1-L2); there were 13 females and 29 males, ranging in age from 12 to 31 years. This was a pre-existing sample, whereby the vertebrae were originally collected for an age estimation study in 1992. For each

individual in the sample, a vertebral column “wedge” was cut at autopsy; longitudinal cuts were made on the lateral aspects of the centra with transverse cuts at T1 and L2 enabling a wedge to be removed intact. Once removed, vertebral column wedges were macerated, and each vertebra was labeled. Two researchers independently and blindly (i.e., sex and age were unknown) measured in millimeters the maximum height of each centra at midpoint using GPM sliding calipers, and data were analyzed grossly and statistically. It should be noted that in some cases, the superior portion of T1 and the inferior portion of L2 were cut midway across the centra due to soft tissue obscurity, resulting in the omission of some height measures for these vertebral centra. This was accounted for during data analysis.

Raw data observations revealed that more cranially-situated thoracic vertebral centra were shorter than caudally-situated thoracic and lumbar vertebral centra, an unsurprising finding which supports anecdotal evidence. When considering age, an *F*-Test Two Sample for Variances showed no significant difference in the age ranges between the female and male samples. Mean vertebral centra height values were calculated for each individual in the sample, and these mean values were correlated with known age. Pearson’s correlations for age and mean vertebral centra height values showed no clear relationship ( $r = -0.45$  for females, and  $r = 0.44$  for males). Thus, the variation found in vertebral centra height was thought to be more related to sex rather than skeletal maturation or aging effects. Since stature was unknown, it remains possible that some differences attributed to sex could very well be a function of living height. Nonetheless, when exploring sex differences in centra height, ANOVA tests were highly statistically significant among all vertebral types (T1-L2).

Descriptive statistical results indicated that females and males had similar low and high centra height values: females had a low of 14mm (T1) and a high of 30mm (L1), and males had a low of 14mm (T1 and T2) and a high of 31mm (L2). Although these low and high height values were comparable, the mode in centra height for the different vertebrae showed variation between the sexes. For nearly each vertebra type, the male mode was consistently about 2mm higher than the female mode. Mean height values were calculated for each vertebral centra type (T1-L2) in the female and male sample; and observations of the raw data (i.e., comparing the means visually) showed that males were consistently higher than females for all vertebral types, although these differences were not statistically significant. Yet, when centra height for each vertebral type (T1-L2) for each individual in the female and male samples was compared, Student’s *t*-test results indicated statistically significant ( $p$ -value < 0.05) sex differences in all vertebral types except T1, T12, L1, and L2. There was a possible small sample size effect on T1 inasmuch as only 17 out of 42 sets of vertebrae contained T1s that were not cut off superiorly at autopsy when the sample was collected.

For each vertebral centra type (T1-L2), the range of low to high centra height measures was calculated in the female and male samples. For example, the range in height for T1 in the female sample was 14-17mm and in the male sample it was 14-20mm. Next, the magnitude of the ranges was calculated for each vertebral centra type in the female and male samples. Thus, based on the example above, for T1 in the female sample the magnitude of the range was 3mm (14-17mm is a 3mm magnitude of difference) and in the male sample it was 6mm (14-20mm is a 6mm magnitude of difference). Results of a Student’s *t*-test indicated a statistically significant sex difference in the magnitude of the ranges ( $p$ -value < 0.05). Gross observations of the raw data showed that the magnitudes of the ranges were wider for males when compared to females for all vertebral types except L1 and L2. This finding seemed to suggest that males exhibit greater variability in vertebral centra height than females, yet with some greater variability becoming apparent in L1 and L2 for females. Moreover, in females, the magnitude of the range of centra height increases from T1-L2 while in males it fluctuates, peaking at T7 and T8 and becoming slightly less than the females at L1 and L2. Findings from this preliminary investigation into sex differences in vertebral centra height provide some initial insight into normal human variability in this area of the skeleton. The goal of this study is that this study will stimulate further research to help explain the reasons for the sex differences in centra height

as well as encourage additional studies of the relationship between vertebral centra height and overall stature as linked to sex.

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#### Vertebrae, Sex Differences, Human Variation

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### H40 Using a Portable XRF to Detect the Transfer of Material from the Prior Use of a Saw in Cutting Bone

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After attending this presentation, attendees will have a better understanding of how saw blades when used to cut bone can transfer evidence beyond typical saw class characteristics.

This presentation will impact the forensic science community by demonstrating that an XRF can be used to detect prior use of a saw in dismemberment.

The principle of transfer evidence is a fundamental tenet of forensic science. Within forensic anthropology the assessment of bone trauma often hinges on the transfer of tool or instrument characteristics to bone.

A protocol was designed to test whether a saw blade used to cut a copper pipe could transfer minute particles of copper when subsequently used to cut through bone. Copper was chosen to simplify the procedure by focusing on a single element and to reduce the likelihood of environmental transfer. Two saw blade classes were chosen: 18 teeth per inch raker set and 18 teeth per inch wavy set. Copper concentrations were measured using an Innov-X Systems handheld X-ray Fluorescence (XRF) unit. This XRF detects 23 elements between titanium and lead on the periodic table. The x-ray exposure is software driven, set by the manufacturer and was identical for each sample. A shielded test stand was used for testing ensuring that the distance from the XRF to the bone was the same for each sample. Each bone sample, control or test, received at a minimum of one XRF exposure session. Larger bones required multiple exposures to ensure that the complete surface had been exposed. This was performed for all cut surfaces. All non-human samples consisted of long bones of *Odocoileus virginianus*. Thirty-one uncut human and non-human bones formed one control set. These included contemporary and prehistoric elements. A second control of 25 non-human bones were saw cut using new blades without prior use. The test samples consisted of 31 non-human bones. Prior to each test cutting the saw blade was used to cut through a piece of 25mm copper tubing. Immediately after cutting the tubing each bone was completely cut through and then analyzed using the XRF. The saw blades, used and unused, were also tested for the presence of copper.

Among the non-cut controls no bone had a detectable copper level. The control cut group of 46 sample sessions from 25 bones yielded detectable copper in four samples in four bones (16.0%). Levels ranged from 62ppm to 129ppm, with an average level of 91ppm. The prior use test cuts consisted of 31 individual bones with 84 sample exposures. Copper was detected in 31 of the 84 sample sessions. This translates to 24 individual bones, or 77.4%. Copper levels ranged from 27ppm to 321ppm with an average of 100ppm. Detectable copper was present in each blade sample, used or unused. Levels were consistently high and ranged from 663ppm to 2,088ppm with an average level of 1,168ppm. A test of the average copper levels of the cut control and test cut groups, 91ppm and 100 ppm respectively, was not significant ( $t = 0.2573$ ,  $p$ -value = 0.7896). However, in terms of absolute numbers the test cuts had a much higher total percentage of bones with detectable copper, 77.4% vs. 16.0%.

As no uncut bone had detectable copper the environmental transfer of copper can be ruled out as a possible source of copper presence. The transfer of copper from the prior use saw blades was confirmed microscopically. This demonstrates that copper was directly transferred from the copper tubing to the bone. It is assumed that the transfer of copper in the cut control samples was from the blade alloy. Therefore the true indication of prior use transfer cannot be ascertained using the XRF. While

copper was transferred from the saw blade to the bone during the cutting process the XRF cannot distinguish between the prior use transfer and incidental transfer from the saw blade itself. The latter scenario may have equal utility in using an XRF in assessing saw cuts in dismemberment.

#### **XRF, Dismemberment, Transfer**

### **H41 Pair-Matching of Human Skeletal Elements: Evaluation of Morphological Features for Visual Examination and Statistical Tables for Metric Assessment**

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The goal of this presentation is to provide attendees with a better understanding of both the visual and metric techniques of the process of pair-matching human skeletal elements.

This presentation will impact the forensic science community by presenting a description of which morphological features of individual skeletal elements are most useful for making a visual pair match and by presenting a new statistic (M) that can be used to metrically assess whether homologous bones are a possible pair match.

The process of matching paired skeletal elements (e.g., left and right femurs) is a technique used in forensic anthropology, paleodemography, and other osteological studies to determine if two homologous bones could have originated from a single individual. It is commonly used to resolve commingling issues from mass graves or disaster scenes, but can also be used in individual forensic cases where partial remains are found in different areas or at different times. When dealing with a closed population (e.g., aircraft accident), pair-matching is an easier task for the anthropologist than when dealing with an open population, when the anthropologist must decide whether the skeletal homologs can be matched to the reasonable exclusion of all other individuals.

There are two methods of pair-matching: visual pair-matching, a subjective technique which uses gross visual evaluation of similarities in bone morphology and taphonomy to match pairs; and osteometric sorting, a quantitative technique which allows statistical evaluation of size similarities between homologs to evaluate possible matches. This presentation covers both methods.

Visual pair-matching has been discussed as a reliable process; however, the details of how the exercise of visual pair-matching is done and what skeletal features are used have been seldom explored. The work presented here evaluates discrete morphological features of the major postcranial paired skeletal elements as described by White and Folkens (2005). Examination of morphological features included pairwise comparisons of 26 skeletons (which led to 231 comparisons for each feature) from the Robert J. Terry collection, housed at the Smithsonian Institution's National Museum of Natural History. Postcranial skeletal elements were examined, including the clavicle, scapula, os coxa, and all long bones. Cranial bones as well as smaller and less diagnostic bones (i.e., ribs, patellae, hand and foot bones) were excluded from this study. This effort provides anthropological investigators with a list of features that are the most consistent between the left and the right side, which can be used for the identification of matched pairs. Perhaps more importantly, it also provides a list of features that show variation between the left and the right side, which should not be used as means for the exclusion of possible pair matches.

This presentation will also address metric evaluation of possible pair-matches. Previous studies have produced statistical tests to resolve commingling, which can be used for pair-matching purposes, but also apply to osteometric sorting of other skeletal elements, and therefore are more

laborious than necessary for use in matching single pairs. These techniques usually require multiple measurements for each bone, which may be impossible in the case of fragmentary remains. Data from a database of standard skeletal measurements (Byrd and Adams 2003) are used to produce a statistic (M), which is designed to capture the range of variability between the left and right elements within human individuals to aid in the metric assessment of possible pair matches. This presentation will include an introduction to reference data tables that show the maximum value, as well as the 90th and 95th percentiles of the M-statistic. These values are shown for 52 standard measurements for paired elements and are applicable to fragmented bones. These tables allow a simple metric test of the null hypothesis that homologous bones originated from the same individual.

#### **Pair-Matching, Osteometric Sorting, Commingling**

### **H42 A Bone to Pick: Obtaining Modern Human Skeletons for Education and Training**

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After attending this presentation, attendees will understand that some whole body donor programs associated with medical schools in the United States have the ability to prepare human skeletons for use in education and training at forensic science programs.

This presentation will impact the forensic science community by increasing awareness of resources available for obtaining and using modern human skeletons for education and training in forensic science and forensic anthropology. This presentation will also inform the forensic community that skeletonization services are beneficial to body donation programs because they allow for the use of donors that may otherwise be excluded. Some exclusionary factors include obesity, disease, and surgical history.

Thousands of people each year become donors to whole body donation programs throughout the United States. For example, the Body Donation Program at the University of California, Davis School of Medicine receives an average of 125 whole body donors per year. Whole body donors are primarily used to teach human anatomy to medical, undergraduate, and allied health students. To complement anatomy education, medical students are often given a "bone box" to learn human osteology. This holistic approach aims to provide students with a robust understanding of all the systems that comprise the human body. In contrast, education in forensic science and forensic anthropology is often limited by access to skeletal material. Many available skeletal collections do not contain modern human skeletons or many educational institutions simply do not have access to human skeletons. At best, students in these situations are limited to learn from casts of modern human bone.

Resources exist whereby forensic science and forensic anthropology programs can obtain access to modern human skeletons. Forensic science institutions interested in obtaining human skeletons for education can contact their local medical school and ask if they conduct skeletonization services. The process of preparing the skeletons is relatively straightforward. After a donor is selected for skeletonization the soft tissue is removed manually. Skeletons are then placed in a dermestid beetle colony to remove the remaining soft tissue. Oils and fats are then removed using acetone baths. The skeleton is placed in a water bath to clean out residual beetle artifacts and to remove traces of acetone. Lastly, skeletons are air dried at room temperature. Skeletons can be whitened at the request of the end user using hydrogen peroxide. For forensic applications, they are rarely bleached; exposure to the natural color of bone is beneficial.

At present, relatively few whole body donor programs are asked to conduct skeletonization services. For example, the University of California, Davis School of Medicine Body Donation Program has

prepared 12 skeletons since 2003. The relative lack of interest in skeletonization services may be due to the idea that many forensic science programs are not aware of the possibilities that exist for obtaining modern human skeletons. Questions regarding the ethical use of whole bodies for science may further reduce the use of human remains by forensic researchers.

Regardless, the lack of interest is certainly not due to the unavailability of human specimens. A more likely explanation is that forensic science programs are unaware of where to request skeletonization services. Furthermore, skeletonization services can benefit body donation programs because they allow a use for donors that may be otherwise unsuitable for medical research or medical education. Medical research and medical education typically require specific characteristics such as average height/weight, no previous surgery, or a narrow age range. In contrast, forensic science education and training benefits from skeletal variation because it accurately represents the skeletal material discovered at crime scenes.

To conclude, the acquisition of skeletons from whole body programs has several benefits. For the forensic science and forensic anthropology programs it provides opportunities to educate and train using modern human skeletons that accurately represent what practitioners will encounter at a crime scene. Skeletonization services are beneficial to whole body donor programs because they allow for the use of donors that are potentially unsuitable for medical research and medical education.

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**Anthropology, Body Donation, Skeletonization**

### **H43 Detecting Submerged Remains: The Application of Side-Scan Sonar to Forensic Contexts**

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After attending this presentation, attendees will understand the application of side-scan sonar to forensic contexts, and specifically how this technology is used to detect submerged human remains. Successful case studies will be presented so that attendees will be introduced to both the advantages and the limitations of utilizing side-scan sonar to detect submerged bodies.

This presentation will impact the forensic science community by providing a better understanding of the application of side-scan sonar to the search for submerged bodies and associated evidence.

Forensic anthropologists and archaeologists frequently work with law enforcement to search for and recover bodies and associated evidence. At the same time, they are continually involved in improving various search methods, particularly with the use of geophysical tools for grave and buried evidence detection. The challenges of forensic searches can be even more confounded when dealing with submerged bodies. While traditional search methods involve multiple divers, side-scan sonar is increasingly replacing divers for the initial search. Side-scan sonar has been an important search tool for locating sunken ships, downed airplanes, and associated debris. Recently, improvements in the resolution of side-scan sonar have enabled the detection of smaller targets such as submerged bodies and evidence. As a result, more law enforcement agencies have acquired and incorporated side-scan sonar into their search and recovery protocols; therefore, forensic anthropologists should be familiar with this remote sensing technique and be involved with the recovery of submerged remains, particularly with mass disasters such as plane crashes resulting in body fragmentation. The goal of this paper is to discuss the methodology of side-scan sonar while emphasizing how this technology can be utilized to detect submerged human remains by drawing from forensic case examples.

Side-scan sonar consists of a towfish, containing the transducer, connected by a cable to a monitor with an attached differential global positioning system (GPS) unit. The operation of the sonar involves a boat dragging the towfish, which emits repetitive pulses into the water. The returning echoes are received by the towfish, which are converted into a digitized signal to discern features on the bottom surface. The sonar operator uses the differential GPS to plot the path of the sonar and ensure that the entire search area is covered. Once a feature is detected, a surface float is used to mark the location so that divers can then investigate. The experience of the sonar operator is paramount when interpreting the sonar data. Targets, such as submerged bodies, are recognized by the combination of the shape and shadow formation, with confirmation of the approximate target size. Additionally, the operator must distinguish between features and aspects of the terrain that reduce visibility, such as thick vegetation, irregular bottom surfaces, and debris. There are numerous advantages for using this technology for water searches. These include decreasing the time involved in searches, reducing the number of divers as well as the risk to the divers, and increasing the area searched. Additionally, since the submerged remains are located quicker than traditional methods, the taphonomic effects to the body are decreased.

Side-scan sonar has been used by the Orange County Sheriff's Office Marine Unit since 2007 to search for submerged remains resulting from homicides, plane crashes, car crashes, boat accidents, and accidental drownings. Two successful submerged human remains cases are highlighted to demonstrate the value of this technology for locating submerged bodies. The first case involved a boating accident that resulted in a drowning. Witness statements reported a specific location of the victim, but the victim was located once the search area was expanded. Since the search continued after dark, this case illustrates the utility of side-scan sonar for night-time searches. This case provides an example of ideal conditions resulting in clearly discernable features. The second case discussed involved a jet-ski accident. In this case, the witness accounts provided an accurate location for the search, but the terrain of the lake bed produced additional challenges and highlighted the importance of operator experience. Additionally, this case demonstrated the difficulty involved with data interpretation when the terrain is irregular. Both of these cases illustrate the application of side-scan sonar for forensic contexts and demonstrate the advantages and limitations of this technology when searching for submerged human remains.

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**Submerged Bodies, Forensic Geophysical Searches, Side-Scan Sonar**

### **H44 The Contribution of the Artificial Radiocarbon Dating Method in Determining the Medicolegal Relevance in Skeletonized Human Remains Cases: Experiences in Chile**

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After attending this presentation, attendees will appreciate the significant value of radiocarbon analysis in forensic cases.

This presentation will impact the forensic science community by showing how time-since-death of skeletonized remains obtained using radiocarbon analysis, can help establish their medicolegal relevance.

The estimation of time-since-death (TSD) of skeletal remains is one of the most important tasks in the forensic field; by defining the age of a skeleton, forensic anthropologists can determine the medicolegal relevance of the finding. Moreover, regarding the particular history of Chile, this determination provides evidence for the probable link between some remains and some of the victims of the military dictatorship (1973-1990) who are still missing.

This presentation discusses the experience of the Unidad Especial de Identificación Forense, of the Servicio Médico Legal of Chile, in relation to the estimation of this parameter using the Modern Radiocarbon Method

(post-bomb). This method is based on the dramatic increase in the concentration of atmospheric carbon-14 between 1950 and 1963 as a result of nuclear tests. Although these levels have declined steadily in recent decades, they have never been as low as those known before 1950.

Since humans incorporated the atmospheric carbon-14 during this period, the concentration of this element in the different tissues can be measured. Depending on their particular rate of cell renewal, some tissues will express atmospheric carbon-14 concentrations in close to the birth date of the individual, while others will show the concentration of this element near the time of death. Next, the radiocarbon values reflected in the different tissues are compared with the annual averages of the atmospheric carbon-14 concentration curve defining the time during which that person lived.

In 1990, 20 bags containing mummified human remains were recovered from a mass grave within the Pisagua cemetery, in the north of Chile. They were identified as 19 victims executed during Pinochet's dictatorship. However, there was one bag with different characteristics, called "Bolsa N° 20", which could not be associated to the same inhumation context. The uncertainty surrounding this case lingered for over 15 years until 2008 when a radiocarbon analysis showed that these corresponded to a prehistoric inhumation.

Since this case, the Unidad Especial de Identificación Forense has sent several samples such as hair, teeth, and bone for radiocarbon analysis; these samples were related to more than thirty cases without a clear temporal context. Through this method, it has been possible to define time-of-death with a high level of accuracy, including cases within the temporal framework of forensic relevance, or excluding cases that belong to periods of prehistoric or historic colonial interest.

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**Artificial Radiocarbon, Time of Death, Skeletonized Human Remains**

## **H45 Craniometric and Non-Metric Assessment of Skulls of Hispanic Descent**

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The goal of this presentation is to provide attendees with some initial guidelines for determining the ancestry of skulls of Hispanic descent. After attending this presentation, attendees can also expect to gain a greater awareness of the difficulties associated with conducting ancestry assessments on skulls of this type.

This presentation will impact the forensic science community by demonstrating the need for and potential application of further research in ancestry assessment of individuals of Hispanic descent, encompassing individuals from a wide range of geographic and genetic backgrounds.

The common methods of estimating ancestry are through visual assessment and osteometric analysis. However, these methods become less accurate when dealing with individuals of Hispanic descent.<sup>1</sup> In the United States, the term 'Hispanic' is usually associated with Spanish-speaking people of North, Central, and South America who genetically are an admixture of European, African, and Native American ancestry.<sup>2-6</sup> The discrepancies between actual genetic background and perceived genetic background can make definitively assigning Hispanic origin to skeletal remains more difficult, as well as creating incongruities between the ancestry determined by a forensic anthropologist and the ancestry listed on official documents such as a missing person's report.

The goal of this project is to provide methodology for accurate and reliable determination of ancestry for skulls of Hispanic descent by identifying traits that are consistent and unique to skulls from this ancestral group. This study examines the metric and non-metric features seen in a sample of 40 Hispanic skulls from the William M. Bass Donated Skeletal Collection, located in the Forensic Anthropology Center in the Department of Anthropology at the University of Tennessee, Knoxville (UTK) and the Pima County Office of the Medical Examiner (PCOME) in Tucson, Arizona. Non-metric data were collected and scored numerically.

Craniometric data were obtained and analyzed using FORDISC 3.0.<sup>7</sup> Following the FORDISC 3.0 analyzes, the ranges and averages for each measurement across the 40 skulls were compiled and compared to similar datasets of African, European, and Native American individuals.

The results of the study indicate some significant metric differences exist between Hispanic skulls and skulls of other populations. Out of 24 cranial measurements, fourteen were found to show significant differences between Hispanics and Europeans, ten were found to show significant differences between Hispanics and Africans, and four were found show significant differences between Hispanics and Native Americans. Only one measurement (Biauricular Breadth) was shown to significantly separate the Hispanic sample from all three of the other populations.

In addition, several combinations of non-metric traits were observed more often in the Hispanic skulls than in other ancestral groups. The most common traits observed were absent post-bregmatic depressions (observed in 88.9% of the sample), prominent anterior nasal spines (observed in 83.3% of the sample), closed but visible supranasal suture (observed in 72.2% of the sample) and intermediate nasal aperture widths (observed in 66.7% of the sample). Due to the diversity of individuals from Hispanic backgrounds, future research is needed to determine the applicability of these findings to all Hispanic groups. In addition, future studies should include a larger sample size of Hispanic individuals, encompassing individuals from multiple geographic locations and including both sexes.

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**Forensic Anthropology, Hispanic, Ancestry Assessment**

## H46 Integrating the Differential Global Positioning System and Geographic Information Systems for Mapping and Analysis of Skeletal Dispersals

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After attending this presentation, attendees will have a better understanding of the benefits of implementing the Differential Global Positioning System (DGPS) and Geographic Information Systems (GIS) in the mapping and presentation of skeletal dispersals.

This presentation will impact the forensic science community by comparing different data collection techniques using the DGPS in the mapping of a simulated skeletal dispersal and to discuss the benefits of mapping these scenes using the DGPS and integrating GIS for data analysis and presentation.

Scene mapping is an integral part of processing a scene with scattered skeletal remains. By utilizing the appropriate mapping technique, investigators can accurately document the location of human remains and maintain a precise geospatial record of this evidence and additional features at the scene. The determination of the appropriate mapping technique can be influenced by the extent of the skeletal dispersal as well as the environment. While baseline and grid mapping methods are typically used for smaller scenes, compass survey or total station methods may be used for mapping skeletal dispersals. Another mapping option is DGPS, as common units now provide decreased positional error suitable for mapping skeletal dispersals. As forensic archaeology is becoming more integrated into forensic anthropology, controlled research is essential to determine the benefits of this technology. The purpose of this presentation is to discuss the accuracy and practicality of using DGPS in mapping scattered human remains. Also, recommendations concerning data collection and the integration of DGPS scene data into a GIS will be discussed.

GPS is a satellite-based positioning system involving twenty-four satellites circling the earth. A GPS receiver uses positional information from the satellites to calculate the position on earth. A DGPS is a more accurate enhancement of a standard GPS that requires two receivers; one remains stationary while the other records positional data. The stationary receiver, a base station, relates all of the satellite measurements onto a single local reference. The base station measures the timing errors and provides correction information to the other receiver. In differential post-processing, the base station information can be obtained via the internet and then compared to the mapped point data for increased positional accuracy. The GPS geospatial data is commonly integrated into a GIS program which allows the user to display and analyze the mapped scene.

A simulated scene was assembled with a widely scattered partial skeleton in an urban environment. A Trimble GeoXH GeoExplorer 2008 Series DGPS with a Trimble Zephyr antenna, which can produce up to 10cm accuracy with post-processing, was used to map the scene. The first data collection used an average of 50 readings at one-second intervals, and the second used an average of 100 readings at one-second intervals. The data were then post-processed using GPS Pathfinder Office and exported into ArcGIS 10. After data were processed, the average corrected difference was 126.95cm for the 50-second collection time and 115.35cm for the 100-second collection time. Areas with tree cover demonstrated a corrected difference of 173.25cm for the 50-second collection time and 148.56cm corrected difference for the 100-second collection time. Areas without tree cover showed a corrected difference of 113.05cm for the 50-second collection time and 105.38cm corrected difference for the 100-second collection time. Overall, the most accurate method was using processed data with an average collection time of 100 seconds for both tree cover obstructed and unobstructed areas. However, the 50-second collection time was sufficient in unobstructed areas for mapping a skeletal

dispersal. Furthermore, the distance between bones is a consideration when mapping individual bones or clusters. It is recommended to map individual features when bones are at least 25cm apart, and map clusters of two or more bones that are less than 25cm apart as one feature.

Generating GIS maps with DGPS data has numerous benefits for mapping skeletal dispersals. Aerial maps are easily added to the mapped scene data as a base layer, and site features such as trees, sidewalks, and structures can be included on the map for scene context. The DGPS software (TerraSync 3.0) also allows recording of attribute data for features through preset data dictionaries, such as bone type and side that can be accessed in a GIS using an attribute table. The user may then label the map with the desired information. Furthermore, distance between features can be easily calculated with a measuring tool. This may be useful in a court setting where the distance between bones and scene features can be easily determined while testifying.

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**Mapping Skeletal Dispersals, Differential Global Positioning System, Geographic Information System**

## H47 Changes to the Integrity of Bone Marrow during Decomposition

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After attending this presentation, attendees will be familiar with the microscopic changes that occur to bone marrow throughout the postmortem interval.

This presentation will impact the forensic science community by introducing a potential semi-quantitative indicator of time-since-death from skeletal remains.

The objective of this study was to investigate the potential utility of histological analyzes of bone marrow for use when estimating the time-since-death.

The impetus for this pilot study is a response to recent legal decisions (the *Daubert* criteria), the National Academy of Sciences (NAS) Report on forensic science research, and best practice recommendations from the Scientific Working Group for Forensic Anthropology (SWGANTH) that have encouraged increased quantitative assessment of forensic techniques.

The purpose of assessing the histological appearance of bone marrow throughout the postmortem interval is to determine if bone marrow biopsy can be used as a quantifiable predictive indicator of time-since-death. Six pigs (*Sus scrofa domesticus*) carcasses were acquired from the Cummings School of Veterinary Medicine of Tufts University in Grafton, MA. All animals were euthanized at the same time by captive bolt, an AALAC and USDA approved technique for euthanasia. Each animal was placed in a wire cage, to limit scavenger access, on the ground at the Boston University Outdoor Research Facility in Holliston, MA during September, 2010. Experimental carcasses were placed approximately 15 feet apart. Two bone marrow plug biopsies were obtained from each pig on a graduated schedule, beginning with daily collections during earlier decomposition and ending with one in the fourth and last week of the experiment, as the pigs had skeletonized. Jamshidi Biopsy needles were used and all samples were immediately placed in small bottles containing 10% buffered formalin. The schedule was devised with the hypothesis derived from previous literature that the early decomposition process would exhibit the fastest changes and would gradually slow throughout the decomposition process.

A total of 122 bone marrow biopsy samples were collected over a 30-day period. They were rinsed in distilled water, decalcified in a rapid decalcifying solution, embedded in 5% gelatin and cut using a vibratome. The 40mm-thick sections were then microscopically assessed and scored based on three criteria: Cellular diversity and appearance, osteocyte abundance and appearance, and cancellous bone structure appearance. The three criteria were assigned a numerical value for each sample and then

correlated with the known time-since-death to determine the changes that are observed in the biopsies as decomposition proceeded. The average summary scores of the daily samples were also correlated with the average temperature and accumulated degree-days (ADD).

The results showed a positive linear correlation between the scored traits and both time-since-death and average temperature with the exception of the cancellous bone structure. In addition, the results suggest that a strong relationship exists between bone marrow appearance and integrity and the postmortem interval. These observations are encouraging and indicate this approach may have utility as a semi-quantitative predictive tool for estimating the time-since-death from skeletal remains.

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**PMI, Histology, Bone Marrow**

## **H48 Estimating the Postmortem Interval from the Pattern of Staining on Skeletal Remains**

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After attending this presentation, attendees will understand the value of bone staining in estimating time-since-death from skeletal remains, the necessary procedures for the successful application of this method, and the types of predictions that can be made regarding soil and environmental characteristics, soft tissue concentration, and time-since-death.

This presentation will impact the forensic science community by providing a new method for identifying the causal factors that affect bone staining to provide an estimate of time-since-death in medicolegal death investigations.

Taphonomic analyzes have become fundamental aspects in forensic anthropological investigations, especially with reference to the estimation of time-since-death for medicolegal and identification purposes. This study examines the causes and sequence of one such taphonomic process, bone staining. While staining has been reported in the literature (Calce and Rogers; Huculak and Rogers; Jagers and Rogers; Sauer), detailed quantitative data on its causes and sequence are limited.<sup>1-4</sup> Therefore, the patterning, timing, and properties of bone stains were analyzed to determine if estimates of the postmortem interval could be accomplished. The appearance and progression of bone staining were examined utilizing 45 fleshed and defleshed juvenile pig long bones that were placed in a burial context at the Forensic Anthropology Research Facility at Texas State University-San Marcos. Half of the sample retained a large amount of tissue (fleshed group), while the other half was processed to remove as much tissue as possible (defleshed group). The remains were buried and then collected at one-week intervals over a period of four months and extensively photographed. Soil samples were also taken at deposition and collection times and analyzed for changes in pH, nitrogen, phosphorous, and potassium levels across these two time points. Additionally, ambient temperature, humidity, and soil temperature were included to account for any influence of environmental factors on the staining process. A Miniscan XE Plus<sup>®</sup> color scanner from Hunter Laboratories was utilized to quantitatively and objectively measure the color of the stains present on the bones.

It was determined that staining occurred as early as two weeks post-deposition. Statistical analysis of the data revealed that differences existed between the fleshed and defleshed remains in soil chemistry, color, and environmental variables. Specifically, pH and nitrogen levels increased only in the fleshed group due to decomposition and the chemical byproducts associated with that process (Carter and Tibbett 2008; Hopkins, Wiltshire and Turner 2000; Rodriguez and Bass 1985) and not the soil environment.<sup>5-7</sup> The color analyses demonstrated that the fleshed remains exhibited darker, redder, and yellower coloration than those in the defleshed group. In the defleshed group, the surrounding environment, including the soil, ambient temperature, and humidity levels were responsible for the coloration seen on those remains. The correlational data between color and the environmental variables indicated that temperature (both soil and

ambient) and humidity levels had more of an influence on the defleshed remains than on the fleshed ones. It is hypothesized that the lack of copious amounts of tissue allowed the soil to interact more directly with these bone compared to the fleshed remains, where decomposition was the primary staining agent.

This study demonstrated that an understanding of the process of bone staining and the agents responsible for its occurrence is critical for establishing a depositional and chronological sequence. Even though time-since-death could not be estimated past two weeks, these results highlight the fact that taphonomic processes, such as bone staining are not limited to single causes, but result from a multitude of sources. While in its infancy, bone staining research has the potential to make important contributions to both forensic anthropology and other medicolegal professions. For medical examiners, death investigators, and forensic anthropologists, awareness of the potential causes, especially decomposition, climate, and depositional context can provide some information regarding the circumstances of an individual's death. Future research may be able to expand on these results to extend time-since-death estimates from two weeks to whole seasons.

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### **Taphonomy, Time-since-death, Bone Staining**

## **H49 An Investigation on the Relationship of Postmortem Interval to the Microbial Biomass of Bone**

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After attending this presentation, attendees will be informed about current research and development of methods for providing estimates of postmortem interval (PMI). The methods being evaluated are based on microbial biomass extracted from bone of varying postmortem intervals.

This presentation will impact the forensic science community by demonstrating how multidisciplinary approaches, which fall under the heading of forensic taphonomy, may be used to estimate time-since-death. The testing and analysis in this project are based on changes in the microbial community found in bone over a four-year PMI.

Bacteria and fungi contribute to the decomposition of a corpse and their taphonomic effects on the body and bone are well recognized and presented in the literature. This research investigates the temporal trends in bacterial and fungal community composition isolated from bone. The null

hypothesis is that as the quality of the resource (i.e., the corpse) decreases with advancing time-since-death, the bacterial load will also decrease, and the relative abundance of fungi will increase. If the hypothesis holds true, then the ratio of bacteria to fungi will decrease over time as well.

To test this hypothesis, a cross-sectional sampling of lower ribs from 14 bodies in different stages of decomposition was performed. The stage of decomposition varied from active decay with skeletal exposure at 10 days postmortem to dry remains at 48 months postmortem. Each recovered rib was thin sectioned, mounted undecalcified, and analyzed for signs of microbial bioerosion. Subsequent to histological preparation and analysis, 200mg of bone powder was pulverized and DNA was extracted. Extracted DNA was purified using the Qiagen MinElute PCR Purification Kit following the manufacture's procedures.

A quantitative real-time PCR for the evaluation of bacteria and fungi from each bone sample was performed. Standard curves, inhibition tests, and primer efficiencies were evaluated through a series of SYBR Green assays using PCR products of the 16s rRNA gene from *E. coli* and an Internal Transcribed Spacer region (ITS), which is a highly conserved fungal rRNA gene, from *Fusarium solani*. Five serial dilutions of known concentration of PCR products were generated and run in triplicate for a single standard curve and used to estimate DNA concentration from unknown samples. Amplification of test specimens were completed using previously reported universal bacterial primers targeting a 200 base pair fragment of the bacterial 16S rRNA gene and fungi were amplified using the ITS primer sets.

Bacterial universal primers were efficient for detecting bacteria in mixed samples according to the obtained qPCR efficiency of 99-100%, while ITS primer sets gave a 10% lower efficiency than the bacterial primer set. Inhibition issues occurred in the early amplification step, but were resolved partially by double purification using the Qiagen purification kit. Given the analytical conditions provided, the amount of bacterial and fungal DNA found in the samples varied from 0.2 to 17.0pg/uL for bacterial DNA and 0.0 to 3.0pg/uL for fungal DNA. Based on the results, bacterial concentrations were highest in the samples of 9, 11, and 12 months postmortem, while the highest concentration of fungi were found in samples with a PMI of 12, 18, and 21 months. The relative abundance of bacteria to fungi, determined by the ratio of total bacteria to total fungi concentrations suggested an overall increase in the ratio over the four-year postmortem interval. This finding is contrary to our hypothesis, suggesting instead an early abundance of fungi relative to bacteria, which changed to a greater relative abundance of bacteria to fungi during the 12 to 18 month time period.

Quantitative PCR is an efficient tool to study a variety of microbial communities in different types of samples. However, primer specificity and inhibition are the most common issues that encounter PCR-based analysis methods. Further research is required that is directed at using primer sets with more specificity for bacterial and fungal groups, rather than using universal sets.

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**Forensic Taphonomy, Postmortem Interval, Microbial Biomass**

## **H50 Stages of Decomposition of Human Remains in a Subtropical Humid Environment**

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After attending this presentation, attendees will gain an understanding of the stages of decomposition of human remains in a subtropical humid environment, similar to that found in Louisiana, Mississippi, Alabama,

Georgia, most of Florida, South Carolina, and portions of North Carolina. Attendees will understand how the physical changes within the stages of decomposition differ from those in temperate, arid and cold climates. Attendees will be introduced to a new two-tier system of categorizing physical changes that occur during the decomposition process.

This study will impact the forensic science community by emphasizing to attendees through the present study how human decomposition is regionally specific and that some characteristics seen in advanced decomposition in temperate environments occur in the beginning of early decomposition in a subtropical humid environment.

Medical examiners, law enforcement agents, and forensic specialists who deal with human decomposition have based their assessment of the postmortem interval on stages of human decomposition established in temperate, cold, and arid environments (Bass, Galloway, Komar, Weitzel).<sup>1-4</sup> Four stages of decomposition have been established from the work of Bass and Galloway. The stages include fresh, early decomposition, advanced decomposition and skeletonization. The descriptions within these stages are based on various colors and general physical characteristics. Although helpful, the present study found that color should not be a predominant factor in the assessment and that there are more physical characteristics present that should be used in the determination of the decomposition stage. In addition, due to the subtropical, humid environment of southeast Texas and the regional scavengers and insects the decomposition process is quite different than temperate or cold climates.

The present study was conducted at the Southeast Texas Applied Forensic Science Facility (STAFS) at Sam Houston State University, Huntsville Texas, a human decomposition research facility.

Over a two-year period human cadavers were placed on the surface, in a natural outdoor facility and allowed to decompose to the stage described in previous research as "skeletonization."

Subjects were placed, unclothed, in areas that received both sun and shade throughout the day. Cages comprised of wooden frames and galvanized mesh wire were placed over some of the individuals in order to prevent scavenging activity. Other subjects were not caged and were accessible to scavengers. The subjects were photographed and observed daily. Climatic data were also collected daily.

Results show that in both sun and shade the skin mummifies. Scavenging is predominantly done by vultures and scavenging begins in early decomposition and extends through advanced decomposition but ceases at skeletonization. In addition, desiccation begins in early decomposition, and once the remains are desiccated, they stay arrested in that state indefinitely and rarely progress to skeletonization. The skeletal elements of subjects that were not accessible to scavengers were blanketed by mummified tissue with minimal hair loss. Skeletal elements in some subjects accessible to scavengers were categorized as reaching skeletonization, but only as a result of vultures pulling the soft tissue away from the bone. Although the bones were exposed, the soft tissue was laying in the vicinity of the body.

In early decomposition, none of the subjects in this study could be described as "pink-white appearance with skin slippage and some hair loss." Nor could any subjects be categorized in the advanced stage as "moist decomposition." In the trunk region, the decomposing tissue does not "sag" as described in advanced decomposition, but rather crinkles as a result of desiccation of the tissue.

These findings show that descriptions of the physical characteristics occurring during decomposition need further clarification and detail. The findings also show that decomposition is regionally specific and the need for research in various climate zones is necessary.

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**Forensic Anthropology, Human Decomposition, Subtropical Humid Climate**

**H51 A Group-Generic Stature Estimation Equation from the Calcaneus and Talus**

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After attending this presentation, attendees will learn of a new stature calculation technique based on a group-generic equation from the calcaneus and talus. The sample from which this equation was developed is a composite sample of African and European Americans, and Japanese.

This presentation will impact the forensic science community by verifying the findings of Holland (1995) with an Asian sample: lengths of the calcaneus and talus have a linear relationship with stature compared to major long bones, which are affected by ancestral, sexual, and secular differences. Therefore, when remains are missing or too fragmentary to determine ancestry and sex, and long bones are not available, forensic practitioners have a viable alternative using the calcaneus and talus.

Measurements for the three independent variables: maximum length (MCAL) and posterior length of the calcaneus (PCAL), and maximum length of the talus (MTAL) were obtained following the methods of Holland (1995). African Americans (males: n=85 and females: n=58) and European Americans (males: n=85 and females: n=52) are comprised of individuals from the Hamann-Todd Human Osteological Collection (HTH) and JPAC-CIL, while the Asian sample is represented by Japanese (males: n=69 and females: n=31) individuals from the University of Chiba, School of Medicine (UCSM) and the University of Jikei, School of Medicine (UJSM). All individuals were born during the 19th or early 20th Centuries.

Before the equation was formulated, multivariate normality, multicollinearity, and residual tests were performed using SPSS 15.0. All three variables are highly ( $r > 0.7$ ) correlated with each other; therefore, they were combined (summed) as a single independent variable to avoid the effect of multicollinearity (Adams and Byrd, 2008). Next, Mahalanobis distance was adjusted from the critical value of chi-square distribution in order to achieve multivariate normality. Four outliers were removed as a result. MANOVA was performed to examine the impacts of sex and ancestry on stature.

The results demonstrate that the main effect of both sex and ancestry are statistically significant: Wilks'  $\Lambda = 0.589$ ,  $F = (2, 361) = 136.964$ ,  $p\text{-value} < 0.001$ , multivariate  $\eta^2$  (partial eta squared) = 0.431 and Wilks'  $\Lambda = 0.479$ ,  $F = (4, 722) = 80.309$ ,  $p\text{-value} < 0.001$ , multivariate  $\eta^2 = 0.301$  respectively. The interaction of sex and ancestry was not significant; only 0.9% (multivariate  $\eta^2 = 0.009$ ) of variance was explained by these variables. The distributions of the residual plots in all cases appeared random, which indicate that the general model performs equally well for all three groups. The SPSS interaction profile plots demonstrated a nearly parallel relationship among ancestry and sex. The formulated equation (Living Stature (L.S.) =  $5.826 * (MCAL+PCAL+MTAL) + 54.062$ ) demonstrate a SEE of 5.24 cm,  $r = 0.874$ , and  $r^2 = 0.763$ .

After formulating the group-generic regression equation for estimating stature, 28 control samples consisting of a combination of individuals from the UCSM, UJSM, HTH, and the JPAC-CIL were tested. There were no statistically significant differences between predictions from the group-generic equation and the Holland equations (combined sex and race:

African and European Americans):  $F(4, 28) = 0.76$ ,  $p\text{-value} = 0.55$ . While Holland equations using MCAL and MTAL overestimated living stature by an average of 2.01cm and 3.83cm, respectively. Using Holland's (1995) combined sex and race (African and European Americans) for PCAL underestimated L.S. by an average of 0.51cm. Using the new equation presented here which includes three ancestral groups underestimated L.S. by an average of only 0.63cm. These results illustrate that a new group-generic stature equation, which includes Asian samples accurately estimates living stature. These results also support Holland (1995) suggesting the foot elements are more linearly related to stature than other long bones, which have demonstrated an allometric scaling relationship to stature.

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**Group-Generic Stature, Calcaneus and Talus, Biological Profile**

**H52 Distinguishing Features of Thermal Destruction on Fleshed Wet and Dry Remains**

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After attending this presentation, attendees will have knowledge of differences in burn characteristics found on fleshed, wet, and dry burned bone. These features may be used to speculate the condition of human remains (fleshed, wet or dry) prior to a burn event.

This presentation will impact the forensic science community by contributing to knowledge regarding the condition of remains prior to the burn event.

The discovery of burnt bone often evokes questions about the condition of the body prior to burning. Although several observational and experimental studies (Krogman, Baby, Binford, Symes *et al*) have defined features on bones that were burnt in fleshed, wet, or dry states, contradictions in interpretations exist.<sup>1-4</sup> Color change, heat-induced fracture patterns, joint shielding, as well as shrinkage and warping have been found most useful in distinguishing burned, fleshed and wet bone from dry burned bone.

The purpose of this study was to score traits attributed to thermal damage on burned skeletal elements as a means to evaluate the reliability of these traits and to assess the relationship of these features to the body's condition (fleshed, wet or dry).

For the current study, 94 skeletal elements from 23 forensic cases (2 fleshed, 9 wet, 12 dry) were used. The South African Police Service had brought these remains to the Department of Anatomy, University of Pretoria for skeletal analysis between 1998 and 2009. Traits that are associated with burned bone were scored as either 0 (absent) or 1 (present). These included: greasy surface; joint shielding; defined tissue border; white tissue border; brown tissue border; predictable cracking in tissue border; minimal cracking around burned area; a heat line; cortical delamination; calcined bone; charred bone; and decomposition staining. Three observers independently scored these traits on each bone. A chi-squared test was performed to evaluate inter-observer error and differences in bone condition (fleshed, wet or dry).

No statistically significant difference was observed among observers. This demonstrates reliability in scoring these traits in a binary form. When all ten traits were compared, a statistically significant difference was noted among fleshed, wet and dry bone ( $p\text{-value} < 0.001$ ) and between fleshed and wet bone ( $p\text{-value} < 0.001$ ). The presence of joint shielding, defined tissue borders, white border, predictable cracking in tissue border and charred bone were found more often in fleshed than wet bone. As expected, wet bone was more likely to have a greasy surface, defined tissue border, white tissue border, predictable cracking in tissue border, and cortical delamination than dry bone ( $p\text{-value} < 0.001$ ).

Differences between fleshed, wet, and dry bone are observable and can be easily quantified among researchers. Fleshed skeletal remains show a normal burn pattern as previously defined by Symes *et al.*<sup>5</sup> Whereas, wet bone, which has retained enough organic content and moisture, presents with similar changes in warping and shrinking as fleshed bone but results in a burn pattern that is different from fleshed and dry burned bone. Dry bone has lost all its organic components and moisture; thus, responding differently to thermal alteration. Warping of dry bone does not occur and therefore thermal related fracture patterns differ in comparison to fleshed or wet bone. Ultimately, the condition and organic composition of the remains at the time of burning affect the manner in which a bone will burn.

Further research is needed to assess sequential changes in the burn patterns with the progression of decomposition. This can aid in the taphonomic profile of unidentified skeletal remains and possibly in the estimation of time-since-death.

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#### Burned Skeletal Remains, Taphonomy, Observational Study

### H53 Subadult Age-at-Death Estimation From Human Metatarsals

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The goal of this presentation is to inform attendees about new methods for estimating the age-at-death of human subadult remains using linear measurements taken on the developing metatarsals, as well as transition analysis from epiphyseal union of the metatarsal heads.

This presentation will impact the forensic science community by demonstrating two simple methods for estimating subadult age-at-death from the developing human metatarsals.

Accurate estimation of age-at-death from the human skeleton can be especially difficult in cases of fragmentary or incomplete remains. While developmental age estimates from subadults are generally both precise and accurate, they tend to rely on certain skeletal regions, which, when unavailable hamper the ability of the practitioner to generate an age estimate. There is currently no method for estimating the age-at-death of subadults from the growth (size) or development (epiphyseal fusion) of the metatarsal (MT) bones of the human foot. The fusion of the epiphyseal heads of MT2 - MT5 is said to occur during the mid-teens, while the fusion of the head of MT1 occurs much earlier (Scheuer and Black 2000 and references therein).<sup>1</sup> Previous research has demonstrated a strong association between metatarsal size and fetal age (Ayres de Vasconcellos and Ferreira) as well as metatarsal development and age in primates and hominins (Susman *et al.*).<sup>2,3</sup> The approach taken here is similar to that of Passalacqua (In Press) regarding subadult age estimation from the calcaneus.<sup>4</sup>

The present sample consists of 67 European American and African American males and females with ages ranging from 1-25 years from the Hamann-Todd Collection. Transition analysis using a cumulative probit model was conducted on the timing of epiphyseal union of the calcaneal epiphysis using Nphases2 (Konigsberg).<sup>5</sup> Fusion was scored as (1) *unfused* (no bony bridging); (2) *fusing* (presence of bony bridging between epiphysis and calcaneal body); or (3) *completely fused* (obliteration of epiphyseal line). This method allows for the mean age-of-transition from one phase to the next to be determined in addition to associated standard deviations using a maximum likelihood method (Boldsen *et al.* 2002; Langley-Shirley and Jantz 2010 and references therein).<sup>6,7</sup>

Results indicate that bony fusion of the metatarsal head epiphyses to the metatarsal bodies for MT2-5 (transition from score 1 to 2) occurs at 12.5 +/- 10.2 years and complete fusion (transition from score 2 to 3) occurs at 15 +/- 10.2 years (2S). The bony fusion of the metatarsal head epiphysis for MT1 occurs much younger at 2.5 and 5 +/- 4.6 years (2S); however the posterior epiphysis for MT1 fuses at similar ages to the metatarsal heads of MT 2-5. The wide standard deviations for the ages of fusion for the metatarsal heads for MTs 2-5 are likely a factor of limited sample sizes for teen individuals in the present sample.

In addition, maximum length for each of the metatarsals was collected from a sub-sample of individuals where the metatarsal heads were not completely fused. In order to determine if there are any significant differences in bone growth/size related to sex or ancestry, an ANCOVA was performed. No significant differences (*p-value* < 0.05) were found for sex or ancestry, and all individuals were pooled into a single sample (n=33). Because the subadult metatarsals may not be distinguishable in younger individuals, especially in the context of incomplete sets of remains, the lengths of MTs 2-4 were pooled and averaged to create a more applicable method.

Linear regressions against age (in yrs) resulted in *r*<sup>2</sup> values of 0.92 for MT1, 0.90 for pooled MTs 2-4, and 0.88 for MT5 all with standard errors of the estimate lower than 1.8 years.

Results demonstrate that the linear regression models fit the data well and allow for age estimates with narrow standard errors. Additionally, transition analysis results for the fusion of the MT heads roughly correspond to age ranges cited in Scheuer and Black (2000) though it should be noted that Langley-Shirley and Jantz (2010) have demonstrated shifts in age-of-fusion of the medial clavicle due to secular changes.<sup>1,7</sup> The Hamann-Todd Collection (utilized here) is no longer contemporary and secular changes in the timing of epiphyseal fusion may be present, necessitating further research on a contemporary sample before widespread forensic application can be recommended.

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#### Metatarsals, Subadults, Age-at-Death Estimation

## **H54 The Role of Forensic Sciences in Humanitarian Operations: Lessons Learned by the International Committee of the Red Cross**

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After attending this presentation, attendees will understand how the implementation of forensic sciences, including forensic anthropology, in humanitarian operations often pose unique challenges that require innovative approaches to resolving and preventing the tragedy of persons missing as a result of armed conflict and major catastrophe. Experiences and lessons learned from the International Committee of the Red Cross (ICRC) will be used to highlight how some of the distinctive characteristics of forensic sciences can be applied to humanitarian investigations.

This presentation will impact the forensic science community by preparing practitioners to adapt the application of their scientific skills to the unique challenges faced in broader humanitarian contexts.

The International Committee of the Red Cross (ICRC) is a Swiss-based international humanitarian organization founded in 1863, which provides protection and assistance worldwide for victims of armed conflicts, and other situations of violence and catastrophes, in a neutral, independent, and impartial way. The mandate of the ICRC stems from international humanitarian law (IHL), especially the four Geneva Conventions and their Additional Protocols. In 2003, the ICRC organized an International Conference on “The Missing and Their Families.” This saw the adoption by the international community of a set of recommendations for preventing and resolving the tragedy of the missing. The recommendations are framed by international humanitarian law provisions, making them universally applicable. Many of these recommendations relate to forensic best practices for the management of human remains and the identification of the dead. In that same year, the ICRC acquired its own forensic capacity to help promote and implement those recommendations worldwide. The activities of the ICRC’s Forensic Department are based on IHL provisions, and they center on families’ *right to know* the fate of their missing loved ones and on State parties’ obligations related to the proper and dignified management of the dead from armed conflicts.

The use of forensic sciences in humanitarian operations for resolving and preventing the missing from armed conflicts and major catastrophes pose a number of unique challenges for practitioners and concerned institutions involved in these investigations. Some of these factors include security, and logistical and resource constraints, which affect the work of forensic investigations. If the number of missing persons and unidentified remains is large, needs often exceed available resources for all stages of the process. As a result, existing structures often cannot cope with the large caseload, and cases are either not investigated appropriately or the process moves painfully slowly for the families. Even in contexts where international actors are able to provide assistance, it is difficult to maintain local and international support, including funds, if the process extends for years, or sometimes decades. Also in contexts affected by armed conflicts, the normal (i.e. peace-time) domestic legal and operational frameworks - which provide for the necessary checks and balances and for the smooth running of forensic investigations respectively - may be frail or absent. In addition, the political will and institutional commitment required to address the issue of missing persons is frequently limited. Families of missing persons as a result of armed conflicts and large catastrophes often experience unique legal, economic, and psycho-social needs, which forensic practitioners involved in those investigations should be aware of.

Many forensic investigations into the missing are long-term projects which require local capacity building and ownership of the process for ensuring their sustainability. This requires appropriate planning, resources, and specialists, including forensic archaeologists and anthropologists, who play a key role in investigations involving decomposed and skeletonized remains. These factors impact the efficiency and feasibility of the forensic

recovery and identification process in humanitarian operations. To the concerned forensic practitioners, they pose particular dilemmas which may be rarely encountered in normal domestic settings.

The lessons learned by the ICRC’s Forensic Department have confirmed the validity and usefulness of the recommendations from the International Conference on The Missing and their Families, for resolving some of the dilemmas faced by practitioners and for implementing forensic best practices in challenging contexts related to humanitarian operations.

**International, Humanitarian, ICRC**

## **H55 The Importance of Proper Data Management: General Considerations and Grassroots Implementation in Guatemala**

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The goal of this presentation is to increase awareness in the forensic science community on issues related to proper management of data on missing persons following armed conflicts. Implementation of a data management strategy in Guatemala is used as a case example.

This presentation will impact the forensic science community by broadening perspectives on data management for practitioners working with large numbers of missing persons and human remains, particularly in situations of violations of international law.

The International Committee of the Red Cross (ICRC) is a neutral, impartial, independent humanitarian organization that provides protection and assistance to victims of armed conflicts, other situations of violence and catastrophes. The ICRC Forensic Department participates in activities worldwide related to the promotion of scientific best practices on the management of human remains and identification of the dead, in contexts such as Central and South America, the Balkans, the Caucasus and the Middle East.

Proper data management involves the organized collection, handling, archiving and analysis of data, recognizing issues of chain-of-custody, data protection and confidentiality, access rights, etc. In situations involving large numbers of missing persons and unidentified remains, proper data management is integral to any strategy to identify human remains and fulfil families’ right to know the fate of their missing loved ones. Data management can be enhanced with a centralized database in which to process varying types of data. Therefore, the ICRC developed the Antemortem/Posmortem Information Management Tool (AM/PM), an electronic database application, which is freely distributed to authorities, forensic practitioners and other parties involved in the management of data on missing persons. The AM/PM includes modules for multiple types of data (AMD, PMD, Events, Field data, etc.) as well as tools for analysis of that data.

During the 1960-1996 internal conflict in Guatemala, approximately 40,000 individuals went missing, the majority belonging to one of the indigenous Mayan ethnic groups. To date there is no centralized missing persons register, and it is difficult to estimate the actual number of persons missing from the conflict. Dozens of NGO’s have developed following the conflict, many of them devoted to the issue of missing persons. These NGO’s range from professional human rights organizations offering legal and counselling services for families of missing persons, to scientific NGO’s recovering and analyzing remains and small grassroots associations of families of missing persons. These organizations possess a wealth of information on missing persons in varying degrees of detail, quality, and format. However, this information is difficult to access and in danger of loss as it is stored mainly in old paper files in unsecured offices. In addition,

a lack of standardized format and terminology has hindered efforts to exchange and consolidate the information between the various organizations.

In order to help organize, archive, update and consolidate the existing dispersed information, in 2010 the ICRC began a data consolidation project with 15 Guatemalan NGO's. The first phase of this project aimed at organizing the individual archives and digitizing them in a standardized format. For this purpose, the ICRC provided the AM/PM database, together with intensive training and coaching, and facilitated discussions on standardization of terminology and data entry. As a result, a consensus on national data entry standards has been developed, and the various organizations now have compatible digital archives of missing persons' information. The large number of organizations involved, their varying degree of organizational and educational levels, and very limited resources have posed challenges to the project. In addition, the need for clear and enforced data entry Standard Operation Procedures became evident early on and thus were integrated into the project. The second phase will focus on data cleaning and consolidation among the institutions in order to reach a consolidated list of missing persons for Guatemala. Such a list will enable authorities and NGOs' to access all the necessary available information and better focus their investigations.

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**Data Management, Humanitarian, ICRC**

## **H56 Working Towards Unified Forensic Protocols for Mexico**

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After attending this presentation, attendees will learn about the problematic issues concerning the unidentified dead in Mexico, particularly the problem of deceased migrants and the efforts to achieve better management and eventual identification of their remains.

This presentation will impact the forensic science community by creating a better understanding of the challenges of forensic human identification in a context of large-scale migration and of the importance of regionally standardized protocols and centralized data management, and of the ICRCs endeavours to support authorities in developing such tools.

Thousands of persons from various - mainly Central American - countries enter Mexico every year to cross the country and migrate to the United States and Canada. Many die on the way, due to accidents, environmental hazards, and attacks and killings by armed groups and drug trafficking gangs.

Having entered the country without any documentation or bureaucratic trace, the identification of these persons poses a major problem. While the individual medicolegal services around the country may have autopsy reports and information that might help identify those dead, there is so far no mechanism for exchanging this information and no standardized format. As a result, bodies usually end up as undocumented no name (NN) burials in mass graves, without possibility of further identification and restitution to their loved ones. Families looking for a missing person, on the other hand, do not have a clear contact point on where to report a missing person and where to start their search. They depend on informal networks, and those who can afford it often end up travelling from one morgue to the other in order to see if they can recognize the bodies. Only in a few high profile cases, which created a lot of publicity and thus alerted families, who in turn contacted the authorities in the respective countries, was it possible to identify a large majority of the bodies, such as in the case of the Tamaulipas massacre.

Mexico has 32 different state medicolegal systems which depend on different structures, each one with varying resources, and working protocols. In 2010 the ICRC sponsored a first national meeting of Medico Legal Services (SEMEFOs - Servicios de Medicina Forense), in which the

lack of standard protocols and centralization of information was identified as one of the main challenges in the identification of the dead in Mexico.

In order to tackle this problem, the Mexican SEMEFOs, in close cooperation with the General Prosecutors Office and with the support and advice of the ICRC, have started to develop a unified protocol for the management, documentation and identification of the dead in Mexico. The protocol consists of a manual and standardized data collection forms. It takes into account the international recommendations on management of dead bodies and forensic human identification while being adapted to the reality of the country. Pilot projects have been started to digitize the information collected in these unified formats into a standardized electronic database (the AMPM, provided by the ICRC), for later integration into a nation- or region-wide centralized system. Unified and centralized information, both on unidentified bodies and on missing persons is easily accessible for those who require it, and adherence to standards of best practice will be an important step towards decreasing the number of bodies being buried without names, and will help bring answers to those families trying to find out about the fate of their loved ones.

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**Protocols, Migrants, ICRC**

## **H57 Building Forensic Capabilities in Iraq**

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After attending this presentation, attendees will be aware of the current forensic capabilities in Iraq and of the various efforts to build and improve them since 2003.

The presentation will impact the forensic science community by providing a view of the challenges faced by forensic practitioners dealing with large numbers of missing persons and thousands of human remains still being recovered from mass graves in Iraq.

Since 2003, hundreds of mass graves have been located throughout Iraq. The Iraqi authorities have undertaken numerous steps to clarify the fate of missing persons, which have included the creation of specialized teams to manage information, collect data, and to recover and analyze human remains.

In relation to the clarification of the whereabouts of missing persons, the Iraqi authorities and the ICRC are involved in tripartite mechanisms related to two international conflicts (Iran-Iraq, Iraq-Kuwait). The development of forensic capacities has been supported since 2005, with resources and training provided to the national bodies involved in the management of information and identification of human remains.

The number of persons unaccounted for from the various conflicts that have plagued Iraq over the past few decades is estimated to be between one and two million. The Ministry of Human Rights is the national entity responsible for the collection and management of information and for the recovery of human remains from mass graves. They have one specialized archaeology team which carries out exhumations throughout the country. The Medico Legal Institute (MLI) of Baghdad, which depends on the Ministry of Health, is responsible for the analysis of human remains. Both bodies coordinate efforts for identification. The work load for these two national agencies is exceptionally large, ranging from one to several hundred sets of human remains per month.

With the support of several international organizations, including the ICRC, the MLI has continuously increased their capacity to deal with these cases and invested in the enhancement of their premises. Because of the large caseload, the MLI created a specialized forensic anthropology team and is in the process of developing a dedicated DNA laboratory to handle the samples taken from these remains and from relatives of missing persons who approach them. However, as happens in many other countries affected by international and internal conflicts, the Iraqi forensic services face many challenges these include: limited resources, few opportunities for high level education in forensic sciences, including forensic anthropology, lack of local standards for the analysis of skeletal remains, as well as no specific

legislation regarding missing persons, data protection, large scale management of human remains and genetic databases.

The MLI and the Ministry of Human Rights in Iraq still face an enormous task which encompasses the development of their capacities, the establishment of standard procedures, the creation and continuous education of specialized teams, the creation of specialized laboratories and the creation of solid mechanisms to provide answers to the thousands of families that continue to approach them.

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**Forensic Capabilities, Missing Persons, Iraq**

## **H58 The Missing From the South Caucasus: Perspectives From the Georgian Context**

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After attending this presentation, attendees will have an understanding of the implementation of humanitarian forensic sciences in the post-Soviet Georgian context.

This presentation will impact the forensic science community by discussing the challenges that are faced in forensic human identification projects and lessons learned regarding implementation of identification efforts in this region.

As a result of the conflict in 1992 and 1993 in Abkhazia, there are approximately 1,750 Georgians and 114 Abkhaz that remain unaccounted for. This conflict is unresolved, and it is a region where territory distinctions, the political environment and different levels of forensic capacity have made it difficult for the authorities to systematically address the issue of missing persons. As a result, families of the missing are left not knowing the fate of their missing loved ones.

While there have been several attempts in the past to implement identification efforts related to this conflict, there have been consistent obstacles to establishing a long-term, systematic identification process. However, in 2010 a new coordination mechanism was established, which included Abkhaz and Georgian representatives with the ICRC acting as the neutral intermediary. Under the auspice of this mechanism, some progress in determining the fate of missing persons from the 1990's has been made. A project has been initiated incorporating both the Georgian and Abkhaz forensic structures with the goal to exhume, analyze, and identify human remains following best practices, while adapting to the specific challenges of the context and increasing local capacity to address the different aspects of forensic human identification. This includes the collection and management of information such as antemortem, gravesite and postmortem data, as well as actively coaching local scientists in exhumation and analysis techniques.

Within this context there are significant trust issues between the parties that greatly influence the working modalities that are acceptable by all. While efforts can be time-consuming, confidence building between the parties is an important aspect of this project. In addition, the lack of local infrastructure has been a significant obstacle from the level of where to store and analyze human remains, to finding an acceptable location for genetic analysis. The set-up of the regular forensic facilities is not such that they can incorporate the additional workload and the needs for physical space associated with the identification process. On-going discussion and negotiation at different levels has been necessary in order to develop the infrastructure required by this project.

Data collection, from antemortem to postmortem and the management of this data have also presented many challenges. The context highlights the need for in-depth training of data collectors and close monitoring of the data that is collected. Furthermore, having experienced people coordinating the process and conducting quality control of the information throughout the identification efforts is fundamental to having reliable and usable data. While these have been new areas of work for many of the people involved in the project, the coaching approach has been successful in terms of building local capacity.

Working very closely with each set of authorities bi-laterally as well as within the coordination mechanism has been essential in order to tailor the project to the specific needs of each yet maintaining a process where the information that is gathered is consistent and the approaches systematic. Experience has shown that theoretical lectures alone are not sufficient to train local scientists in forensic archaeology and anthropology. Close working relationships, hands-on, tailored coaching, and the presence of a consistent team working on the issue with a multi-faceted approach have been essential to the process thus far.

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**Identification Project, Humanitarian, ICRC**

## **H59 Mechanisms to Address Missing Persons: The Sub-Working Group on Forensics in Kosovo**

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After attending this presentation, attendees will become aware of the complex nature of the recovery, analysis, and identification of human remains of missing persons, particularly in situations of armed conflict. This presentation will highlight the long-term nature of such projects, and the inevitable challenges that are faced throughout such a process. The main goal at this presentation will be to highlight the need for a mechanism to effectively coordinate the various participants in the field to successfully address these complex challenges; examples of such mechanisms and the lessons that can be learned from these models for the future will be provided.

This presentation will impact the forensic science community by highlighting the need for having a long-term perspective and plan to address the problem of missing persons. In addition, by making attendees aware of how work carried out at the start of a project can have serious ramifications later on, the need for continuity throughout the process will be demonstrated.

The issue of missing persons is a worldwide problem and the integral importance of forensics in trying to successfully address this matter continues to grow. The Western Balkans, in particular, Bosnia, Croatia, Serbia, and Kosovo, has seen a massive involvement of international and national forensic experts to recover, analyze, and identify persons who went missing as a result of the 1990's armed conflicts in this region. However, following all these efforts, approximately 14,000 persons remain unaccounted for (Bosnia @ 10,000, Kosovo @ 2,000, Croatia @ 2,000). Some of the main challenges today, are limited information on new gravesites, poor coordination between the actors early on in the process, many different actors involved at different times in the process, potential misidentifications, and large numbers of unidentified human remains stored in facilities in Kosovo (300-400), Bosnia (3,000), and Croatia (900). The reasons for these unidentified remains are numerous and complex, and to address them successfully will require a coordinated strategic approach.

While mechanisms have tried to address the challenges that the process faces from a forensic point of view, it is also important to highlight the need to involve the families and the wider community in assisting in the resolution of these issues. One example of a mechanism that has focused on these technical challenges is the Sub Working Group on Forensic issues in Kosovo. In 2005, the Working Group on Persons Unaccounted for in Connection with Events in Kosovo, realized the need for the involvement of forensic specialists and better coordination of concerned actors, established the Sub Working Group on forensic issues (SWG). The main goal of this forum is better management and acceleration of the forensic process.

For the construction of future mechanisms it is important to evaluate the successes and failures of this mechanism. Many challenges still face the process today; indeed these are not exclusive to Kosovo; across the Western Balkans similar challenges are being faced concerning the recovery, examination, and identification of missing persons from the conflicts of the 1990's. The need for a regional approach to these issues must be considered.

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**Mechanisms, Missing Persons, ICRC**

## H60 Advances in Victim Identification in Colombia

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After attending this presentation, attendees will understand how victim identification is achieved in Colombia through a multidisciplinary team approach and how the construction of a new human identification center aims to streamline the identification process by increasing training, implementing research as a major goal, and introducing country-wide protocols into the process.

This presentation will impact the forensic science community by demonstrating how a country with serious human rights and victim identification challenges is attempting to meet its responsibilities through practices geared towards scientifically accurate identifications using a team approach.

Colombia has been ensconced in internal conflict with illegally armed groups for the past 40 years which has resulted in tens of thousands of victims, many of whom are buried in clandestine graves, buried by the authorities as John or Jane Does, or interred by family members in plots not reported to the government. Additionally, human rights abuses exist in situations where the armed forces kill poor civilians so they can pose them as armed combatants, increasing their kill quota for those illegal groups.

Eighteen years ago, the Colombian government began hiring anthropologists to help in the effort towards identification of the victims from the different conflicts. Today there are four different government agencies that employ forensic anthropologists with a total of about 50 practicing anthropologists. Each agency has a slightly different function and protocols in use for human identification and field exhumations differ between the agencies. Efforts have been made over the past few years to streamline the process between the agencies in terms of viewing the skeletal analysis as a forensic autopsy, which involves a team of a pathologist, anthropologist, and odontologist who examine the remains. The anthropologist constructs the biological profile and analyzes the skeleton for taphonomy while the odontologist analyzes the jaws and teeth. The pathologist and anthropologist work in tandem to analyze pathology and trauma. The pathologist ultimately determines the cause and manner of death, when possible, and issues the death certificate. Once analysis has been completed, a report will be generated that is signed by all individuals involved with the analysis. At that point, DNA samples are processed.

After this analysis process, a dedicated team works on determining whether or not an identification has been made based on the forensic autopsy report and DNA report. This overall teamwork approach has been adopted by the two agencies currently involved in skeletal analysis, but there is still much to be done in terms of training and protocol development.

The Human Identification Center is a joint undertaking between ICITAP-Colombia, the Colombian Attorney General's office (CTI-Fiscalia), and the Colombian national morgue (National Institute of Legal Medicine and Forensic Sciences). It will be the first center of its kind in Colombia and possibly Latin America. The center has been developed to process the large number of human remains that derive from clandestine burials and other illegal methods of body disposal. While laboratories doing this type of work already exist in Colombia as mentioned above, the backlog is typically 12 months or more, the labs are short on space, and the workers are taught within the culture of the agencies that quantity is more important than quality. The center's goal is to combat all of these issues.

The Center is a morgue that has been designed specifically for forensic autopsies of skeletal remains. In addition to the forensic autopsy floor, the center includes a bone pulverization lab for DNA purposes. Further, three key goals of the center are protocol development, training, and research. In addition to casework, each scientist on staff will be expected to generate research and participate in the training of their colleagues on rotation at the

center. Further, the Center will be compliant with the ISO 17025 accreditation standard, the first laboratory analyzing human remains in Colombia to do so. The collaborative effort of the different government agencies involved and the different scientific disciplines involved in creating the center demonstrates a renewal in the commitment that the Colombian government has towards identifying victims of the Colombian conflict.

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Colombia, Victim Identification, Human Identification Center

## H61 Burial Patterns during Times of Armed Conflict in Cyprus in the 1960s and 1970s

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After attending this presentation, attendees will understand the patterns of burial during times of armed conflict in Cyprus, as learned from the Committee on Missing Persons in Cyprus (CMP).

This presentation will impact the forensic science community by introducing the mission of the CMP, the CMP Bi-Communal Forensic Team's (BCFT) archaeology program, and the observations and patterns of burial practices during armed conflict that occurred in Cyprus in the 1960s and 1970s.

Between 1975 and 1979, the United Nations General Assembly implemented three resolutions, and as a consequence, an investigatory body was established to determine the fate of approximately 2,000 people that were registered as missing from the inter-communal violence of the 1960s and the events of July 1974 and onwards. The Committee on Missing Persons in Cyprus (CMP) was established in 1981 under the auspices of the United Nations following an agreement between the Greek-Cypriot and Turkish-Cypriot communities. The CMP project is unique, as it is the only institutionalized bi-communal program where individuals from both communities, which were once fighting, are now working together at every stage of the project. The goal of the BCFT is to maximize the recovery of skeletal elements and document the spatial relationship of all physical evidence as to provide as much information as possible that would help in the final identification process. As such, the BCFT uses standard archaeological methods and techniques that have been adapted to the specific conditions of the "Cypriot recovery scene."

To date, 555 sites have been investigated after obtaining information from witnesses from the two communities. Of those, 213 contained human remains that are being analyzed by the bi-communal Anthropology and DNA teams for identification. The BCFT has excavated multiple burial sites across Cyprus, where specific patterns of burial during armed conflict have emerged. Thus far, BCFT excavations can be consolidated to five main types based on similarities in archaeological context, which include: (1) *in situ*, primary burials; (2) burial in wells; (3) deposition in stream beds; (4) surface deposition on mountain tops; and, (5) burial inside or next to abandoned cemeteries.

From the outset of the project, many informants and witnesses came forward to provide information on burial location. The first sites to be excavated were those with persistent and reliable witness statements, which invariably involved primary *in situ* burials. Of the 555 sites, 51 involved *in situ* primary burials.

In the mid-twentieth century, much of Cyprus moved to municipality water supply and in the years that followed wells became redundant and fell out of use. During the events of the 1960s and 1970s wells became a convenient place to dispose of bodies. Consequently, the BCFT has excavated 82 wells, of which 24 contained human remains.

Seasonal streams were also popular locations to dispose of bodies. The formation processes that dominate streambeds ensure that human remains would be dispersed and difficult to find. The BCFT has excavated

38 streambeds, of which 15 contained human remains. The remains in most cases consisted of isolated skeletal elements.

According to historical information, intense fighting took place on the Kyrenia Mountains in July and August 1974 resulting in a large number of missing persons. Many were left unburied resulting in dispersion by various taphonomic processes. The recovered remains typically consist of isolated skeletal elements that are often sun-bleached. Since the start of the project 46 sites have been excavated in the Kyrenia Mountains, of which 20 contained human remains.

The BCFT has excavated 22 sites in abandoned cemeteries, of which five contained remains related to the inter-communal fighting of the 1960s or the events of July and August 1974 and onwards. The archaeologists assigned to these cases must determine the forensic significance by drawing on their understanding of the various burial practices of an island where Christian and Muslim religions have dominated for centuries.

In excavating the island's past the forensic archaeologist becomes aware of the various burial practices that prevailed during armed conflict, which become informative for locating future burial sites. The five main categories of CMP recovery sites are reflective of the environmental and cultural parameters that characterized Cyprus during the 1960s and 1970s. Today, in conducting excavations for the CMP, the forensic archaeologists from the two Cypriot communities contribute to the overall mission for the recovery and identification of missing persons, in order to find a reconciled future.

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**CMP, Burial, Post-Conflict**

## **H62 Recovery of Missing Persons in Cyprus: Methods and Techniques of Complex Well Excavations**

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After attending this presentation, attendees will learn the methods and techniques that were developed by the bi-communal forensic teams of the Committee on Missing Persons in Cyprus (CMP) in order to recover remains of missing persons buried in deep wells at several sites across Cyprus during the 1960s and 1970s.

This presentation will impact the forensic science community by sharing best practices of the CMP forensic archaeologists to overcome the difficulties and safety risks associated with excavating human remains from deep wells.

The Bi-communal Forensic Team (BCFT) of the CMP has been conducting excavations since 2005 in order to find persons reported missing from the inter-communal fighting between the years 1963 and 1974. As a result of the violence during those times, a total of 494 Turkish-Cypriots and 1,493 Greek-Cypriots were officially reported as missing by both communities to the CMP. From our experience over the past five years, 145 individuals have been recovered from 82 deep-well excavations.

Well excavation methods vary according to the type, the depth, and the type of soil surrounding the well. The wells in Cyprus are mostly wheel wells for drawing water, dry wells, drainage wells, and reservoir wells, which are typically shallow wells. The depth of the wells excavated thus far varies from 5 meters to 30 meters. The well depth depends on the soil type and water level of the area. As a result, the BCFT establish an excavation plan, which includes decisions about what type of heavy machinery will best serve the excavation process. Management of the excavation process begins with gathering historical and circumstantial data related to the missing person(s), and dominant landforms, land use, and geomorphology of the excavation area. These data inform the necessary excavation methods and techniques.

Due to the prevalence of well excavation sites, the CMP forensic archaeologists developed a system of access ramps for heavy machinery,

pockets, and pools, which are excavated next to the mouth of the well to manage excess ground water. Initially, a well feature is opened on three sides by an excavator, not only for safety and timeliness, but also to allow access for the team to recover remains and additional evidence in situ. The excavator does not disturb the sediment inside the well; rather it makes a ramp with the assistance of a wheel loader, which transports large amounts of loose soil out of the excavation area. This method enables CMP archaeologists to excavate the deepest wells successfully by maintaining provenience of recovered evidence, while reducing excavation times and keeping costs low. For example, the CMP archaeology team excavated and recovered remains from wells in Gökhan/Voni and İskele/Trikomo that were 28 and 31 meters deep, respectively.

Over the past five years, the CMP Bi-communal Forensic Team has developed methods and techniques for excavating deep wells, which enables efficient excavation and recovery of remains. In doing so, the CMP forensic archaeologists overcome archaeological, logistical, and physical difficulties to reach human remains of individuals that went missing as a result of the inter-communal across Cyprus during the 1960s and 1970s.

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**Forensic Archaeology, CMP, Well**

## **H63 Thinking Outside the Box (of Bones) in Forensic Anthropology: Revisiting Roles, Components, and Naming Rights of the Discipline**

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After attending this presentation, attendees will fully understand the relationship between forensic archaeology and forensic anthropology and the significance of this role in the determination of a wide range of issues critical to the medicolegal investigation of the outdoor death scene.

This presentation will impact the forensic science community by discussing the diversity of roles forensic anthropology plays beyond just the determination of biological profile from human bones.

Forensic anthropology was formally defined in the early 1970's as a laboratory-based discipline focused almost exclusively on aiding the process of establishing the identity of the victim. Typically, law enforcement and coroner/medical examiner officials brought human remains to the forensic anthropologist only after other mainstream identification efforts such as analysis of soft tissue features, dental comparisons, and even clay reconstructions failed.

The bulk of these cases involved, therefore, skeletal remains or bodies altered or decomposed to a point at which the forensic pathologist could get little information from the soft tissues. As is the case today, most of these remains came from environments in which the body had gone undetected for long periods of time: burials and outdoor scenes. While their indoor counterparts were subjected to increasingly sophisticated mapping, documentation and information retrieval techniques, outdoor scenes were deemed by law enforcement as too altered and complex to receive the same treatment. Animals, weather conditions, and other natural processes would have erased most of the evidentiary information originally present at the scene. The absence of walls and other fixed spatial references made indoor mapping techniques impracticable, and burials were shoveled or backhoed to retrieve the body in order to send it ASAP to the morgue.

It was in this environment of hastily processed outdoor scenes and laboratory-based anthropologists that the field of forensic anthropology was defined and incorporated into the AAFS as an independent section. At the time, many forensic anthropologists saw the potential utility of archaeological techniques to process outdoor scenes and burials. A few of them took an active role in the recovery of the body and associated contextual information. However, for a variety of reasons, the initial

acceptance of archaeologists into the Physical Anthropology section of AAFS was delayed.

During the last four decades, the evolution of the field has resulted in a completely different reality. Paralleling a similar trend in paleoanthropology, forensic anthropologists gradually realized the need to collect and analyze scene information in order to answer many of the old and new questions posed by investigators and tribunals. For example, interpretation of skeletal trauma may be confounded by taphonomic and site formation processes, which cannot be reconstructed without carefully recorded scene data. Accurate methods of bone detection and recovery also required the involvement of an osteologist in the analysis and processing of the recovery scene. Archaeology and paleontology methods were demonstrated to be superior over conventional criminalistics techniques, and their adaptation into the medicolegal context translated into the development of forensic archaeology.

The application of archaeological techniques and methods to crime-scene recovery resulted in increased analytical capabilities that go well beyond the assessment of the biological profile for identification purposes. Forensic archaeology and taphonomy allowed for the reconstruction of events surrounding death, including factors such as original position and location of the decedent, and the estimation of postmortem intervals.

Today, forensic archaeology has been embraced and is an integral part of the work of forensic anthropologists. In addition, archaeologists have taken an active part both in the research and development of new forensic archaeological techniques, and in their application in forensic investigations. This presentation argues in favor of the necessity of acknowledging this reality within the Forensic Anthropology Section of AAFS, by accepting and incorporating forensic archaeologists into the section and renaming it accordingly.

**Forensic Archaeology, Scene Context, Forensic Taphonomy**

## H64 The 40th Anniversary: Research Trends in the Physical Anthropology Section of the AAFS

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After attending this presentation, attendees will gain an understanding of the trends and depth of research that has been conducted in the Physical Anthropology Section of the AAFS during its 40 year history.

This presentation will impact the forensic science community by identifying forensic anthropological research trends over time. Specifically, this presentation will outline what forensic anthropologists do and how much effort they put into particular types of research.

In 1972, the AAFS welcomed a new section of forensic investigation into its organization: the combined Odontology/Physical Anthropology section. A few years later this combined group split forming their own sections. Cooperation with other sections was common during the first twenty years, where combined sessions highlighted interdisciplinary collaboration. More recently, as the field of forensic anthropology has become more established and welcomed in the forensic community, and as AAFS itself has matured, the number of presentations in a given year has increased dramatically. In 1972, anthropologists presented two papers, today the Physical Anthropology Section averages over a hundred oral and poster presentations. In addition, the Physical Anthropology section regularly hosts workshops and breakfast/lunch seminars, as well as presenting research in other AAFS sections.

This presentation, given on the Physical Anthropology section's 40<sup>th</sup> anniversary, is timely. Twenty years ago the section hosted a session recalling the advances within the discipline. It was a look back at the last twenty years of work anthropologists have conducted in the AAFS. Recently, members of the section have expressed an opinion of possibly expanding our ranks with the inclusion of other branches of anthropologists (archaeologists, cultural anthropologists, and linguistic anthropologists). Now on the 40<sup>th</sup> anniversary, instead of looking back on the history out of

curiosity, the research trends should be reviewed with an eye towards truly understanding all the things the discipline is involved in. How much of the presentations and publications actually include archaeological research? How do the fields of cultural and linguistic anthropology influence research? Are we incorporating enough of our sister disciplines to acknowledge their contribution and inclusion into the Physical Anthropology section (if they so desire)?

This review of Physical Anthropology history in the AAFS builds upon Benedix and Belcher (2006), who presented a poster on the subject. Some of the categories that they originally included in their study have been modified, the recent AAFS meetings have been added, and years that were not represented in their original study have now been included. Ten categories of research have been identified: biological profile, identification, case studies, taphonomy, archaeology, trauma, pathology, human vs. non-human identification, discipline/history of the Physical Anthropology Section, and other (a loose collection of research that does not fit with the other categories). The specific details of each category will be outlined in the presentation.

A total of 1,976 presentations have been made in the Physical Anthropology section meetings of the AAFS between 1972 and 2010. The following table displays the ten research categories, the number of presentations that have been made in each category, and the percentage that category represents in the history of the section.

Category	Number	Percentage
Biological Profile	565	28.5%
Identification	252	12.8%
Case Studies	191	9.6%
Taphonomy	248	12.6%
Archaeology	96	4.9%
Trauma	147	7.4%
Pathology	20	1%
Human/Non-Human	23	1.2%
Discipline/History	260	13.2%
Other	174	8.8%

Through an examination of the annual AAFS meetings presentations and anthropology publications in the *Journal of Forensic Sciences*, a discussion will be presented on the trends in the Physical Anthropology Section and its ever expanding research directions. It is hoped that with this history in mind, the attendee will have a clear understanding of the research done and will thus be better prepared to discuss the future direction of the Physical Anthropology Section.

**Physical Anthropology, Trends, History**

## H65 Roles of the Forensic Anthropologist at the New York City Office of the Chief Medical Examiner

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After attending this presentation, attendees will have a greater understanding of the growing role of the forensic anthropologist in medical examiner/coroner systems with particular attention to anthropology at the New York City's Office of Chief Medical Examiner (OCME-NYC).

This presentation will impact the forensic science community by discussing the expanding role of the forensic anthropologist and demonstrate the holistic approach needed in forensic anthropology casework.

T. Dale Stewart classically defined forensic anthropology as "that branch of physical anthropology which, for forensic purposes, deals with

the identification of more or less skeletonized remains known to be, or suspected of being, human” (Stewart 1979:xi). By this definition, the forensic anthropologist is a specialist in the reconstruction of the biological profile from skeletal material. Over the past 30 years following Stewart’s definition there has been a paradigm shift in the conceptual framework by which the forensic anthropologist operates with specific reference to forensic taphonomy, forensic archaeology, and trauma analysis (Dirkmaat et al. 2008). This shift has increased the need for anthropological analyzes in the medical examiner’s setting and expanded the role of forensic anthropology. As noted by Austin and Fulginiti (2008), anthropologists within the medicolegal system frequently perform roles in addition to standard forensic anthropological analyzes. The Forensic Anthropology Unit at New York City’s Office of Chief Medical Examiner (OCME-NYC) has developed the largest dedicated staff of forensic anthropologists outside of the Department of Defense and currently consists of eight full-time anthropologists. In this setting, the roles and responsibilities of the forensic anthropologist are diverse and span into several operational areas beyond the classic laboratory based analyzes, specifically as they relate to field operations and decedent identification.

With expertise in archaeological techniques and methods, forensic anthropologists can assist medicolegal investigators with scene investigations where decomposed, skeletonized, fragmentary, burned, or buried remains are discovered. An archaeological approach to crime scene documentation is used to thoroughly and efficiently assist with the search and recovery of human remains and associated evidence. This approach also provides contextual details that may contribute to a better understanding of the crime scene. Scene maps provide a valuable addition to the case file and at the OCME-NYC, hand drawn maps are produced but specialized mapping technologies (GPS, total station, etc.) may also be employed when appropriate.

Forensic anthropologists at the OCME-NYC are also taking an increased role in the identification of decedents. No unidentified case is released for burial without an anthropological estimation of age. Analysis of sex, ancestry, and stature are performed as needed. Furthermore, electronic data are compiled and entered into national missing/unidentified databases such as the National Missing and Unidentified Persons System (NamUs) and the National Crime Information Center (NCIC).

Outside of daily operations, the forensic anthropologists are a critical component of OCME’s disaster response team. In response to a disaster, the forensic anthropologist may fill standard roles such as mapping the scene of a mass fatality incident or assisting at the anthropology section of the disaster mortuary. Non-traditional roles are also evident in a disaster response. For instance, the forensic anthropologist may be involved with the Family Assistance Center, dissemination of information to family members, review of antemortem information, and coordination of the identification process. The forensic anthropologist may also take a leadership role with the body recovery effort. Similar roles are currently being assumed by the OCME’s forensic anthropologists with the on-going efforts surrounding the World Trade Center disaster, both in field recovery operations and identification efforts.

It is apparent from the current work performed at the OCME-NYC, as well as by the increased employment opportunities over the past decade for forensic anthropologists that forensic anthropology has grown beyond the scope of its classic definition. As the result of this growth, the required education/training and qualifications of those pursuing a career as a forensic anthropologist is also changing. A comprehensive anthropological background is necessary considering that forensic anthropology draws methods and theory from physical anthropology, archaeology and cultural anthropology. Forensic anthropologists benefit from this broad training and expertise within these sub-fields of anthropology.

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#### **Forensic Anthropology, Roles, Medical Examiner**

## **H66 Redefining the Scope of Forensic Anthropology: Embracing Archeology Would Help to Guide the Development of Best Practice for Field Recoveries of Human Remains**

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After attending this presentation, attendees will understand: (1) why forensic anthropologists should formally recognize archeology as a subfield of forensic anthropology; and, (2) why anthropologists must more actively advertise their field recovery skills to the medicolegal system.

This presentation will impact the forensic science community by clearly defining the five basic skill sets required for field recoveries of human remains and presenting a “best practice” argument for an increased reliance on archeologists in such cases.

Traditionally, forensic anthropology has been defined in terms of laboratory skills in skeletal analysis and human identification. Less appreciated by the medicolegal system is the fact that most American anthropologists are trained broadly in multiple subfields, including archeology. As a result, many jurisdictions continue to rely on crime scene investigators to process outdoor human remains scenes. However, compared to other types of forensic cases, decomposed human remains are relatively rare. The typical CSI will have little practical experience with these cases, and few agencies will invest the resources to properly train their personnel in all aspects of field recovery. CSI’s tend to come from criminal justice and other non-science backgrounds and have little formal training in field methods. Short continuing education courses do not provide enough theoretical background or experience for them to conduct adequate recoveries, particularly of buried remains.

Five major skill sets are necessary to conduct controlled field recoveries. First, one must identify and inventory human bones and decomposed soft tissues in any condition so that missing elements can be noted and found before the scene is released. This skill requires coursework in osteology and anatomy. Second, one must interpret soil stratigraphy and understand how various processes have altered the soils, helping in the search for clandestine graves and the exclusion of irrelevant areas from continued investigation. This skill requires coursework in geology and sedimentology. Third, one must understand the techniques of controlled archeological recovery to minimize damage to the remains, to maximize evidence recovery, and to prepare effective maps. This skill requires coursework in archeological methods, surveying, and an intensive archeological field school. Fourth, one must recognize unusual forms of evidence, such as plants and insects that can link a suspect to the crime scene or help to estimate the postmortem interval. One must also trace all evidence back to specific soil strata so that its temporal association with the remains can be firmly established. This skill requires coursework in biology and the laboratory analysis of archeological materials. Fifth, one must recognize and interpret phenomena that alter the crime scene through time, including scavengers, water action, and agricultural practices, so that the final distribution of remains and evidence can be understood. This skill requires coursework in taphonomy, ecology, and site formation processes.

Only anthropologists with formal training in archeology are likely to possess all of these skills. At a time when the qualifications of all forensic practitioners are falling under scrutiny, the question must be asked whether the inconsistent, unregulated approach to human remains recovery in this country constitutes best practice. Systematically extending our established expertise in field recovery techniques into the forensic realm can significantly improve the quality of death investigations and increase the sophistication of interpretations drawn from the available evidence. Otherwise, we may witness more situations where, under informed cross-examination, a police recovery “expert” may not be able to render opinions regarding assailant activities, the original deposition point of the body, how

the scene was altered in the postmortem interval, and how the evidence helps to explain the circumstances of death. Worse, crucial evidence may be impeached for lack of proper contextual associations.

Anthropology has an opportunity to increase its public impact by encouraging its professional archeologists to participate more frequently in local medicolegal investigations. We must partner with law enforcement to find ways of educating archeologists in the unique problems and specific needs of forensic casework. In support of these initiatives, we should expand the traditional definition of "forensic anthropology" to include the subdiscipline of archeology, formally define minimum professional qualifications for field recoveries, and establish an appropriate certification process in forensic archeology.

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**Forensic Archaeology, Field Recoveries, Decomposed Remains**

## **H67 An Atypical Burn Pattern Associated With Forensically Significant Human Remains**

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The goal of this presentation is to demonstrate patterns of heat alterations on fresh bone, and more specifically the identification of atypical characteristics of heat alteration to human remains that decomposed for approximately two weeks before being burned. In addition, this presentation present a more typical burn pattern and color to fresh bone while in a pugilistic pose to decomposed remains configured in a tightly flexed position.

After attending this presentation, attendees will understand typical burn patterns on fresh bone including the initial, secondary, and final areas to burn on bone, the color range on burned bone, and how to identify possible trauma caused to an individual prior to heat alteration.

This study will impact the forensic science community by serving as an example of an atypical burn pattern seen in a homicide case and demonstrate the effects that heat has on bones.

Forensic anthropologists analyze burned remains to aid medical examiners in determining the manner in which an individual died. Burning human remains can destroy or alter evidence and is used to attempt to obscure the identity of an individual. Proper recovery of burned skeletal remains is important for identification of the individual and possible trauma sustained antemortem. Heat can cause bone fractures making it difficult to distinguish trauma from heat altered fractures. Recovery and thorough reconstruction of the skeleton is helpful in determining if there was any trauma to the bones prior to the body being burned (Herrmann and Bennett).<sup>1</sup> Heat altered bones also exhibit a range of colors indicative of the amount of time the bone was exposed to heat in a sequence (from the highest exposure to heat to the lowest) of calcined, charred, border and heat-lined (Ubelaker).<sup>2</sup> Teeth exhibit a similar range of colors which are categorized (from highest heat to lowest heat exposure) as incinerated, charred, scorched and intact. Some teeth may burst apart from high amounts of heat as well (Delattre).<sup>3</sup>

This presentation is based on an analysis of a decedent that was decomposing in a large garbage can for two weeks and then set on fire in an attempt to cover up a homicide. The remains were analyzed at the Southeast Texas Applied Forensic Science (STAFS) Facility in Huntsville, Texas. During the recovery process some skeletal elements were recovered approximately fifteen feet from the concentration of remains. In the laboratory, the skeletal remains were reconstructed, placed in anatomical position, and inventoried. In the anatomical position the burn pattern of the skeletal and dental remains was evaluated. The burned bone ranged from no burn to calcination. The burn pattern of "initial, secondary and final areas to burn on bone" was not consistent with that typically seen on fresh bone in the pugilistic pose. In addition to the atypical burn pattern, a triangular-shaped unburned bone fragment, from the left parietal bone, measuring approximately 23mm on each side, was recovered. With the

exception of the posteroinferior tip of the bone no surfaces, including edges, were burned. When reconstructed the edges of this bone were part of radiating fractures that extended from a tool mark located approximately 15mm anterior to the unburned bone fragment. The tool mark, radiating fractures, and the unburned parietal bone are consistent with blunt force trauma prior to thermal alteration.

In fresh bone burn patterns of the skull, the squamous portion of the frontal bone is typically one of the first areas to burn while the occipital is one of the last areas to burn. In the present case, the occipital was calcined while the frontal bone was charred with small areas of unburned diplöe. Other atypical burn patterns of the bone and dentition will be compared to the more typical heat-altered pattern. The results of this presentation will give insight into the importance of proper recovery and reconstruction and the recognition of an atypical burn pattern as a result of unusual body positioning during burning. The information provided in this study will be helpful to forensic anthropologists and medical examiners presented with burned human remains.

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**Forensic Anthropology, Heat Altered Human Remains, Atypical Burn Pattern**

## **H68 Contaminated Cremains? Evaluation of Biological Characteristics Derived From Ash Weight**

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After attending this presentation, attendees will gain a better understanding of the value of anthropological assessments of cremated human remains. The goal of this presentation is to highlight methods used to evaluate whether suspected cremated human remains match the biological profile of the decedent.

This presentation will impact the forensic science community by providing awareness of the value of cremains weight in estimations of sex and assessment of other attributes of the biological profile.

The growing popularity of cremation in the United States has increased the likelihood of civil litigation involving commercial crematories and funeral homes.<sup>1</sup> Forensic anthropologists are often consulted to determine whether alleged cremains are in fact cremated bone and if aspects of the biological profile are consistent with the decedent. There are numerous limitations to the forensic analysis of cremains, due to the standard practice of pulverizing bone into small, unidentifiable fragments following cremation. Despite these challenges, several studies have documented a significant sex difference in average cremains weight, with males weighing approximately 1,000 grams more than females.<sup>2-5</sup> Sectioning points generated from cremains weights reported in these studies have shown reasonable predictive accuracy for sex estimation, although significant variation in average weights has been documented between regions.<sup>2,5</sup>

In 2011, the California State University, Chico Human Identification Laboratory (CSUC-HIL) was contacted by an attorney to examine a cremains case suspected of contamination. The family of the decedent notified the law office that a funeral home had misplaced an urn containing

the remains of a relative (a 60 year-old male). After further legal inquiry, the funeral home claimed to have relocated the urn. Due to the family's suspicions, the law office contacted the CSUC-HIL to analyze the contents of the urn to determine whether the cremains are consistent with that of the decedent.

Upon receipt, the urn contained a conglomerated block of material, which was removed from the plastic bag liner and placed into aluminum trays. Using geological sieves (1/8 inch mesh) and a strong magnet for small ferromagnetic metal items (screws, staples, etc.), the contents were separated into either osseous or non-osseous material. The majority of the urn contents were non-osseous material having the consistency of damp sediment (e.g., concrete dust, sand, cat litter). Following removal of the osseous remains from the sieves, radiographs of the remaining material showed very little cremated bone had passed through the 1/8 inch mesh. None of the cremains showed diagnostic anatomy; thus, it was not possible to determine if the cremains were human or nonhuman in origin.

Several lines of evidence supported the initial suspicion of contaminated cremains. First, the urn's contents weighed 544.3g more than originally recorded in the cremation log. Second, the urn contents weighed 4,173.5g, with non-osseous material (e.g., sediment, metal, stones) comprising 86.4% (3,604g) of the total weight, the majority of which (85.7%; 3,575.3g) was sediment. Only the remaining 569.5g (13.6%) represented cremains, well below even minimum reported weights for both sexes. Third, based on the reported stature of 154.9cm for the decedent, cremains weight is predicted to be 2,021.8g using equations reported in Warren and Maples.<sup>4</sup> The predicted weight for the decedent's stature is less than one-half of the actual weight of the urn contents. Additional testing, including x-ray fluorescence and x-ray diffraction analysis, will soon be undertaken to chemically characterize the urn's contents.

The results of this study highlight the value of cremains weights in evaluating aspects of the biological profile, as well as in settling legal disputes regarding potentially contaminated cremains. Estimations of sex and other biological parameters in cremation litigation cases should be applied cautiously due to reported regional differences in mean cremains weights. Further, additional studies, such as x-ray fluorescence and x-ray diffraction, can also be used to aid in the distinction of bone from other non-osseous materials.

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#### Cremains, Biological Profile, Forensic Anthropology

## H69 Validation Study of Microscopic Analysis of Saw Marks in Bone

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The goals of this presentation are to review the current method of microscopic analysis of saw marks in bone, showcase the variables utilized in the analysis, and present the results of an independent validation study of the method.

This presentation will impact the forensic science community by increasing the general knowledge of the method of microscopic analysis of saw marks in bone and providing specific information regarding interobserver error and the potential error rate associated with the method.

Microscopic analysis of saw marks in bone is a well published, generally accepted, and commonly used method. The strength of the method is that it is based on the use of standard laboratory equipment, a stereomicroscope, to analyze easily recognized qualitative and quantitative characteristics of a saw mark. Despite the method's attractiveness, it has not been independently validated, nor has the potential error rate been defined.

In 1975, Bronte published a seminal article on microscopic analysis of saw marks. He expanded the then current method of measuring the width of a saw mark to the analysis of the shape and pattern of striations observed in the walls of the saw mark.<sup>1</sup> He disproved the hypothesis that saw marks on bone destroy themselves with each consecutive stroke of the saw and showed that several class characteristics of the saw are recorded in the mark. In 1978, Andahl expanded on Bronte's work. He divided a saw mark into two components: the floor and the wall.<sup>2</sup> Andahl found features recorded in the floor of the mark, both in partial cuts and on the breakaway spur of complete cuts, that reflected the number of teeth per unit length, tooth set, degree of wear, direction of cut and condition of the blade. Symes contributed to previous works in his doctoral dissertation.<sup>3</sup> He evaluated experimental saw/cut marks made using 26 types of saw blades and serrated knives. Through microscope analysis, Symes observed and described numerous features of the marks that reflected the class characteristics of the tool.

Harris County Institute of Forensic Sciences Anthropology Division designed a validation study of microscopic analysis of saw marks in bone. Experimental saw marks were examined by three analysts following the current method as described by Symes.<sup>3,4</sup> Experimental saw marks were made using three hand saws and one power saw. The hand saw blade types were an 18 teeth per inch (TPI) wavy set, an 18 TPI raker set, and an 8 TPI raker set. The power saw was a reciprocating saw with a 10 TPI raker set blade. The four saws were used to create 58 samples in four human femora. Each sample consisted of a false start and both surfaces of a complete cut. The samples were separated using a Stryker saw and each Stryker saw cut was scored to differentiate it from the experimental cut surfaces. Each sample was analyzed using a Keyence digital microscope and indirect fiber optic light. Fourteen variables were evaluated on each sample. Eight variables were quantitative: minimum kerf width, tooth width, trough width, floor dip length, pull out striae interstriation distance, number of directional changes of striae, inter-tooth hop distance and inter-harmonic distance. Six variables were categorical: breakaway spur, kerf wall shape, kerf flare, trough morphology, entrance shaving and exit chipping.

Initially, a pilot study was conducted with 10 randomly chosen samples. Consistency among the three analysts for each feature was measured. The analysts agreed on the kerf wall shape and exit chipping six out of 10 times, consistency of cut seven out of 10 times, presence of pull out striae eight out of 10 times, kerf flare, trough morphology and entrance shaving nine out of 10 times, and harmonics 10 out of 10 times. The difference in minimal kerf width measurements ranged from 0.01 – 0.67mm and the difference in inter-tooth hop distance ranged from 0.14 – 2.54mm.

Not all variables showed equal sensitivity. Harmonics were found to be absent in all of the samples. The floor morphology of the false start was found to be flat in all but one of the samples. Also, entrance shaving was found to be absent in all but one of the samples.

Following the pilot study a review of the variable definitions and results of the study was conducted. The complete study was initiated and is ongoing. The statistical analysis of the complete data set will show the discriminative value of each variable, the associated interobserver error, and the potential error rate of the analysis.

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#### Validation Study, Saw Marks, Bone

## H70 A Prospective Study of Hyoid Fractures in Cases of Fatal Blunt Force Injuries to the Upper Body

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After attending this presentation, attendees will better understand the relationship of hyoid fractures to mechanism of injury, sex and age-at-death, stage of bone union, and soft tissue hemorrhage.

This presentation will impact the forensic community by providing a clearer understanding of hyoid fractures, which will affect the manner in which forensic anthropologists and pathologists interpret this type of trauma, especially when the context of the injury is unknown and soft tissue has been lost.

The cause of hyoid fractures and its contribution to assessments of cause and manner of death are unresolved issues in forensic sciences. Neck compression injuries, strangulations and hangings, are often examined for fractures of the hyoid, as well as thyroid and cricoid cartilages. Due to an increase in death from motor vehicle accidents, anecdotal statements have been made that injuries associated with rapid deceleration, such as hyperflexion or hyper-extension of neck, are more likely to fracture the hyoid than either strangulation or hanging. Isolated hyoid fractures have been reported in sports injuries, falls, and profuse vomiting (Padgham 1988; de la Grandmaison 2006; White 2010). While copious literature is available on the incidence of hyoid fractures with various mechanisms of death, the results are contradictory and do not provide a systematic approach to the assessment and interpretation of throat organ fractures. The purpose of this study is to assess the relationship of hyoid fractures with six mechanisms of injury, sex, age, bone fusion, and presence and location of soft tissue hemorrhage.

A total of 276 hyoids associated with motor vehicle accidents (MVA) (79), pedestrian vehicle accidents (PVA) (74), falls from heights (44), strangulations (15) (manual strangulations six; ligature strangulations six; and unknown two), and hangings (64) were examined. The sample was predominantly male (n=209) with 56 females; mean ages were 36.29 years and 38.05 years, respectively. Cases were examined during autopsy at the Forensic Pathology Services (FPS) in Johannesburg, South Africa from September 2009 to June 2011. The University of Witwatersrand authorized ethical clearance (R14/49; MO90728).

Neck structures were dissected in situ and in accordance with the Gordon method, a standard dissection technique for the neck. Presence and location of hemorrhage was recorded at autopsy. The hyoid bone was retained and processed free of soft tissues. The probability of obtaining a fracture with the above-mentioned independent variables was tested with logistic regression.

Fractured hyoids comprised 24% (n=67, 55 males and 12 females) of the sample. Fractures were noted on the greater horns; at the articular facets; and on the body, and were associated with two strangulations; eight falls; 14 PVAs; 20 MVAs and 23 hangings. Mechanism of injury, sex, and hemorrhage did not increase the probability of a bone fracture and did not show statistical significance. But, persons older than 50 years had a 4.3 increased likelihood of a fracture than younger persons (*p-value* < 0.003). Likewise, unilateral fusion of the greater horn on the left or right side were 2.9 and 2.7 times more likely to fracture than un-fused or completely fused bones (*p-value* = 0.019 and *p-value* = 0.041, respectively).

Although inconsistent with forensic anthropology and pathology literature, it appears that mechanism of injury, sex, and hemorrhage are disassociated with the presence of fractures in the hyoid. The interpretive circumstances surrounding a neck injury is likely biomechanical in nature, i.e. magnitude, direction, duration, and location of force. While older persons demonstrated a greater incidence of fractures, horn failure should not be associated with complete fusion of the greater horns of the hyoid to the body. The unique configuration of a unilaterally fused U-shaped structure, the histological composition of the bone, and the composition of muscle and cartilage may contribute more to fractures in older persons. While an association with hemorrhage and fracture indicates injury during life, an equal number of cases with fractures (n=27) and without fractures (n=30) presented with hemorrhage to neck structures. At autopsy, only 27% (n=18) of the above-mentioned fractures were recorded.

Recognition, physical examination and correct interpretation of trauma from the skeleton are invaluable tools to understanding total body trauma in violent deaths. This can only be achieved with accurate contextual information and a closer examination of fractured or presumed fractured bones.

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#### Trauma Interpretation, Neck Structures, Mechanism of Injury

## H71 San Bruno Gas Pipeline Explosion: Responding to a Neighborhood-Wide Disaster

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The goal of this presentation is to examine the complexity of applying forensic archaeological methods to a neighborhood-wide disaster. This presentation will include an overview of the recovery efforts following a gas pipeline explosion in San Bruno, California and highlight the challenges of working within various local, environment, and personnel constraints.

This presentation will impact the forensic science community by providing an example of a complex forensic recovery and the collaboration with various local first responders. As disasters requiring the assistance of forensic anthropologists are difficult to anticipate, this presentation addresses the need for outreach efforts to connect forensic scientists to other individuals and agencies involved in the recovery efforts before, during, and after a disaster occurs.

On September 9, 2010, a Pacific Gas and Electric (PG&E) natural gas pipeline exploded in a neighborhood located in San Bruno, California. The explosion occurred at 6:11 p.m. and quickly engulfed surrounding houses in the residential area. Escaping natural gas was ignited for more than an hour as PG&E personnel worked to shut down valves above and below the line rupture. Fires associated with individual homes were brought under control within 24-hours of the initial explosion. In total, the explosion destroyed 38 homes, damaged 70 other residences, and resulted in eight fatalities.

The California State University, Chico Human Identification Laboratory (CSUC-HIL) was contacted on September 11, 2010 to assist in the recovery efforts. A team of six faculty and staff members from CSUC-HIL and San Francisco State University, and 13 California State University, Chico students and alumni arrived on-site on September 12, 2010. Other

agencies involved in recovery operations for the San Bruno area included the National Transportation Safety Board, the San Mateo County Coroner's Office, San Bruno Fire and Police, San Mateo County Search and Rescue, and personnel from PG&E. The focus of the CSUC-HIL forensic recovery was a single residence near the scene of the explosion.

The CSUC-HIL recovery efforts included locating and mapping the remains of three individuals found in the residence. As a result of the exposure to intense and prolonged heat, the remains recovered were highly fragmented and calcined, making the distinction between the osseous material and the surrounding matrix extremely difficult. The residence, like all the homes in proximity to the initial blast site, was completely destroyed with no standing structures except for the chimney and an exterior concrete fence. The matrix from the collapsed structure was contained in the footprint of the house and was between one and two feet in depth. No interior walls or supporting structures survived the fire.

Several factors encountered added to the complexity of the recovery operation. First, the destruction from the gas pipeline explosion was a neighborhood-wide disaster with dozens of residences completely destroyed. As a result, the recovery operations took place in a large area with no existing infrastructure, such as clear streets, electricity, phones, or running water. Second, the length of time of the fire and the intensity of the heat from the ignition of the natural gas main created a large debris field up to three feet high across the neighborhood. The attempts to control the fire in the two days prior to the recovery included several fire departments and aerial fire suppression efforts, which greatly disturbed this scene. Third, the nature of the fire created a scene unlike those normally encountered by local fire personnel. As a result, early efforts to recover victims of the house were halted when the highly fragmented condition of the remains became apparent. The subsequent recovery by the CSUC-HIL team included examining remains in situ within the residence, and also identifying which elements had been recovered the previous day during the initial search for remains. This ensured maximum recovery of human remains from the site. Lastly, the lack of surrounding infrastructure and the duration of the recovery effort hindered the acquisition of proper safety equipment for the CSUC-HIL team. This limited the number of personnel the CSUC-HIL team could actively have conducting the forensic archaeological operations at any given time, which became a concern given a timeframe of less than 10 hours for the recovery.

This presentation will discuss the approach and recovery methods used by the CSUC-HIL team and will examine the lessons learned from the limitations encountered. The collaboration with local personnel will also highlight the role of forensic anthropologists in communicating proper recovery methods to a larger community of first responders.

**Forensic Anthropology, Forensic Archaeology, Fire Scene Recovery**

## **H72 Determination of Impact Direction Based on Fracture Patterns in Human Long Bones**

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After attending this presentation, attendees will have a better understanding of how to determine the direction of impact on long bones based on fracture pattern. The main goal of this research is to show that under controlled laboratory conditions, fracture patterns in human long bones display consistent characteristics that allow the determination of impact direction.

This presentation will impact the forensic science community by clarifying the relationships between characteristic features of fracture patterns and directionality of impact on human long bones.

The current forensic literature suggests that the direction of blunt force trauma to human long bones can be determined by the presence and orientation of a butterfly fracture. However, a recent study by Thomas and Simmons (2010) using a sheep bone model suggest that the butterfly pattern was an inconsistent finding. They then conclude that blunt force direction should not be assumed based on the presence or orientation of a butterfly fracture pattern. A series of experimental studies using 550 human long bones (Kress, 1996) also generated various patterns of fracture, including the typical butterfly, inverted wedges, oblique and transverse fractures. However, a variety of impact loading conditions and devices were used. Therefore, it remains unclear whether a butterfly wedge is a consistent finding during blunt force trauma, or, when present, if its orientation accurately predicts direction of loading.

Based primarily on the mechanics literature, this study hypothesized that under controlled laboratory conditions of impact loading and defined constraint of the bones, a consistent set of fracture features will result that can be used to predict impact direction on human long bones. While previous research typically focuses on the presence of butterfly fractures to determine impact direction, the current study examined the presence of complete as well as incomplete fracture characteristics to determine directionality of blunt force trauma to a long bone. Since it is believed that human long bone fracture initiates on the tension side and then propagates along lines of shear on the compression side to form a butterfly fracture, the current study also examined the location of fracture initiation and the pattern of fracture propagation.

In this study, 15 dry human femora were impacted with a rounded 2.5-inch cylinder perpendicular to the long axis of the bone. The anvil was connected to the hydraulic actuator of a material's testing machine (MTS, model 810). The ends of the bones were potted in room-temperature curing epoxy resin and inserted into cylindrical cups that allowed translational and rotational motions. The femora also had an axial load of 100 lbs. applied during the three-point bending tests. Impact loads transverse to the long bone axis were applied in the anterior-posterior and posterior-anterior directions by controlled displacement of the anvil at 0.2 m/s. In numerous experiments, fracture initiation and propagation were captured with a high-speed camera at 10,000 fps.

Results indicate that on a gross scale, 9 of 15 (60%) fractures were oblique, 4 of 15 (27%) fractures were transverse, and 2 of 15 (13%) were comminuted. But the gross fractures did not tell the whole story. A lesson learned is that one must also inspect the incomplete fractures. Closer examination of the bones revealed four common long bone fracture patterns: (1) incomplete butterfly fractures (tension wedges) in 80% of the cases; (2) transverse fractures on the tension (initiation) side in 80% of the cases; (3) failure angle shifts from approximately 45° to parallel (to the long axis) in the oblique gross fractures in 87% of the cases; and, (4) breakaway spurs on the compression side in 73% of cases. These common fracture patterns represent criteria that enabled us to determine the blunt force impact direction in 14 of 15 experiments.

In summary, the current study indicated that in a controlled laboratory setting, examination of distinct fracture characteristics help determine impact direction on human long bones. Specifically, a set of four criteria, outlined above, can be evaluated and used to quite accurately determine the direction of traumatic forces on tubular bone. The results also show that fracture consistently initiated on the tension side of the bone, opposite to the side of blunt force trauma, and that fracture propagation typically followed theoretical lines of maximum shear stress.

**Fracture Pattern, Blunt Force Trauma, Directionality of Force**

## **H73 Citrate Content of Bone for Time-since-death Estimation: Results from Burials with Different Physical Characteristics (Wooden Coffins Versus Plastic Body Bags) With Known PMI**

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After attending this presentation, attendees will better understand how determining the content of citrate in bone of skeletal remains can help estimate time-since-death or postmortem interval (PMI) and how covering the body in a plastic body bag during the burial also affects the outcome of these determinations.

This presentation will impact the forensic science community by increasing knowledge, competence, and performance by describing how the citrate content determination in bones can be used to estimate the PMI and how to apply the proposed method properly. Problems arising from different physical characteristics of the burial will be identified. Finally, a protocol to implement this method in forensic practice will be recommended.

The reliability of methods for PMI determination of skeletonized human remains found at crime scenes is still far from satisfactory; these methods include macroscopic reaction to UV light, an analysis of the histological quality of cross-sections, chemical determination of nitrogen, carbon or amino acid content or the reaction of bone tissue to luminal. Recently, a new, extremely promising method was introduced, analyzing the citrate content of bone and establishing an equation for calculating the PMI.<sup>1</sup> In this study, it was postulated that there has to be direct contact between bone and soil in order to initiate and prolong the process of decreasing citrate content in the bone over time. Therefore, our study was conducted using skeletal remains with known PMIs but with different physical burial characteristics. One group was buried in conventional wooden coffins and the second group was additionally covered in plastic body bags, which should hypothetically lead to an underestimation of their PMIs.

The study included a total of 20 individuals (10 females and 10 males) of known age, ranging from 54 to 83 years. They were exhumed at Vienna Central Cemetery during a routine transfer of remains following the removal from their graves. Their documented PMI ranged from 29 to 52 years and they were buried at a depth of 1.4 to 3 meters in a uniform loess soil. All bodies studied were clothed and buried in wooden coffins as standard. Half of them were also covered before their burial in air and water-tight plastic body bags. This kind of burial was a common way of preventing the escape of decay gases when awaiting burial between the 1950s and the 1970s in Vienna.

In most cases, clothing remains were still present at the time of exhumation. None of the exhumed bodies had been in contact with ground or backwater. In the "normal" burial group, the coffin walls were largely destroyed and the skeletal remains were completely embedded in the ground. Soft tissue and ligaments were completely degraded. In the "body bag" group, the situation was similar because decomposable back panels were used with the body bags, which meant that body bags lying in the earth over time could be penetrated. Thus the soft tissue and ligaments had completely decomposed and the corpses skeletonized. However, in almost every case, the extremities showed traces of grave wax formation. Bone samples were taken from the midshaft of the femora and from the temporal bone of the skull of every individual. Citric acid content was determined according to the procedure described by Schwarcz and his colleagues.<sup>1</sup>

A clear trend of decreasing citrate content was observed. As time-since-death elapses, the process seems to accelerate. There were only slight differences in the behavior of the citrate decomposition between the burials in the body bags and the wooden coffins. This might be explained by the decomposable back panels which allowed the buried bodies to decompose faster.

The results of this study have shed new light on the impact of burial environments on the possibility of the citrate content of human skeletal remains to decrease. Even partial contact with the surrounding soil seems to be enough to initiate the process. Nevertheless, with this new method forensic science appears to have gained access to a powerful tool for estimating the PMI of skeletonized human remains found at crime scenes.

### **Reference:**

- <sup>1</sup> Schwarcz HP, Agur K, Jantz LM. A new method for determination of postmortem interval: citrate content of bone. *J Forensic Sci* 2010;55(6):1516-22.

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### **Postmortem Interval, Skeletal Remains, Citrate Content**

## **H74 The New Kid on the Block: Trials and Tribulations of Building an Outdoor Research Facility and the Preliminary Results on the Rate and Pattern of Decomposition in Southern Illinois**

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After attending this presentation, attendees will gain knowledge of the decomposition rate and pattern sequence of buried and surface remains over a one-year period in southern Illinois. Scientists and law enforcement officials involved in human remains investigation will benefit greatly from both the data and visuals presented. Furthermore, attendees will experience the announcement of the newest open air forensic research facility, the Complex for Forensic Anthropology Research (CFAR) at Southern Illinois University-Carbondale. Information on the establishment and maintenance of an open air facility such as CFAR will be given, citing political and social issues that have arisen during formation.

This presentation will impact the forensic science community by instituting a baseline of information for the decomposition of soft tissue in the southern Illinois region. The findings of studies at CFAR will prove more applicable to forensic cases in climatological and environmentally similar regions than those from any other comparable facility.

CFAR is located within the city limits of Carbondale, in Jackson County, IL and is a unit of the Department of Anthropology at Southern Illinois University - Carbondale. This location represents a geographic area which is located farther north, has the lowest average temperature, the most acidic soil, the worst soil drainage, and is the second lowest in elevation of all forensic anthropology research facilities in the United States. CFAR is roughly 1/3 an acre of fenced lightly-wooded grounds that provide intermittent periods of areas of shade and sunlight. The open-air location is supplemented by a freestanding office.

Unlike the most recently developed research facilities, CFAR is a bottom-up endeavor, with the driving force of the facility being the founders, a graduate student, and a first year faculty member. Political and social obstructions have played a significant role in the formation of CFAR. Due to university constraints, the location of CFAR has changed twice. City ordinances of animal and human rights were investigated and law enforcement was notified of the project. University administration meetings have been undertaken up to the Chancellor level. Even through this barrage of potential stumbling blocks, CFAR has been approved to carry out animal and human experiments, the public has been notified of the facility, and the facility has hosted two law enforcement training seminars.

Currently, 10 pigs (*Sus scrofa*) are being assessed to establish baseline rates and patterns of decomposition at CFAR. Seven of these subjects were buried at varying depths (25-46cm) and five have been placed on the surface in both sunny and shaded areas. The surface subjects have been covered with 18-gauge wire fencing to prevent scavengers from removing them. Research subjects were deposited at two experimental conditions (October and December 2010). iButton Link thermochrons (DS1921G)

have been placed at CFAR to monitor temperature at the site for use of accumulated degree days as the method of quantifying decomposition rate. Observations of the decomposition stage for each subject were collected daily following the method of Megyesi et al., using the Total Body Score (TBS) for each subject. Motion-activated cameras were used to record still photographs and video of research subjects in the absence of the authors. This proved extremely useful in identifying the types and activities of avian and mammalian scavengers in the region.

The recorded data show significant differences in both the rate and pattern of decomposition when CFAR is compared to other facilities. CFAR has exhibited a range of scavengers, such as opossum and turkey vultures, which consume buried and surface interments from one day to six months after burial. Furthermore, CFAR has exhibited slower progression in time between decomposition stages, and several of the subjects mummified unexpectedly. Preliminary results suggest the postmortem interval at CFAR may be delayed by as much as three weeks when compared to other regions, although this may be the direct result of cooler temperatures in late fall. Further research is necessary and ongoing.

**Outdoor Research Facility, Taphonomy, Forensic Anthropology**

## **H75 Taphonomic Impacts of Small and Medium-Sized Scavengers in Northern New England**

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After attending this presentation, attendees will better understand the impact small and medium-sized scavengers can have on postmortem processes and on the estimation of postmortem interval (PMI).

This presentation will impact the forensic science community by emphasizing the role of regionally specific scavenger guilds in outdoor cases.

Published models of decomposition generally assume a progressive skeletonization that includes fresh, bloat, early decay, late decay and skeletal phases, a process that includes insect involvement and excludes mammalian and avian scavengers. However, when scavengers remove soft tissue, they can truncate this process, skip bloat and/or decay phases, and move directly to skeletonization.

Apart from increasing the skeletonization rate, scavengers may disarticulate, scatter and consume elements, altering PMI estimations. Furthermore, insect PMI indicators may be limited or absent in scavenged cases. First, when scavengers remove flesh, although insects may be attracted to the odor, there is little left for larval stages to feed on. Second, in colder climates, much of the year is too cold for insect activity, whereas carnivorous scavengers are active year-round.

Three pig cadavers were placed in similar forested, highland environments on October 20, 2010, in northern New England, about one mile apart. Replicating modal forensic cases, pigs were placed 15-30 meters off unpaved access roads. Cadavers were clothed to provide human scent. Each site was equipped with two trail cameras for 24-hour surveillance. Temperature and humidity data loggers at each site were set for hourly monitoring. Sites were visited approximately weekly and at that time, close-up photographs were taken of the cadavers.

Between October and early April temperatures at all three sites (Sites M, N, and O) remained below 5°C. From late December to early April the sites were snow-covered. All three cadavers were scavenged, but scavenger patterns differed. The Site M cadaver was unscavenged until late April, when turkey vultures consumed and scattered the remains. Insect infestation was concurrent. Sites N and O had no insect infestation.

At both Sites N and O, when snow was approximately 2.5 feet deep, raccoons built snow tunnels to access cadavers. The Site N cadaver,

unscavenged until February 17, was then defleshed in 60 days, while snow-covered. Scavenging at Site O began in early December, prior to snowfall, and continued throughout winter, defleshing the remains by April 15. Ravens, turkey vultures, and a bobcat also utilized the snow tunnels.

The scavenger guild included pine marten, raccoon, bobcat, porcupine, skunk, ermine, turkey vultures, crows, ravens, blue jays, and chickadees. Coyotes, bears, and eagles visited the sites, but did not feed. Insect infestation and bloating did not begin until six months postmortem. Two of three sites had no insect involvement, but were entirely defleshed during winter, mostly while under snow cover.

By June, scatter extended approximately 15 meters at each site. Most bones were still present and there was very little bone chewing. The original body location at Sites N and O were identifiable due to the presence of clothing. At Site M clothing is associated with a decomposition island. Sites N and O have characteristics that may have influenced scatter. The N Site was located on a gentle slope, and scattering of this cadaver was dispersed down-slope from the original deposition. The O site, located on flat terrain, had a game trail; scatter extended primarily along this trail.

These sites exhibit modification and scatter from small and medium-size scavengers in a wooded environment from late fall through spring. More research is needed to explore whether these patterns are typical of these taxa in this environment.

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**Taphonomy, Scavenging, Decomposition**

## **H76 High Soil Acidity Associated With Near Complete Mineral Dissolution of Recently Buried Human Remains**

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After attending this presentation, attendees will better understand how highly acidic soil can almost completely dissolve human bones within a short postmortem interval (8-10 years).

This presentation will impact the forensic science community by providing a well-documented postmortem interval for extreme microbiotic and chemical environments where the acidity is high. In addition, this research may help preliminary review and search teams in Colombia narrow down the list of graves to be excavated when similar conditions are present given the possibility of complete bone dissolution.

This research explores the potential causes of complete diagenetic bone mineral dissolution in graves of unidentified individuals buried between 2000 and 2002 in Colombia. The bodies of unidentified persons were documented and buried by an undertaker in a cemetery at the request and authorization from the local government agency of the municipality of La Macarena in the department of Meta, Colombia. The bodies were reportedly buried in coffins and wrapped in cloth or covered with plastic. A team of forensic scientists was sent by the government in February of 2010 to the cemetery in order to investigate reports of a possible mass grave in the area. However, they were able to determine that instead of a mass grave, there were approximately 450 single interments of unidentified individuals, each with its own grave marker. The investigation was initiated by complaints from community members looking for their loved ones.

The investigators were surprised to find that most of the skeletal material found was nearly completely eroded and destroyed by the action of unknown taphonomic agents. The location is described as an artificial terracing on a plain, surrounded by trees of medium height, grasses, and shrubs. The weather is mostly hot and humid year round in this location.

During the excavation, the anthropologists excavated an archaeological test trench in order to detect changes in the soil and determine the exact location of individual graves. The trench revealed partial grave outlines of four graves. Many of the grave outlines could only be distinguished by discoloration of the soil and coffin nails outlining the graves. Of the graves that the trench revealed, it was observed that clothing remained in some of the graves, but the bones were almost completely absent. Only one grave was completely excavated in order to detect the alteration suffered by the bones.

At the time of excavation, soil samples from different levels within the cemetery were collected and stored in plastic containers. The chain of custody was maintained as the samples were sent for testing at the Institute of Geography Agustín Codazzi, Department of Agrology - National Soil Laboratory in Bogotá, Colombia. The soil samples were tested for fungus, bacteria and chemical solvents. The samples do not exhibit a high level of flora that would indicate that the samples had dissolved as a result of the organic processes, especially phosphates that are normally active in this type of sample. The analysis did yield several genera of fungus: *Penicillium*, *Fusarium*, *Aspergillus*, and *Sclerotinia*. The most unique signature of this soil, however, was the extreme acidity. The abnormally low pH levels in the samples ranged from 4.2 to 4.5. According to the soil analysis from the laboratory, “highly acidic” soil ranges from 5.1 to 5.5. This acidity range is much lower than the “extremely acidic” range. As the pH scale ranges from 0 – acidic to 14 – alkaline, the samples here are even more acidic than what the laboratory reports as “highly acidic.” Such a low pH can facilitate the degeneration of the bone mineral (hydroxyapatite) and facilitate the structural dissolution by opening the microscopic canals in bone and increasing the available surface area so it is subjected to greater taphonomic activity.

This research provides evidence of a taphonomic process of the near complete dissolution of human osseous material within a very short and well documented postmortem interval (8-10 years – burial to excavation).

This presentation will impact the forensic community by providing a well-documented postmortem interval for extreme microbiotic and chemical environments where the acidity is high. In addition, this research may help preliminary review and search teams in Colombia narrow down the list of graves to be excavated when similar conditions are present; given the possibility of complete bone dissolution.

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#### **Taphonomy, Soil Acidity, Human Skeletal Remains**

## **H77 Where Are the Missing Bones? Structural and Material Properties of Bone and Differential Survivability**

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After attending this presentation, attendees will be able to understand how various bone properties can affect bone preservation and skeletal element representation, which is essential in order to make a more robust and thorough assessment of commingled assemblages.

This presentation will impact the forensic science community by providing baseline data regarding human bone structural density and survivability in forensic contexts. Of the most useful contexts are those that are common in forensic settings – that of commingled remains.

While many intrinsic properties of bone affect its potential for preservation, the property that has received the greatest amount of attention in taphonomic studies has been bone density. The definition of “bone density” has been the source of significant confusion among anthropologists and archaeologists. This confusion is warranted, given that

this term has been used to refer to different properties or other similar terms (e.g., “bone mineral content,” “bone mineral density,” “structural density,” “bulk density,” “true density,” “apparent density”) have been used in the same context, and that “bone density” has been measured using several different methods that vary in accuracy. What one may typically think of as “bone density,” the (dry) mass of a bone divided by its volume, is also known as its apparent density (Martin et al.) or structural density (Lyman).<sup>1,2</sup>

Bone density data have been collected for a number of animal species and for humans, and density has become, by far, the most commonly used proxy value for a bone’s potential to survive destructive processes. Bone density studies with a zooarchaeological focus have far outnumbered those conducted with a forensic objective on human material. This reflects the comparatively high frequency in which zooarchaeologists encounter large bone assemblages that include commingled remains of multiple individuals often representing multiple species. However, bone density data for human are of particular relevance to forensic studies by providing guidance as to how to most accurately determine the number of skeletal elements (and individuals) present in the assemblage. Although previous studies have documented bone mineral density in humans (Galloway et al., Willey et al.), these data have not been widely used to explain the actual skeletal element representation. This study offers a larger sample size (MNI=432) discovered from various macro-environments, such as South East Asia, Korea, the Pacific Islands, and Northern Europe.<sup>3,4</sup>

In forensic and archaeological cases, in which the remains of large numbers of individuals are commingled, the determination of the number of individuals is a common research objective. Archaeologists seek to determine the minimum number of individuals (MNI), with the tacit assumption that an accurate determination of MNI provides a plausible estimate for the actual number of individuals represented. Ubelaker noted the relevance of zooarchaeological methodology to the study of commingled human remains.<sup>5</sup> In particular, he cited the observation that, when long bones are consumed by carnivores, the shaft fragments are more likely to survive than the epiphyses and would therefore provide the highest counts for long bone elements. In fact, reconstruction of shaft fragments has allowed a more accurate understanding of prehistoric diet and procurement activities (Bunn; Bunn and Kroll).<sup>6,7</sup> In forensic cases, the use of DNA analysis can be an important tool in determining the number of individuals represented in a commingled assemblage. Where DNA analysis is constrained by financial limitations or poor preservation, the determination of MNI rests largely on the morphological assessment. Under these circumstances, an understanding of the patterns of bone density and their effect on bone preservation is essential.

Skeletal remains recovered by the Joint POW/MIA Accounting Command (JPAC) provide multiple assemblages to test the relationship between bone structural density and survivability in human bones; specifically, this study tests the hypothesis that more dense bone will display increased survivability regardless of contextual background. In most cases, bone structural density and survivability show statistically significant correlation, which indicate the presence/absence of certain skeletal elements can probably be best explained by the natural process of bone degradation. Particular case studies originate from the Democratic People’s Republic of Korea (DPRK; aka “North Korea”), related to human remains originating from the Korean War, between 1950 and 1953. One specific commingled case originates from a secondary, fluvial deposit from a POW holding area. The structural density and bone survivability showed significant correlation, and the MNI count of nine was based on the femoral shaft. The DNA data then confirmed that there were nine individuals. This example shows the importance of correctly identifying the most dense long bone shafts to calculate the most accurate MNI. Other examples come from a large commingled assemblage of remains that were turned over by the DPRK to the United States government, collectively referred to as the K208.

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#### **Taphonomy, Bone Density, Commingling**

### **H78 Taphonomic Signatures of Animal Scavenging: The Benefits of Using Remote Recording Equipment to Monitor Scavenging Activity**

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The goals of this presentation are: (1) to provide an assessment of regional variation in relation to animal scavenging behavior; (2) to provide an evaluation of the benefits of using remote recording equipment; and (3) to encourage a discussion using actual experimental data including surface deposit and burial scenarios.

This presentation will impact the forensic science community by providing a critical evaluation of taphonomic research and present the benefits of using remote recording equipment to enhance taphonomic analysis.

Historically anthropological research was based on direct observation of the research subjects. The practice of direct observation causes minute changes to the test sites and test subjects. Further, the continued presence of human activity at a site can discourage the natural scavenging and decomposition progression. Daily observation introduces human scent, and human presence affects an accurate picture of activity at the site. This tradition can be modified using remote recording equipment to monitor scavenger behavior. The taped video recordings and motion activated still images provide actual observations of scavenger behavior and feeding as it occurs. This research was conducted in ecological reserve in rural northern California and in a wooded area of suburban northern Virginia.

The California portion of the study was conducted in October 2009 and November through December of 2011. Common northern California scavengers include the black bear, western spotted skunk, gray fox, coyote, raccoon, as well as the domestic dog and cat. Digital game cameras were positioned at six sites within the Big Chico Creek Ecological Reserve (BCCER) to monitor scavenger activity on a single adult mule deer, and five 100 pound pig carcasses. Two pictures were taken each time the motion sensitive laser was triggered, with a delay of one minute between pictures. Sites were monitored daily and the camera was repositioned if the carcass had been moved out of the cameras' field of view. The carcasses were placed on the surface and to prevent immediate removal from the site location, each carcass was tied down to rebar stakes with lengths of wire wrapped around the forelimbs and hindlimbs. Documented scavengers include: black bear, gray fox, turkey vulture, red-tailed hawk, golden eagle, and common raven.

The Virginia portion of the study occurred in three phases, from May 1999 through July 1999, May 2000, and from November through December 2000. Common scavengers in this area of northern Virginia include coyotes, domestic dogs, turkey vultures, red foxes, opossum, and the common crow. Site scenarios included surface deposit and shallow burial of less than one foot involving child-sized pigs (approximately thirty pounds). The 1999 study used four high-resolution video cameras with an infrared light source. Cameras used on site focused on a site overview and/or on a close up of the remains. The cameras were set to record for various lengths of time. The researcher also monitored the sites physically. Documented scavengers include: turkey vultures, crows, red foxes, raccoons, striped skunks and opossums.

The use of remote recording equipment allowed researchers to document a clear division of diurnal or nocturnal predilection amongst the scavengers, as well as a hierarchy within the specific scavenger niches. This study also highlights the differences in large-body scavengers (black bear) versus small-bodied scavengers (raccoon and opossum). Although there are now several studies regarding the effect of scavengers on human remains, direct evidence of scavenging is still scant. Traditional anthropological techniques can be updated using modern technology, and regional studies should be conducted to create contextual information regarding scavenger behavior across the country.

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#### **Taphonomy, Scavenging, Remote Recording**

### **H79 Hanging in an Outdoor Context: An Actual Perspective Using Human Cadavers**

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After attending this presentation, attendees will appreciate how hanging changes the decomposition process to include the colonization of particular arthropods, the amount of biomass reduced, and the subsequent effects these factors have on estimating the postmortem interval.

This presentation will impact the forensic science community by describing and defining the decomposition process of hanging cadavers in the outdoors, the entomological activity associated with the hanging of human cadavers, the resulting skeletal deposition, and how these relate to time-since-death estimations of outdoor hangings.

Most suicidal hangings occur indoors, but outdoor hangings often create more questions than answers for investigators (Komar et al., Spitz and Fisher).<sup>1,2</sup> Shalaby et al. are one of the first studies to evaluate how hanging can affect decomposition using a carrion model.<sup>3</sup> This research highlighted how the pattern of decomposition in a hanging versus surface deposition followed similar patterns; however, they did not explain the long-term preservation of hanging remains or the lack of biomass reduction. Komar et al's. retrospective study, on Edmonton medical examiner cases, indicated a high rate of mummification and a large quantity of desiccated tissue being recovered in hanging contexts.<sup>1</sup> Despite these studies, few researchers have systemically evaluated how the stages of decomposition are affected by hanging a human cadaver.

The goals of this project were three-fold: to document the basic stages and differences in decomposition of cadavers that are hanging compared to those on the surface of the ground; to document the entomological activity associated with a hanging scene (including defining the "drip zone"); and to determine the final skeletal deposition related to the hanging environment. A 10-foot high wooden device was built with a pulley/crank system to ease the force required to lift individuals into the hanging position approximately one to two feet above the ground surface. The number of variables, such as clothing or knot type, was limited so that seasonal differences could be appreciated. Eight cadavers from the University of Tennessee's Forensic Anthropology Center's body donation program were used in this experiment (8 males ages 50-60, weighing less than 200 lbs).

Four cadavers were hanged and four were placed on the surface about 10 feet from the hanging contraptions at the Anthropological Research Facility, the University of Tennessee, Knoxville, in the summer and fall 2009.

Similar series of decomposition events including oviposition patterns were observed for the control and hanging cadavers; however, significant differences, including the rate of decomposition, and biomass reduction were observed. These differences can primarily be explained by the fact that hanging cadavers maintained ambient temperatures while surface cadavers were able to maintain more constant and significantly higher temperatures even in the winter months. Coupled with limited continual larval activity, the hanging individuals exhibited putrefaction and advanced decomposition much later than the control/surface individuals. This corresponds with previous surveys indicating that decomposition rates are significantly slower in hangings at higher heights as opposed to individuals partially on the ground (Komar et al.).<sup>1</sup>

The most significant difference in the decomposition process occurred between the individuals hanged in the summer as opposed to the late fall months. The summer trials experienced rapid decomposition and subsequent purging of the internal cavities through the loss of the perineum, while the late fall trials had a settling of the internal materials, but no loss of the perineum. Instead of being hollow, these bodies maintained biomass for a longer period of time, falling from the noose prior to those in the summer trials. In fact, the summer trials have been hanging for over two years.

Expanding our understanding of the decomposition process and subsequent skeletal deposition in situations other than an individual lying on the ground is necessary for the continued development of our field. The limited nature of published research evaluating hanging scenarios, both indoor and outdoor, necessitates the continuation of this project over the long term, as there is potential to determine characteristic markers to differentiate between skeletal remains that decomposed on the surface as opposed to those from a hanging situation.

This research was supported by the William M. Bass Endowment.

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#### Human Decomposition, Hanging, Forensic Anthropology

## H80 Multi-Factorial Estimation of Skeletal Age-at-Death Using the Sugeno Fuzzy Integral

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After attending this presentation, attendees will understand the basic principles of how to use the fuzzy integral to obtain a multifactorial age-at-death estimation for a single skeleton.

This presentation will impact the forensic science community by providing a new standardized method for combining multiple indicators of age along with their respective accuracies, into a single, accurate, age-at-

death estimation. It will also cover a feature-based method to determine the degree to which age-at-death fuzzy set results belong to different anthropological classes. The final topic is an OWA-based contrast approach to measure the amount of specificity in an age-at-death fuzzy set.

Accurate and precise estimations of chronological age-at-death based on skeletal remains are vital in forensic anthropological analyzes to help narrow the search for potential missing persons and to aid in the identification of the skeleton. Combining multiple indicators of biological age (multifactorial method) from different regions of the skeleton provide a more accurate estimation of chronological age than using any single indicator. However, most currently published multi-factorial methods are not appropriate for forensic anthropology because they cannot be applied to a single skeleton, do not provide a confidence in the point estimate or prediction interval, or are restricted to a certain types of age indicators. Currently there are no "best practice" guidelines in forensic anthropology for combining multiple indicators of age. As a result, forensic anthropologists frequently develop their own guidelines for combining multiple indicators, often based on their past experience and the skeletal remains present for a specific case. A standardized method for combining multiple indicators of age from a skeleton into a single, accurate, and repeatable age-at-death estimation is needed in forensic anthropology.

A novel multifactorial approach is presented that uses the Sugeno fuzzy integral to analyze skeletal age and takes into account as much information as possible, including the accuracy of the method and the quality of the bone, to reach a decision about a hypothesis. Fuzzy integral acquired fuzzy sets are then used to provide results about the age-at-death estimation that are reproducible and can be understood by different scientists. Using this approach, forensic anthropologists obtain an age-at-death estimation, a measure of the confidence in the estimation, and additional results (numeric, graphical, and linguistic) regarding the type of graph and degree of specificity of the age-at-death estimation. This method has multiple advantages over other multifactorial methods. The procedure allows investigators to use nearly any well established and tested age-at-death indicator methods and fuse the information about the accuracy of the methods with other types of quantifiable information that cause uncertainty in the age-at-death estimation. No other method allows for the fusion of additional information such as the quality of the bone, the appropriateness of the method for the target age group, or inter-observer error in the methods used. Other advantages of the fuzzy integral method are that it does not require the use of a population so it can be easily used for a single skeleton, it can be used for both adult and immature skeletons, it can be customized to meet the investigator's needs on specific cases, and it provides informative graphs and a standardized reproducible way to generate linguistic descriptions of age-at-death estimations.

To demonstrate the use of the fuzzy integral method, we apply it to three aging methods commonly used by forensic anthropologists (pubic symphysis, auricular surface, and cranial suture closure) on a known-age skeletal sample from the Terry Anatomical Collection. The research shows that the fuzzy integral method produces results that are more accurate with smaller intervals than single indicator methods. Unlike other multi-factorial methods, the fuzzy integral approach allows investigators to estimate age-at-death for a single skeleton by applying the well-established age methods they are comfortable using and that are available to them based on the bones present, the condition of the bones, and the equipment they have accessible. Furthermore, unlike other methods, the fuzzy integral method allows the investigator to incorporate additional information about the quality of the bone or any other quantifiable variable about the uncertainty of the method.

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#### Forensic Anthropology, Age-at-Death, Sugeno Fuzzy Integral

## H81 A Comparison of Site-Specific Versus National Weather Service Temperature Data and its Applicability to Estimation of Postmortem Interval Using Accumulated Degree Days

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After attending this presentation, attendees will gain knowledge on how retroactively collected National Weather Service (NWS) temperature data from the closest NWS weather station to a recovery site are not necessarily directly applicable to the temperatures occurring at a recovery site, regardless of the proximity of the two.

This presentation will impact the forensic science community by correcting the previous assumption that the closest NWS station to a recovery site provides acceptably accurate temperature data for estimation of the postmortem interval using accumulated degree days. It should serve as an impetus for future research into the correlation of the rate and pattern of decomposition with site-specific Accumulated Degree Day (ADD) data, improving our ability to estimate the postmortem interval accurately.

Following the "best practices" guidelines of the Scientific Working Group for Forensic Anthropology, an increasing number of anthropological studies of human decomposition are utilizing accumulated degree days (ADD) to quantify the postmortem interval (PMI) at given decomposition stages and to estimate the number of ADD required for certain events, such as tooth exfoliation, to occur. This study addresses the utility of applying retroactively collected temperature data from the National Weather Service (NWS) station closest to the recovery site to these calculations, as prescribed in the past. Using iButton Link thermochrons (model DS1921G), hourly temperature readings were collected at 15 sites throughout the eastern half of the United States (Arkansas-one site; Connecticut-one site; Illinois-six sites; Kansas-three sites; Louisiana-one site; New York-one site; Mississippi-one site; Vermont-one site). With the exception of one, all research sites represent private property owned by an individual who volunteered to participate in this study upon the request of the author. There is no intentional bias in the selection of sites, the patterning merely reflecting the extent of the author's social network. The non-private research site is located at the Complex for Forensic Anthropology Research (CFAR) at Southern Illinois University. Each thermochron was placed 25 cm above the ground surface on the north side of a support made of natural fiber, most being attached to trees or wooden fence posts. The thermochron was placed at least 3m from any permanent structure (house, shed, driveway, etc.). This placement protocol was designed to ameliorate the possibility that reflected and radiant heat from synthetic materials may increase the recorded temperatures at research sites. The preliminary analysis reported here is based on data collection periods ranging from 135 to 169 days (mid-December 2010 through May 2011). Data collection is ongoing and the total data set will increase to at least 365 days per site by February 2012. The average daily temperature was calculated by averaging the daily maximum and minimum hourly temperature readings for each site, as prescribed by the NWS. In order to maintain consistency with the average daily temperature as reported by the National Weather Service a day was considered to be the 24-hour period from 0000-2400 XST or 0100-0059 XDT. In 2011, Daylight Saving Time began on March 9<sup>th</sup>. National Weather Service temperature data (F6 data) for the weather station closest to each of the fifteen research sites were retrieved from an official reporting website for the NWS ([www.wunderground.com](http://www.wunderground.com)). The direct linear distance between each research site and the closest NWS weather station averaged 7.0km. The NWS stations ranged between 0.5km and 15.3km from the individual research sites. The site-specific and NWS data were matched by date, and a paired-samples t-test (SPSS 17.0) was used to identify statistically significant differences between the temperature data recorded at the

research sites and those recorded by the National Weather Service. The difference in average daily temperature between NWS data and site-specific data ranged between 0.05°C and 3.03°C. All 15 sites presented high correlations between NWS and site-specific data ( $0.809 \leq r \leq 0.989$ ). The observed differences were statistically significant for 11 of the 15 research sites (73.3%) ( $p\text{-value} \leq 0.021$ ). Four sites exhibited no statistically significant differences between the temperature data recorded by the NWS and the site-specific data ( $p\text{-value} \geq 0.094$ ). These four sites are suburban and rural, far from the NWS station and close, both west and east of the Mississippi River, coastal and mountainous—in short, there is no clear, observable difference in environmental conditions that may cause these sites to be more consistent with the NWS data than other sites. The results of this study demonstrate that utilizing retroactively collected temperature data from the nearest NWS station to a site without investigation into the correlation between the microclimates of the site and the NWS facility is unwise at best and unacceptable at worst, particularly if the intended purpose is the development of methods of estimating PMI.

This project is partially funded by a Lucas Grant from the Forensic Sciences Foundation.

**Forensic Anthropology, Postmortem Interval, Accumulated Degree-Day**

## H82 A Forensic Pathology Tool to Predict Pediatric Skull Fracture Patterns - Part 3: Entrapped Porcine Head Impacts vs. Controlled Head Drops

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The goal of this presentation is to inform attendees about research on fracture initiation and propagation caused by controlled head drop experiments onto the parietal bone in a developing porcine (*Sus scrofa*) model.

This presentation will impact the forensic science community by describing comparisons between controlled head drops and the previous data presented using an entrapped head impacted by a gravity-dropped mass with compliant and rigid interfaces.

Pediatric deaths involving head injury with associated cranial fractures represent one of the greatest challenges to forensic professionals. The ability of the forensic investigator to establish the circumstances of death in these cases is severely hampered by the lack of skull fracture standards for infants and young children. This research aims to understand the basic principles behind infant cranial fractures in the porcine model which may then be used to help guide future human research.

Previously, findings were reported from a porcine head model entrapped in a bed of epoxy that was used for constraint during impact onto the parietal bone using a dropped mass impact interface. Fenton et al. showed multiple fracture initiation sites on the porcine cranium away from the impact site, and more recently Passalacqua et al showed that when the impact energy was doubled, there was extensive fracture propagation in both the parietal bone and into adjacent frontal and occipital bones.<sup>1,2</sup> The phenomenon of remote fracture initiation in this infant porcine model has also been documented using high-speed video (Passalacqua).<sup>2</sup>

Because forensic cases often involve situations in which free fall head drops are suspected, this next phase of research dealt with fracture initiation and propagation in controlled head drop experiments onto a rigid interface at energy levels comparable to the previous study of Powell et al. using the entrapped head model.<sup>3</sup>

In order to compare head drops versus the entrapped impacts, 31 porcine specimens aged 2-17 days were used. To produce the necessary impact energy, the head was attached to a drop tower trolley, which was raised to the necessary drop height. In the experiments a solenoid disengaged, allowing the trolley to fall freely. Upon impact, the head was disengaged from the trolley allowing it to impact a rigid aluminum interface once, by using an electromagnetic solenoid to catch the head after impact. The impact energy levels for various aged specimens in the current study were matched to the energy levels documented in Powell et al. for the entrapped heads. The fracture patterns were compared between experiments using a GIS image-analysis approach, as previously described by Marean *et al.*<sup>3,4</sup>

Results from these controlled head drop experiments demonstrated that the impact duration was significantly shorter than for the entrapped head experiments of Powell et al. ( $p$ -value < 0.001), however the peak impact force data was not different at each specimen.<sup>3</sup> There was significantly less skull fracture at each age for the free fall experiments than for the entrapped heads ( $p$ -value < 0.001). GIS fracture pattern results demonstrated that fracture initiation was located primarily along the anterior parietal bone in all free fall specimens. A simplified, theoretical model analysis of each experiment, using the finite element approach, showed that large tensile stresses develop around the periphery of the entrapped head, near the epoxy constraint, that likely produced extensive fractures remote to the site of impact. The tensile stresses in the head model were lower and located more near the impact site in free fall experiments.

While further research is necessary in order to define any potential relationships between the infant porcine model and the infant human, these results showed that head entrapment provides a significant stress riser that enhances the potential for cranial bone fracture compared to an equal amount of energy to a freely falling head impacting a rigid surface.

This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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#### Child Abuse, Fracture Patterns, Bone Biomechanics

## H83 Geometric Morphometric Analyses From Dental Orthopantomogram Images: A Regional Anatomical Analysis of Sexual Dimorphism in the Adult Mandible

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After attending this presentation, attendees will have a better understanding of a new method of sex assessment through the analysis of clinical panoramic X-ray images.

This presentation will impact the forensic science community by introducing a novel method of sex determination following a skull assessment.

The human mandible has routinely been utilized in forensic assessment of age at death, sex determination and biological affinity. However, such studies have generally utilized conventional assessments of size and shape variables, and as such fail to record the true nature of shape differences due to dimorphism in this functional skeletal element. The research here presented utilizes geometric morphometric techniques to investigate and quantify shape and size variation in the morphology of the mandibular corpus and ascending ramus, and consequently the potential for forensic human identification. The results of a novel morphometric study using clinical panoramic scanning x-radiography to study the extent of morphological variation within a modern Italian sample population are presented.

Clinical digital orthopantomogram images (OPG) were acquired of the upper and lower jaws of 50 male and 50 female participants. Ten type I and type II 2D landmarks were applied to the symphysis, and condylar and coronoid processes. One hundred equidistant semi-landmarks were established along the inferior border of the corpus, and the posterior border of the ascending ramus. The resulting landmark configurations ( $n$  100) were subjected to Generalized Procrustes Analysis (GPA) with Full Tangent Space Projection. Principal Components Analysis (PCA) was applied in order to assess population variation. Factor loadings were subject to Canonical Variates Analysis with stepwise and leave-one-out classification in order to assess the effects of sexual dimorphism on mandibular shape. The results showed individuals to be correctly classified for sex in 89.6% of cases, (males were correctly classified in 90.1% of cases, and females in 85.6%).

Analyses of the mandible were subsequently broken down into anatomical regions based on the mandibular body, the bony processes and the ascending ramus in order to investigate regional functional differences in the expression of dimorphism in mandible. A partial least squares (2-block PLS) method was further applied, in order to examine patterns of covariation between shape variables and the exploration of patterns of functional modularity. Most interestingly the results indicate the greatest level of individual and sex-specific variation is found in the shape-curve and pattern of the inferior corpus, in contrast to that of ramal flexure. Stepwise permutation tests and analyses of regional covariation indicate functional coupling, with a moderate degree of modular integration between the corporal and ramal regions suggesting that functional ties between the units are correlated in influencing sex-based morphological trait expression between anatomical regions, indicating that the geometric relationship between the mandibular corpus and the ascending ramus offers significant power for forensic identification purposes. Consequently such units may be studied together or in isolation, and this may allow for the development of identification criteria based on modular unit shape variables which may be applicable for both whole specimens and fragmented remains depending on the forensic situation. Overall the results are strongly significant and suggest that both dependently and independently that the

shape relationship between the mandibular corpus and the ascending ramus offers significant power for forensic identification purposes. Of particular interest is that inferior corpus border shape offers significant discriminating potential in the assessment of sex, with the effects of allometry being strongly implicated. These and other implications of the shape analysis will be discussed.

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#### Sex Assessment, Geometric Morphometrics, Forensic Anthropology

### H84 A Comparison of Age-Related Macroscopic Traits of the Ilium and Sacrum

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After attending the presentation, attendees will have a better understanding of age-related traits of the auricular surfaces of the ilium and sacrum in terms of age-at-death indicators

This presentation will impact the forensic science community by demonstrating potential differences in the development of three macroscopic traits of the auricular surfaces of the sacrum and ilium as well as the potential significance for estimating age-at-death from these structures.

Accurate age-at-death estimation from human skeletal remains is critical for establishing a comprehensive and forensically significant biological profile of an unknown individual, which in turn facilitates the victim identification process. While many regions of the human skeleton have methods to facilitate adult age estimation, the auricular joint of the pelvis presents a unique structure to compare similar traits across the sacrum and ilium. Interestingly, while both the sacrum (Passalacqua) and the ilium (Lovejoy et al., Buckberry and Chamberlain, Osborne, among others) have age-at-death estimation methods, there has been little work relating the adult degenerative changes which occur on the paired auricular surfaces.<sup>1-4</sup> In fact, Lovejoy et al. state: "The sacral [auricular surface]...does not reflect the age changes described below [in regard to their method of scoring the ilium] and cannot be used to determine age."<sup>2</sup> This presentation tests that assertion.

The primary aim of this study was to assess three degenerative traits (apical changes, microporosity and macroporosity) found on both the sacrum and ilium auricular surfaces and compare the timing of appearance for each trait across the auricular joint. Paired ilia and sacra from the Hamann-Todd (n=380) and Bass (n=234) collections comprise the samples used in this study. Individuals from the Hamann-Todd collection consist of males and females, mainly of African and European ancestry, ranging in age-at-death from 10 to 96 years and individuals from the Bass collection consist of males and females of European ancestry, ranging in age-at-death from 16 to 97 years. Previous research (Lovejoy et al.; Passalacqua) found no significant sex or ancestry differences in regard to age-related changes of the ilium or sacrum.<sup>2,1</sup>

These traits develop over a continuum and each morphological character was scored according to multiple trait variants as described in Passalacqua and Buckberry and Chamberlain.<sup>1,3</sup> However, in order to limit observer error and deal with discrepancies in method scoring procedures, these traits were re-scored on a presence or absence scale (1 and 0, respectively). A Kruskal-Wallis test was then used to assess differences in presence and absence of these traits between the ilium and sacrum. Results suggest that macroporosity appears earlier and more often in the ilium (mean age = 50.65) than the sacrum (mean age = 53.8 years,  $\chi^2 = 82.926$ ,  $df = 1$ ,  $p$ -value = 0.000). However, apical change ( $\chi^2 = 2.145$ ,  $df = 1$ ,  $p$ -value = 0.143) and microporosity ( $\chi^2 = .464$ ,  $df = 1$ ,  $p$ -value = 0.496) show no significant difference in presence or absence frequency.

While Lovejoy and colleagues may have failed to recognize corresponding traits appearing on the sacral auricular surface, our results suggest similar ages of onset for microporosity and apical changes. However, much of the auricular changes of the ilium are more subtly

expressed on the sacrum, likely as a function of thicker cartilage covering the sacral auricular surface (Schunke).<sup>5</sup> While the etiology of macroporosity on the auricular surfaces is unclear, overall it was found in much greater frequency on the auricular surface of the ilium. This could be a function of the degeneration of the ilium's cartilage due to the thinner cartilage covering that portion of the auricular joint; however, further research is required to investigate the cause.

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#### Forensic Anthropology, Age-at-Death Estimation, Auricular Surface

### H85 Metric and Non-Metric Assessments of Sex: Accuracy, Correlation, and Corroboration

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After attending this presentation, attendees will understand the reliability of non-metric methods, as well as their correlation with metric methods, which are commonly employed in biological profile estimation for unknown individuals.

This study will impact the forensic science and anthropology community by demonstrating the relationship between both types of methods, as well as their relative accuracies, in sex estimation from a forensic context.

Sex estimation is a vital component of the biological profile estimation in forensic identification of skeletonized or badly decomposed unknown individuals. While the forensic anthropological community is moving toward the increased use of metric methods, non-metric methods continue to be routinely employed because of their relative ease of use, their perceived reliability, and because they are frequently "passed-down-knowledge." Because of these factors, non-metric methods are often still utilized for biological profile estimation, in conjunction with metric assessments, particularly with the human skull and pelvis. The skull has historically been the most studied portion of the skeleton for both ancestral and sex related differences, while the pelvis, specifically the innominates, is widely regarded as the greatest indicator of sex due to the dimorphism related to childbirth in females.

Non-metric and metric data were collected from all forensic cases conducted from 2009 to present at the Department of Applied Forensic Sciences at Mercyhurst College in Erie, PA. The non-metric methods utilized for sex estimation include: (1) the Walker method for sex estimation of the crania using the expressions of the supra-orbital ridge, the mastoid processes, the mental eminence, the nuchal crest, and the supra-orbital margin of the orbits; and, (2) the Klaes *et al.* (in press) method for estimating sex using ordinal scoring and expressions of the subpubic concavity, the medial aspect of the ischio-pubic ramus, and the ventral arc in the pubic bone as modified from the Phenice method.<sup>1-3</sup> In addition to

non-metric techniques, standard osteometric measurements of the skull and pelvis were also collected for each for individual or case based on the parameters outlined in Buikstra and Ubelaker and were analyzed using FORDISC 3.1 (Jantz and Ousley).<sup>4,5</sup> Graduate students with extensive training in each of the methods collected all measures.

Cases were separated into two groups, those that were positively identified and those that were unidentified at the time of this research. Classification accuracies were calculated for each of the non-metric methods and also for the osteometric measures. This was undertaken in order to evaluate how well the metric assessment corroborates with the results obtained using non-metric methods and vice versa. Additionally, with the positively identified cases, each non-metric method and the osteometrics were examined for accuracy in sex estimation. Lastly, correlation matrices were then used to examine the relationship between standard osteometric measures and non-metric trait expressions.

Preliminary results using the positively identified individuals indicate that metric methods had slightly higher classification accuracies of sex (100% using FORDISC 3.1) than the non-metric methods employed (92.9% combined accuracy for the Walker and Kiales *et al.* methods). However, the non-metric methods and metric methods for sex estimation were found to be highly correlated using the entire sample. Finally, metric methods showed high correlation with the Walker non-metric traits: nuchal crest with nasion-occipital length (NOL), and the expression of the mastoids with mastoid height (MDH).<sup>2</sup> As expected, metric methods failed to show a high correlation with the Kiales *et al.* (in press) and Phenice traits.<sup>2,3</sup> This suggests that metric measures of the pelvis fail to capture these visual sex differences in the pubic bone which may best explain why these non-metric methods are still frequently employed for sex estimation.

Evaluating the correlation between metric and non-metric methods will aid in the understanding of non-metric traits and will also increase the confidence in their use and reliability for sex estimation in forensic cases. Furthermore, this understanding will improve the practitioner's ability to assuredly utilize both types of methods by revealing the factors contributing to each of the non-metric traits and thus result in more accurate estimates.

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#### Sex Estimation, Osteometrics, Non-Metric Methods

## H86 Evaluation of Vertebral Lipping in Age Estimation in a Modern Skeletal Sample of Colombian Individuals

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After attending this presentation, attendees will understand that while one of the signs of osteoarthritis (lipping) in a modern skeletal collection of Colombians is associated with advanced age, it is a weak association. However, it can be useful when assigning fragmented remains to an age cohort.

This presentation will impact the forensic science community by encouraging gross observation of degenerative spinal diseases as an age indicator that may contribute to an overall age-at-death estimate. In addition, it contributes to the literature suggesting that vertebral osteoarthritis is not the most reliable sign of age.

In addition to the typical four aspects of the biological profile, it is essential to record and analyze other individualizing features such as antemortem injuries, diseases, or physiological alterations as these may lead to a presumptive or positive identification. Of these other features that should be taken into account, the most frequently found in the analyzes performed by the Forensic Anthropology Laboratory of the National Institute of Legal Medicine is the presence of a suite of traits compatible with degenerative spinal disease. For Colombia, its frequent presence in forensic cases is anecdotal and has not yet been scientifically studied. Therefore, the purpose of this presentation is to fully document its scope, beginning with the feature of lipping, typically associated with osteoarthritis.

For this research, 127 individuals from the Colombian modern skeletal collection were analyzed. This included 81 males and 46 females, with an age range of 18 to 93 years old; a mean age 47 with a standard deviation of 23 years. Each bone of the vertebral column was analyzed in addition to the first sacral segment. For this part of the project, the investigators focused on the superior and inferior portions of each vertebral body and recorded the presence of lipping on a 0, 1, 2, or 3 scale of severity after Rojas-Sepúlveda *et al.*<sup>1</sup> While data were also collected on osteophytic growths, porosity, and eburnation, this phase of the project focused on the analysis of lipping; specifically, its frequency, severity, and age-at-transition from one stage of expression to the next. For the types of forensic cases that are often analyzed in Colombia, namely, commingled and/or severely fragmented/deteriorated, this type of analysis may help with assigning an age cohort category to remains that are missing other more reliable age indicators. Further, it helps to fully document the extent of degenerative spinal disease in modern Colombian individuals.

Frequency statistics for males and females combined of the severity of lipping were calculated indicating that lipping rates were low overall, with the highest frequency of moderate/severe lipping on the fourth lumbar vertebra (26%). The highest Pearson correlation coefficient for age and lipping was 0.657 for the second lumbar vertebra (L2), indicating that age and lipping are not highly correlated in this research sample. The next step included transition analysis in order to determine the average age-at-transition from one stage to the next using L2. The cumulative probit option in NPHASES2 was used for these calculations. Individuals transition from no lipping to slight lipping at 41 years (standard deviation 23 years); from slight to moderate lipping at 69 years (standard deviation 27 years); and from moderate to severe lipping at 93 years (standard deviation 19 years).

These results are concordant with the findings of Listi and Manhein,<sup>2</sup> which showed that while vertebral osteoarthritis is associated with age, it is a weak association. The large standard deviations in the transition analysis show that an individual can transition from one stage to the next within age categories that are decades wide. Essentially, the use of vertebral lipping as an indicator of age-at-death for identification purposes in Colombia is limited, but will provide useful information if attempting to assign fragmented remains to a general age cohort, and if sorting commingled remains.

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#### Degenerative Spinal Disease, Lipping, Presumptive Identification

## H87 The Role of Oral and Labial Devices in the Identification of Human Skeletal Remains

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After attending this presentation, attendees will recognize the potential of using wear patterns and damage caused by oral and labial dental devices to assist in the identification of human skeletal remains.

This presentation will impact the forensic science community by demonstrating the types of oral and labial devices that create wear and damage to the dentition and how observations of these patterns can aid in the identification of remains.

For purpose of this study, an oral and labial device will refer to any object inserted or implanted into the oral cavity. Oral and labial devices include, but are not limited to the following: bridges, partials, dental implants, braces, fixed or removable retainers, crowns, veneers, night guards, tongue or lip rings, and grills.

This presentation represents the preliminary findings of an on-going study to determine if: (1) oral and labial devices produce discernable wear and/or damage to the dentition; (2) if this wear and damage occurs in a repetitive pattern among individuals; and, (3) if this wear and damage can be used to assist in the identification of skeletal remains. This study was primarily conducted using online surveys. One survey was created for participants with fixed or removable oral or labial devices. A second survey was developed for dental professionals, including dentists, hygienists, and dental assistants. Both surveys were used to collect data regarding the types of devices that may create dental wear and damage, as well as determine the observable nature of this wear and damage to both the patient and the dental professional. Both dental professionals (n=8) and lay participants (n=31) reported observations regarding wear on teeth caused by oral devices. Seventy-one percent of lay participants reported observing wear or damage to teeth and soft tissue as result of an oral or labial device. The devices reportedly causing wear or damage included cosmetic and therapeutic dental devices, as well as oral piercings. One hundred percent of dental professionals surveyed reported observations of tooth and soft tissue changes caused by oral devices. The number of patients represented by the dental professionals totaled 31,952. Of these patients, it was reported that approximately 40% have some type of fixed or removable oral device.

This preliminary report also utilized a case study to demonstrate the effects of oral piercings upon the dentition. The subject of this case study had a metal tongue ring for approximately seven years. The piercing was removed six years prior to the study; however, the effects of the piercing upon the dentition were clear and observable. The individual suffered severe enamel erosion on the lingual surfaces of both anterior and posterior teeth. Significant chipping in both anterior and posterior teeth was also observed. The subject reported an extraction of a molar due to damage sustained from the piercing, as well. In addition to these findings, another piercing under the lower lip induced significant gum erosion that was also observable. The subject had soft tissue scarring of the tongue and upper chin resulting from the piercings. The details of the damage observed upon the dentition and soft tissue were recorded in the dental record provided by the subject's dental care provider, as well.

The preliminary findings of this study suggest there is potential for the use of observable wear patterns on the dentition created by oral and labial devices to assist in the identification of human skeletal remains. It is important to note that dental professionals must be diligent in documenting the types of wear and/or damage present on patients' teeth, as well as the possible causes. All eight dental professionals surveyed reported patients with oral or labial piercings; however, only six reported that they note this information in their patients' records. At minimum, dental wear and osseous changes may allow the forensic anthropologist to speculate that the unidentified had a specific oral or labial device. However, through detailed observation and documentation by the dental care provider, wear and

damage observed in the dentition of skeletal remains could be matched with thorough dental records.

## Oral Device, Identification, Wear Patterns

## H88 Fluvial Transport of Human Remains in the Sacramento River, California

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After attending this presentation, attendees will gain a better understanding of the dynamics involved in the fluvial transportation of human remains in the Sacramento River. The goals of this presentation are to examine key variables involved in the transport of human remains and to evaluate their influence on decomposition and disarticulation rates.

This presentation will impact the forensic science community by outlining a framework for future predictive modeling for search strategies of river victims.

The fluvial transport of human remains has received significant attention by paleontologists and forensic anthropologists over the past several decades.<sup>1-4</sup> Pioneering work with animal carcasses by Voorhies and with human crania by Boaz and Behrensmeier have provided a preliminary framework from which to study the movement of human remains in riverine systems.<sup>1,2</sup> In an early forensic application, Dilen used mannikins to simulate the transport of human remains in the Chattahoochee River, Georgia.<sup>3</sup> More recently, Bassett and Manhein have conducted a retrospective examination of Mississippi River cases to establish a predictive model for locating human remains.<sup>4</sup> While fluvial transport rates for human remains have been studied within a few river systems in the eastern United States, no published research exists for the western United States. More importantly, studies must take into account the specific hydrological factors of a particular river, including seasonal variation in water discharge rates. Focusing on the Sacramento River, this project is the first step in the development of a regional river victim database to evaluate rates of fluvial transport.

The Sacramento River is California's largest river, flowing 335 miles north to south from Mount Shasta to the Sacramento-San Joaquin Delta. The annual average river discharge is 350 m<sup>3</sup>/sec<sup>-1</sup>, which derives from both precipitation and snowmelt (USGS). Discharge rates are also controlled by dams, and vary depending on flood and drought conditions. Each year, the river claims many lives, including boating accidents, drownings, and suicides. It is also a common dump site for homicide victims. An understanding of how the dynamics of this fluvial system influence the transportation of human remains can be used to establish a predictive model for narrowing down search parameters for victims who entered the river on known dates (e.g., date missing).

Modeled after Bassett and Manhein's study, data collection for this project involved two broad categories of variables.<sup>4</sup> The first category focuses on variables relating to the biological profile of the river victims, as well as date missing, date found, location and side of river entry and recovery, distance traveled, and the reported cause and manner of death. The second category includes data on river dynamics such as water temperature, depth, bed load, and average rainfall at the time and location of entry and recovery. Collectively, these variables have been shown to have a relationship with transport rates of human remains in different fluvial systems. Data from this study will also be compared with other rivers to explore variation in fluvial transport rates.

As part of this preliminary study, we highlight two case studies of drowning victims that demonstrate long distance fluvial transport of human remains within the Sacramento River. Case #1, an elderly male drowning victim, was transported intact approximately 180 river miles (PMI=7 months). Case #2 also involved an elderly male drowning victim, but only

an intact foot (within a shoe) was recovered 46 river miles away from the where he entered the river (PMI=19 months). Using average river discharge rates for these locations, estimated fluvial transport intervals for each case were significantly less than the postmortem interval. Both cases show unusual transport distances, but the reported missing dates for each corresponded to peak discharge rates for 2005 and 2007, respectively. In this study, the hypothesis is tested that long distance fluvial transport is correlated with high discharge rates at the time a victim entered the river. The addition of known death and recovery dates from a large sample of case files from multiple counties, in conjunction with river discharge rates, will permit more precise estimation of transport rates for different sections of the river.

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#### Fluvial Transport, Taphonomy, Forensic Anthropology

### H89 Applicability of Common Age-at-Death Estimation Methods in Cold Case Analysis

*Alice E. Fazlollah, MS\*, University of Tennessee, Department of Anthropology, 250 South Stadium Hall, Knoxville, TN 37996*

The goal of this presentation is to explain the statistical comparison of six age-at-death estimation methods commonly used by practicing forensic anthropologists. Additionally, the applicability of these methods to cold case work is explored.

This presentation will impact the forensic science community by highlighting the limitations of these methods and posit modifications of their use when developing a biological profile.

The most commonly employed methods for forensic age-at-death estimation from adult skeletal remains utilize gross morphological changes of the auricular surface of the ilium, the pubic symphysis, and the sternal end of the fourth rib. Due to their frequency of use, age estimation methods should be subject to continued accuracy of use assessments. As such, this research utilized three standard methods of skeletal age estimation (Lovejoy et al., Brooks and Suchey, and Işcan et al.,) as well as, three recently published methods, which modify these standard methods (Osborne et al., Hartnett) to identify the differences in error and accuracy of each method using known aged individuals.<sup>1-7</sup> Additionally, the limitations and applicability of each method for populations with known and unknown age-at-death was assessed. The research was completed with two goals in mind: first, to determine if the more recent methods more accurately assessed the age of a known-age individuals than the older standard methods, and second, to examine the statistical properties of each of these methods to ascertain the practical application of each with regards to an unknown sample.

Forty individuals from the William M. Bass Donated Skeletal Collection were examined using these six aging methods. The data were analyzed for accuracy and error across all six methods. Using the McNemar statistical test, the researcher found that the Osborne et al. method provided correct age ranges significantly more often than the Lovejoy method ( $p$ -value < 0.0001); neither the Hartnett nor the Brooks and Suchey method provided correct age ranges significantly more often ( $p$ -value 0.4142); and the Işcan method provided correct age ranges significantly more often than the Hartnett method (2010b) ( $p$ -value < 0.0001). It was determined that the accuracy of some methods was due to the large prediction intervals for a

given phase.<sup>5,1,6,2,7</sup>

For this sample, the linear regression results were not associated with age using the Osborne et al. and Lovejoy et al. methods (Root MSE 8.58, R-square 0.13, Root MSE 8.65, R-square 0.12, respectively).<sup>2</sup> Results also show that the age phases using Hartnett (2010a; Root MSE 8.63, R-square 0.12) are more closely associated to actual age than Brooks and Suchey (Root MSE 8.99, R-square 0.05).<sup>6,2</sup> Overall, the Hartnett (2010b) method proved to have the closest relationship between phase and age.

These results suggest that when the Işcan et al. method is used to predict age, the inclusion of either the Lovejoy et al. or the Brooks and Suchey methods will not significantly improve the estimated age of the individual in this sample. The same is true for the newer methods; the estimated age cannot be significantly improved by including estimations from the Osborne et al. method or the Hartnett pubic symphysis or rib methods. Finally, the statistical relationship between phase and age for each method was used to develop six regression formulas that could be utilized to calculate age intervals based upon the user's determination of phase for that individual.

Following this analysis, thirty sets of unidentified skeletal remains from the Georgia Bureau of Investigation (GBI) were examined. After sex, ancestry, and stature had been determined, the regression formulas from this study were applied to calculate skeletal age estimation for each individual.

While this research does not ultimately point to a single of the six methods studied as superior to others, it provides a validation for these methods using a modern sample and applies the conclusions to a contemporary morgue sample in order to observe the shortcomings of each of the methods.

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#### Age-at-Death Estimation, Biological Profile, Unknown Sample

### H90 A Radiographic Positive Identification from a Foot Altered by Diabetes Mellitus

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The goals of this presentation are to reveal how quickly destructive skeletal changes can occur in cases of diabetes mellitus and to demonstrate that even in the event of advanced stages of diabetes, comparative medical radiography can be successfully employed to make a positive identification.

This presentation will impact the forensic science community by addressing how the growing epidemic of diabetes may potentially impact forensic casework and strategies for successfully meeting this challenge.

Forensic anthropologists are routinely called upon to positively identify individuals using comparative medical radiography. Such cases are made more difficult when there is a significant amount of time between the antemortem and postmortem films. Since bone is a living tissue, it is subject to continual remodeling over a person's life. Work by Sauer et al. (1988) reported that despite normal progressive bone maintenance and degenerative change over time, it was still possible to positively identify individuals using antemortem radiographs taken ten to twenty-three years prior. What has not been accounted for is the utility of comparative medical radiography in the cases of diseases, like diabetes mellitus, that significantly impact skeletal structures.

The growing epidemic of diabetes in the western world means more individuals will be passing through forensic laboratories afflicted with this disease. Thus, it is imperative that forensic scientists understand the disease process and its timing for appropriate interpretations and analyses. Although the exact mechanisms for skeletal changes are debated in the clinical literature, it is agreed that diabetes can have an impact on bone mineral density.

The complicated nature of such changes is highlighted by a forensic case in which comparative medical radiography was employed to make a positive identification. The decedent had suffered from hypertension and diabetes, with a history of hospitalization for a diabetic coma. The antemortem radiograph was an anterior-posterior film of the left foot taken five years prior with no noticeable osseous pathology. Examination of the postmortem radiographs revealed that the distal half of the first metatarsal and the associated proximal and distal phalanges had completely resorbed. The second metatarsal displayed resorption of its distal aspect in conjunction with prolific ossification around the mid-shaft. The remaining phalanges, with the exception of the proximal fourth and fifth, all showed either degenerative or proliferative changes. These changes are consistent with Aufderheide and Rodríguez-Martin (1988: 342) who report that diabetes induced ischemia may cause bony breakdown of the distal metatarsals and proximal phalanges and, without treatment, may eventually lead to gangrene of one or more toes.

Despite the significant skeletal alterations that had occurred in the five-year period between the decedent's antemortem medical radiograph and the postmortem films, it was still possible to use overall morphology, trabecular patterns, cortical bone thickness, and osseous spurs to make a positive identification.

As forensic practitioners, it is important to recognize the extreme osseous changes that can occur in a relatively short period of time with diabetes mellitus. Although it is possible to successfully employ traditional radiographic identification methods in these cases, they must be applied conservatively and within the bounds of sound practice.

**Positive Identification, Comparative Radiography, Diabetes Mellitus**

## **H91 Sex Determination Based on Clavicles from a Sample of Modern Colombian Mestizos**

*Juan M. Guerrero, BA\*, and Carlos A. Villalobos, BA, National Institute of Legal Medicine and Forensic, Cra. 7A N° 12-61, Bogota, COLOMBIA*

The goal of this presentation is to provide insight into new research on sex estimation that has been recently conducted in Colombia. This project aims to document sexual dimorphism in the modern Colombian population by investigating metric differences of the clavicle between males and females.

This presentation will impact the forensic science community by demonstrating significant sexual dimorphism of the clavicle of modern Colombians. In addition, it presents three discriminant function equations that can be utilized by Colombian forensic anthropologists and potentially by other workers in South America.

Sex determination in forensic anthropology is a critical pillar of the biological profile, as this variable often informs other biological parameters such as stature and age. Considering that Colombian forensic practitioners

frequently receive incomplete, dismembered, and decomposed bodies, metric analyzes of various postcranial skeletal elements are often required for sex estimation. Based on the above scenarios and the need to develop standards specific to the Colombian population, this research developed three discriminant function equations based on four measurements of the clavicle.

This research used a sample of clavicles drawn from the Colombian Modern Skeletal Collection from a total of 102 individuals (38 females and 64 males), with an average age-at-death of 50 years. Four measurements were utilized: maximum length (XLN), anterior-posterior diameter at midshaft (APD), superior-inferior diameter at midshaft (SID), and circumference at midshaft (CIR). In cases where both right and left clavicles were present, each was measured so that bilateral asymmetry could be tested.

The mean measurement values of the left clavicle were as follows: XLN (137.23 mm females; 154.33 mm males), APD (10.18 mm females; 11.82 mm males), SID (8.69 mm females; 9.92 mm males), and CIR (43.02 mm females; 51.43 mm males). The resulting mean measurement values of the right clavicle were as follows: XLN (136.66 mm females; 153.13 mm males), APD (10.54 mm females; 11.99 mm males), SID (9.43 mm females; 10.03 mm males), and CIR (33.23 mm females; 37.12 mm males).

The results obtained show that the differences between the means of male and female individuals were statistically significant ( $p$ -value < 0.05). Bilateral asymmetry was also statistically significant between right and left sides ( $p$ -value < 0.05), which resulted in the calculation of discriminant function equations for both the right and left clavicle. One equation was also calculated that utilized measurements from both the right and left clavicle together.

The following discriminant function equations for sex estimation were developed:

- Left Clavicle:  $(0.082 * XLN) + (0.192 * APD) + (0.363 * SID) - 17.788$ ;
- Right Clavicle:  $(0.085 * XLN) + (0.141 * APD) + (0.073 * CIR) - 16.761$ ;
- Right/Left Clavicle:  $(0.085 * XLNR) + (0.067 * APDR) + (0.079 * XLNL) + (0.361 * APDL) - 16.361$ .

In each of the above equations the sectioning point was 0, with females falling below this value and males falling above it. When the formulae were applied to the sample, the equation for the left clavicle classified 89.0% of cases correctly, the equation for the right clavicle classified 87.9% of cases correctly, and the equation utilizing both right and left clavicle data achieved a correct classification rate of 85.7%. Further testing of these equations on other known Colombian cases is necessary in order to discern a wider applicability of these formulae.

This research is expected to contribute to the improvement of the quality of forensic anthropological analyzes in Colombia by generating population-specific sex estimation criteria. It will expand forensic knowledge at both national and international levels as researchers continue to investigate population variation throughout Latin America.

**Sex Determination, Clavicle, Colombian Population Standards**

## **H92 An Experimental Study of Scavenging in Aqueous Environments**

*Jennifer A. Hertzog, MS\*, 2129 West 25th Street, #2, San Pedro, CA 90732*

After attending this presentation, attendees will have an increased understanding of the process of decomposition in different types of aquatic environments and knowledge of how fast certain organisms consume animal remains in a controlled aquatic tank setting.

This presentation will impact the forensic science community by demonstrating the need for decomposition studies to include macro-scavengers. The presence of macro-scavengers can actually delay decomposition before it accelerates decomposition and therefore are important factors in estimating postmortem interval.

The hypotheses of this study are that tanks with organisms (experiments) would decompose faster than those without organisms (control), that the salt experiment would decompose faster than the fresh experiment, and that both controls would precede at the same rate.

Four tanks (two freshwater and two saltwater) were assembled including sediment, filtration system, air pumps, thermometers, and light control timers. The saltwater experiment tank held six red-legged hermit crabs, one fiddler crab, and six Nassariid snails. The freshwater experiment tank contained six ghost shrimp, one red-clawed crab, and six mystery snails. Two rabbits (sold as food) were weighed, photographed, measured, and quartered. One hind leg was photographed, weighed, and measured for each tank before being placed into a cage secured with zip-ties and lowered into the tank and held into place with suction cups. During two experimental trials, remains were allowed to decompose for 45 days. On a daily basis, tanks were photographed and water quality tests (pH, ammonia, nitrite, and nitrate) were performed. Hourly (minimum of four hours a day and maximum of 12 hours a day) water and ambient temperature readings were taken as well as other observations on the appearance of the remains, color of the water, odor of the water, level of foam and organism activity. At the end of the experiment, the cages were removed, photographed, and opened. The bones and remaining flesh and adipocere were weighed.

During the first trial, the water temperature in the saltwater tanks was lower than the water temperature in the freshwater tanks. The ambient temperature had a broader range during the second trial, but the tanks matched more closely to the ambient. Except for brief periods, the water temperatures in the first trial were higher than the second trial. The appearance of the remains in all four tanks looked obviously different: both freshwater tanks had filamentous algae; whereas the saltwater tanks had encrusting algae, but the controls had more, longer, and thicker algae than the experimental tanks did. Furthermore, gas bubbles that were trapped in the adipocere and algae appeared different between the salt tanks: the control's bubbles were short and flat whereas the experiment's bubbles were tall and peaked. Stages of decomposition were defined as float, total float, floating decay, disarticulation, and sunken remains for this experiment. The presence of scavengers initially delayed decomposition and then decay preceded faster in the experiment tanks than the controls. In the first trial, the freshwater experimental tank hit floating decay a day before the saltwater experimental tank, but in the second trial, the saltwater experimental tank hit floating decay 14 days before the freshwater experimental tank. In both trials, the freshwater remains reached a further stage of skeletonization than the saltwater remains, even though the saltwater remains occasionally entered the next stage first. The remains from the first trial had less flesh, less algae, and less adipocere on the bone than the remains from the second trial.

In conclusion, scavengers affect the rate of decomposition by direct consumption, indirect consumption of the microbial layer, or by disbursing the algae growing on the remains. This research showed that changing the number of macro-scavengers present greatly changed the visible qualitative results of the appearance of the remains.

Despite the problems and limitations with laboratory settings, this experimental study suggests that studies that exclude scavengers are not showing the entire picture and might lead to inaccurate models of decomposition in a specific region.

#### **Aquatic Decomposition, Scavenging, Taphonomy**

## **H93 The Influence of the Topical Application of Cosmetic Products on the Rate of Decomposition and Insect Activity on Pig Carcasses**

*Sophie Radicke\*, and Tal Simmons, PhD, School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, PR1 2HE, UNITED KINGDOM*

The goal of this presentation is to provide attendees with information about the effects of the topical application of three commonly used cosmetic products on the rate of decomposition and insect activity on the carcass and how this might affect the estimation of the postmortem interval.

This presentation will impact the forensic science community by suggesting that the rate of decomposition and insect activity on a corpse is not affected by the application of cosmetic products. As a result the potential presence of cosmetic products on a dead body does not have to be taken into account estimating the postmortem interval.

The total body score (TBS) appearance of decomposition of a cadaver can be helpful to determine the time-since-death. The TBS is a combination of point scores, which are derived from the individual assessment of the decomposition stage of head and neck, trunk and limbs (Megyesi *et al.*).<sup>1</sup> The characteristics of decomposition appear a more or less fixed sequence; however, the rate at which these appear is highly variable. A number of factors have been shown to influence the rate of decomposition. One of the most influential factors is insect access to the cadaver (Simmons *et al.*).<sup>2</sup> Circumstances restricting insect access and activity on a corpse may include its placement, whether it has been wrapped or covered and whether insect repellent substances are present on the body.

Charabidze *et al.* found that the application of HCl, patchouli perfume, insecticide and petrol significantly delayed the arrival of flies to rat carcasses in comparison to the control group.<sup>3</sup> Under laboratory conditions it was also observed that mosquito citronella repellent, HCl, insecticide, petrol and paradichlorobenzene have a repellent effect on female flies (Charabidze *et al.*).<sup>3</sup> A similar insect-repellent effect of insecticides has been noted by Vass, which also led to initial underestimation of the postmortem interval.<sup>4</sup> The previously mentioned research shows that the application of varying substances have an effect on insect activity on the carcass and as a result may influence the rate of decomposition and thus also the estimation of time-since-death.

However, no literature exists that considers the effect of commonly used substances that are applied to a large portion of the body such as cosmetic products. Considering the widespread use of these products, it is important to establish any potential effects on the rate and/or pattern of decomposition that could lead to a miscalculation of time-since-death.

As a model for human cadavers, carcasses of the domestic pig (*Sus scrofa*) were used in this research. Three experimental groups and one control group were set. Each group consisted of four carcasses that were laid out on the ground. In each of the experimental groups, either sunscreen, fake tan or insect repellent was applied to the animal's body surface. The area covered included the head, trunk and limbs, but excluded the anal and groin area. This was done to represent a realistic application pattern as closely as possible.

The stage and pattern of decomposition was assessed using Megyesi *et al.*'s scoring system and carcasses were observed approximately every 50 Accumulated Degree Days (ADD).<sup>1</sup> Insect activity was measured by the body surface area covered with superficially visible maggot masses, which was converted into a maggot mass score (MMS). The MMS increased with the amount of the body surface area being covered. Furthermore, samples of maggots were taken from the maggot masses and reared to the adult stage to determine the insect species attracted to the carcasses.

The experiment ran for a total of 694 ADD during which the rate of decomposition was consistently similar in all four test groups and did not show any significant differences (Mixed effects model, DF = 3, F = 0.556, *p-value* = 0.654). Superficial maggot masses were first noted in all groups

at 141 ADD. As a general trend, the MMS increased until 376 ADD and then dropped again in all groups. The increase in the MMS was, however, not linear and fluctuated until reaching its highest value. At the end of the experiment no superficial maggot masses were observed in most carcasses.

Identification of collected and reared larvae showed the most abundant fly species in all groups was *Calliphora vomitoria*, followed by *Protophormia terraenovae* and *Calliphora vicina*. In contrast, *Lucilia sp.* were observed less frequently.

The results of this study indicate that the topical application of cosmetic products to a large portion of the body surface area does not have any notable effects on the rate of decomposition; neither did insect activity, as measured by the size of superficial maggot masses, differ between the control and test groups. As insect activity has a key influence on the rate of decomposition, these two findings are concordant and consistent with previous research. In conclusion, applied cosmetic products cannot be considered to be a factor that needs to be taken into account when determining the time-since-death.

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#### Decomposition Rate, Cosmetic Products, Entomology

### H94 Human Grave Identification Using GPR and Its Implications for Recognizing Covert Burials of Greater Antiquity

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The goal of this presentation is to investigate the effectiveness of Ground Penetrating Radar (GPR) for identifying longer-term (e.g., cold case) covert human graves in diverse burial environments. By comparing GPR signals of known historic human graves across diverse burial environments and treatment, the effect of these variables on GPR signals can be visualized. This will lead to a clearer understanding of the effectiveness of identification of longer-term covert burials in a modern forensic context.

This presentation will impact the forensic science community by providing guidelines for the use and interpretation of GPR technology in detecting long-term human covert graves. Attendees will learn the significant impact that burial environment and time have on the GPR signal. The outcome of this presentation will be the description of GPR-produced grave images which distinguish more formal grave sites from those potentially produced to hide a criminal act. This presentation will also raise awareness of the utility of geophysical remote sensing in searching for covert burials of greater antiquity.

Ground-penetrating radar is arguably the best geophysical tool to use for covert burial searches in forensic contexts.<sup>1</sup> Several researchers have recently illustrated the potential for GPR to successfully identify covert graves over short to moderate periods of time.<sup>2,3</sup> These studies have utilized pigs as human models and have suggested that pig graves with additional burial treatment (e.g., wrappings, tarpaulin, rocks) showed clearer signals than those without.

This poster examines variation in ground-penetrating radar (GPR) images from human burial sites of greater antiquity and differing burial environment to test the following hypotheses: (a) GPR signals are capable of detecting covert graves of greater antiquity than those created for experimental study; and, (b) GPR signals are capable of identifying graves of individuals who have less “formal” burial preparation (a situation comparable to covert forensic burials) than those with more grave preparation.

The burial sites examined are two historic cemeteries on the R.J. Reynolds Homestead in Critz, Virginia. The Reynolds family cemetery contains 24 marked graves dating from 1849 to 2004, representing individuals from the wealthy and prominent Reynolds family (founders of the R.J. Reynolds Tobacco Company and Reynolds Metals [Aluminum]). Approximately 200 meters north of this cemetery in a wooded area is the second cemetery for slaves and (after 1865) former slaves who worked in tobacco production or as servants for the Reynolds family. At the slave cemetery are 54 probable graves (based on ground depressions and field stones); only four of these manifest marked “formal” stones. These two cemetery samples represent economically and ethnically distinct historic individuals who varied significantly in terms of their burial treatment and environment. While individuals from the Reynolds family cemetery were encased in burial vaults and underwent more formal burial treatment, those from the slave cemetery were in pits with wood coffins and shroud coverings (representing the “less formal” burial more typical of a modern covert grave).

GPR surveys of both cemeteries were conducted in July and November 2008, and for the slave cemetery again in May 2011. All surveys were conducted using a PulseEKKO GPR system, with a 500-MHz antenna being used for the July 2008 surveys and a 100-MHz antenna for the later surveys. The 500-MHz antenna produces better resolution of smaller subsurface targets but has a shallower penetration depth, especially in the clay soils of the survey area. The lower frequency 100-MHz antenna provides lower resolution but deeper penetration (more than two meters).

The 500-MHz scan of the Reynolds Family cemetery identified anomalies correlated with graves; however, some anomalies were inconsistent with the position of above-ground gravestones. The survey of the slave cemetery using this antenna was less productive, due to the damp, forested environment. The soil conditions resulted in expected signal loss, but the scan did identify anomalies correlated with a previous map of grave sites.

The November, 2008 scan of the Reynolds cemetery used the 100-MHz antenna along a 0.5 meter grid, with the instrument reading north-south transects along this grid. Scans produced much clearer images of graves in relation to their markers. Also, the grave of a four-day old child that was not seen in the 500-MHz scan was revealed.

The 2011 slave cemetery 100-MHz scan also produced clearer associations of GPR signals with mapped grave locations in many cases. However, the GPR signals for graves became much more subtle and in several cases disappeared at shallower depths (1.5 meters from the surface) as opposed to 2.0 meters for the signals for the Reynolds family. This suggests a shallower depth and less grave preparation for the slave burials - a scenario common to modern covert burials. This study indicates that GPR surveys for covert graves of considerable antiquity in similar moist, clay-rich soils can be productive if the appropriate antenna is used. However, the signals for such graves will be far more subtle and diffuse than those from a formal cemetery setting.

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**Ground-Penetrating Radar, Covert Human Graves, Long-Term Burial Environments**

**H95 Microscopic and Macroscopic Changes to Peri-Mortem Pediatric Trauma Following Burial**

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The goal of this presentation is to examine the effect of burial on peri-mortem (blunt force and sharp/blunt force) pediatric trauma. This is accomplished by documenting taphonomic changes in the microscopic and macroscopic signatures of traumatized bone in a sample of frozen stillborn pigs undergoing trauma immediately after thawing and burial immediately following trauma. After attending this presentation, attendees will have a clearer understanding of the microscopic and macroscopic temporal changes affecting traumatized subadult remains undergoing burial.

This presentation will impact the forensic science community by enhancing attendee's ability to recognize and interpret peri-mortem trauma in remains subjected to extended burial and the ability to differentiate this trauma from postmortem damage.

This poster is the first in a series of research projects exploring the relationship between time and the microscopic and macroscopic signatures of pediatric trauma. This study examines the effects of extended burial on subadult remains undergoing peri-mortem trauma. SWGANTH guidelines for trauma analysis ([www.swganth.org](http://www.swganth.org)) have called attention to the need for great caution when identifying peri-mortem blunt and sharp force trauma altered by postmortem processes.<sup>1</sup> This characterization is important not only for understanding the effects of taphonomic variables on peri-mortem trauma, but for differentiating peri-mortem and postmortem processes. A previous study by Calce and Rogers found significant alterations in peri-mortem (blunt force) trauma signatures in adult pig skulls in a surface environment in Canada over a period of 12 months, primarily due to the freeze/thaw cycle of this region.<sup>2</sup> The effects of a varied postmortem environment (e.g., burial rather than surface, more temperate climates) on peri-mortem trauma are not known, particularly with regard to subadult remains.

The current study examines the effect of burial over extended periods of time on peri-mortem blunt force and blunt/sharp force traumatized subadult remains. Eleven (unprocessed) stillborn pigs (*Sus scrofa*), frozen at death, were used in the study. Eight of the 11 pigs were subjected to trauma after thawing for 24 hours. Four of these eight pigs underwent blunt force trauma by means of a standardized drop-force mechanism using a concrete cylinder weighing 1,109g. Pigs were placed on a hard substrate (a metal plate which measured impact force) and impacted by the concrete cylinder on both their left and right sides. Right side impacts (two per pig—one focused on the lateral cranium, the other, the lateral shoulder) were delivered via a drop mechanism through a stabilized 50cm long PVC pipe. Similarly, two impacts on the same areas (cranium, shoulder) on the left side were dropped through a 108cm long PVC pipe. The remaining four traumatized pigs were similarly treated, although the impact tool was a sharpened steel wedge (simulating both sharp and blunt force trauma) weighing 2,100g. The final three pigs underwent no trauma.

Within 24 hours after trauma, nine of the eleven pigs (both traumatized and non-traumatized) were individually buried supine in 40cm deep burial pits at a decay facility in the spring season. After four months, six pigs (representing all three treatment categories) were exhumed; after seven months, the remaining three pigs (again, representing all three treatment categories) were exhumed. All exhumed pigs were skeletonized. The final

two pigs (one traumatized with blunt force trauma, the other with blunt/sharp force trauma) were not buried, but processed immediately, serving as controls.

Comparisons were made across the buried and non-buried, traumatized and non-traumatized bone by means of a Keyence VHX 1000 Microscope (using 5 – 50x and 20 – 200x lenses) as well as through visual examination. Variables examined included: number, type, pattern, and dimensions of fractures and fracture lines, fracture surface morphology (e.g., smooth vs. jagged) and angle (e.g., obtuse, right), presence of color differential, hematoma staining, hinging, and inbending/outbending.

Although observed taphonomic damage included microfractures, root etchings, erosion, and split and frayed bone ends, bone samples from pigs undergoing burial after four and seven months retained the characteristic signatures of peri-mortem blunt force trauma, including presence of identifiable inbending/outbending, hinging, radiating fracture lines, and sharp, irregular fracture outlines. Analysis of samples under light microscopy enhanced the identification of these signatures, particularly in samples buried for seven months. Low power magnification of fracture outlines in the extended (seven month) buried samples revealed feathering and serration of fracture edges, making their edges more irregular compared to the non-buried samples (although still recognizable as peri-mortem fractures). Similarly, pigs with blunt force/sharp force trauma retained characteristic signatures of this trauma after seven months of burial.

These comparisons suggest that although microscopic and macroscopic alteration of bone occurs after periods of extended burial, characteristic signatures of blunt force and blunt/sharp force pediatric trauma may not be erased. It is recommended that light microscopy be utilized to examine subadult bone suspected of peri-mortem trauma.

**References:**

1. Scientific Working Group for Forensic Anthropology (SWGANTH). [www.swganth.org](http://www.swganth.org)
2. Calce SE, Rogers TL. Taphonomic changes to blunt force trauma: a preliminary study. *J Forensic Sci* 2007;52(3):519-527.

**Pediatric Peri-Mortem Trauma, Blunt/Sharp Force Trauma, Burial**

**H96 A Method for Standardization of Anatomical Axes and ROIs in Femoral Thin Sections of Unknown Orientations**

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After attending this presentation, attendees will learn how midshaft cortical distribution observable via microscopic slides can be utilized to standardize femoral anatomical axes and histological regions of interest (ROIs) when skeletal orientation is unknown.

This presentation will impact the forensic science community by extending a technique for standardizing thin section anatomical axes and histological regions of interest, which may ultimately help identify areas of the femur most suitable for age-at-death estimation.

Different regions of the femur are in contact with separate lower limb muscle groups and subject to differential biomechanical demands. As both periosteal loads and far-field intracortical stresses influence remodeling—and, hence, osteons, the basic structures forensic histomorphologists quantify for an age estimate—it is imperative for correct anatomical orientation of investigated microscopic slides to be known. When working with fragmentary remains and thin sections removed from dissecting room cadavers, exact determination of anatomical axes locations becomes problematic if the samples are not expressly marked at the time of

extraction. While anterior and posterior surfaces are easily identified through the presence of the femoral linea aspera, medial and lateral anatomical locations are not easily distinguishable.

To address this issue, Goldman and colleagues suggest using Sharpey's fiber insertion direction, and White (2000) suggests the lateral posterior femoral surface is usually more concave than the medial posterior surface. The accuracy of these techniques has yet to be demonstrated.<sup>1,2</sup> Nobel and colleagues extended a metric solution to the problem of identifying medial and lateral femoral cortices.<sup>3</sup> Seeking to identify the best placement for femoral midshaft medical implants, they measured 80 adult femora at their anatomical axes via radiographs. The authors found that the posterior cortical thickness on average was thickest (7.7mm), followed by the lateral cortex (7.6mm), the medial cortex (7.2mm), and finally the anterior cortex (5.1mm). Corroborating these findings, Stephenson and Seedhom (1999) measured 16 adult femoral midshaft and found anterior cortical thickness to be the thinnest, the posterior cortex the thickest, and medial and lateral cortices similar, but with the lateral side slightly thicker on average.<sup>4</sup>

To test the reliability of these observations, 200 femoral midshafts of known orientation were harvested from GWU Medical School Dissecting Room cadavers and processed for histological assessment. A 1200 dpi resolution image of each of the thin sections was obtained using an HP 4850 Scanner. ImageJ (version 1.44) and MomentMacroJ (version 1.2) were used to draw the principle axes of maximum and minimum bending rigidity on each femoral image. To standardize anatomical axes and histological ROI locations when a complete femur is not present for analysis, the A-P anatomical axis was consistently determined by drawing a vertical line, and the M-L anatomical axis by drawing a horizontal line, through the mathematically determined centroid of the section. To distinguish between medial and lateral cortices, each femoral cross-sectional image analyzed by ImageJ with observable axes was additionally placed over a background that standardized cortical width measurement every 22.5°. Analysis of this data suggests cortical width at the 292.5° lateral location is consistently thicker than at 112.5°, the medial location on the axis 180° across. These measurements were determined to be significantly different from each other using a paired comparison test and can therefore be used to distinguish medial and lateral cortices when femoral anatomical orientation is unknown.

Here, a method is extended for standardization of anatomical axes and histological ROIs in femoral thin sections with unknown skeletal orientations, with an additional technique for distinguishing between medial and lateral femoral cortices. While a useful technique when confronted with unlabeled femoral thin sections, standardized ROIs can also help to improve repeatability and reduce operator subjectivity in the field of histomorphology. Further research is underway that utilizes these techniques in an effort to uncover patterns in histomorphometric variation in biomechanical versus anatomical sampling locations in midshaft femoral cortical bones. Discovery of such patterns will be of great assistance to forensic anthropologists who use histomorphometric techniques by suggesting ROIs most suitable for age-at-death estimation.

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2. White T. *Human osteology*. 2nd ed. London and San Diego: Academic Press, 2000.
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#### Standardization, Histomorphology, Regions of Interest

## H97 REFACE: New Features Addressing the Needs of Forensic Identification Professionals for Objective Facial Approximation

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After attending this presentation, attendees will become aware of REFACE, understand how it generates facial approximations, be informed of new features that have been incorporated, and appreciate the usefulness of those features for forensic identification professionals.

This presentation will impact the forensic science community by illustrating the advantages of statistically-based computerized facial approximation (CFA) as well as the possibilities of a system that can quickly and objectively generate multiple images of an unidentified decedent for eventual presentation to the public.

REFACE was developed during a long collaboration between the Federal Bureau of Investigation's Laboratory Division and General Electric Global Research to complement traditional clay modeling techniques with an objective, repeatable, and less labor-intensive means to generate facial approximations based on large, diverse sample sizes, and whose methods could be fully documented for forensic purposes. Literature assessing traditional facial approximations has repeatedly shown that presenting the same skull to different artists can result in very different approximations. Additionally, many tissue depth studies have been based on small sample sizes.

Modern computed tomography (CT) technology allows three dimensional viewing of facial skin, or the underlying bone, or both together. This technology, which links facial and skeletal data, forms the foundation for REFACE. A CT database of approximately 400 individuals of both sexes and four ancestry groups was collected to form the reference database for a statistically-based CFA program. REFACE applies registration, warping, and principal components analysis to the known relationships between skin and bone of each head in the reference database to approximate the skin for an unknown skull that is scanned and imported into the system.

One of the advantages of REFACE is time. Generation of an approximation using an 8-core processor takes 20 minutes. Because of this speed, users can efficiently generate multiple approximations per day, including the option of estimating the face in alternate groups if ancestral attribution is ambiguous. Users can also address cases of mixed ancestry by specifying a different ancestry group for the nose tip regression if recommended by a forensic anthropologist's analysis of the skull. Export formats for 3D approximations include VTK, STL, OBJ, and DXF, as well as two-dimensional JPEG or PNG formats. Users can also record an AVI video of the rotating 3D approximation. These export options allow the user to perform post-approximation artistic modifications with a software package of their choice or to present multiple images/image formats to the public.

Another advantage of a system such as REFACE is documentation. Users assign unique identifiers for each imported skull and generated approximation, and every aspect of user input, approximation parameters, modifications, etc. is automatically saved and documented. Additionally each unknown skull must be CT- or laser-scanned before import into REFACE, providing agencies with permanent electronic storage of each of their unidentified cases. REFACE also has security features that allow an organization's designated administrator to control user access to specific software features and to specific approximations. These features allow

strict control within a facility that may have multiple individuals generating approximations and provide documentation for exactly how each approximation is generated, so that the process can be repeated or presented in court, if either is necessary.

Although nationwide standards for the generation and presentation of facial approximations do not currently exist, features within REFACE allow forensic anthropologists/artists the flexibility to address future developments or changes in the field through the use of multiple export formats and presentation types (3D, 2D, video, multiple images), whether for post-approximation modification, public display, craniofacial superimposition, or for use in facial recognition software aimed at matching unidentified decedents to missing persons. In summation, REFACE provides agencies a way to generate facial approximations objectively and efficiently, the ability to document and secure data for each approximation, and the flexibility to address any changes that may arise within the field of facial approximation.

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#### **REFACE, Facial Approximation, Forensic Identification**

### **H98 The Potential of the Angle of the First Rib, Head to Tubercle, in Sexing Adult Individuals in Forensic Contexts**

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After attending this presentation, attendees will understand the potential application of using the angle of the first rib, head to tubercle, in the successful sexing of unknown individuals, the calculation of the angle of the first rib and the statistical probability of correctly sexing an individual using the angle.

This presentation will impact the forensic science community by providing another tool for sexing unknown individuals using skeletal material, which can aid in identification.

Accurately assessing the sex of an adult human skeleton is fundamental in forming the biological profile used in forensic anthropology. The first rib was chosen due to its distinct shape, compact size and increased sustainability to taphonomic processes. The first rib has been examined in past research, but all focus has been on the sternal end of the rib and none on the angle created between the tubercle and head. This angle is present when the rib is viewed in its non-anatomical orientation. In some cases the angle is not present and the anatomical positioning is necessary to determine the siding for a rib with an angle of 0°. When a rib is sided in anatomical position the head will, in most cases, point downward and the subclavian grooves will be on the inferior surface.

This study was conducted using males and females, both African and European American, from the William M. Bass and Hamann-Todd Skeletal Collections. The left and right first ribs of 286 individuals were measured for total length, internal length, height of head off of a surface and length from tubercle to head. The angle was determined using a sliding caliper to measure the length from the head to the tubercle and the head to a surface, and then calculating the inverse sine to obtain the angle from the measured hypotenuse and height.

The calculated angles were then compared using logistic regression analysis, to determine the likelihood that a given angle was either male or female. Of the 572 measured samples, 555 were calculated, 17 were excluded for missing angle or length measurements, with 266 angles being male and 289 female. Logistic regression showed that angle alone is 60.2% concordant, while angle, total length and internal length combine to yield a 70.5% concordance. These results suggest that the angle can be used to predict sex of an individual, while the addition of total rib length and internal rib length increase correct classification by 10%.

This research shows that the angle of the first rib is able to determine the sex of an individual with a high statistical probability, using several

measurements of the bone that are likely to survive prolonged taphonomic exposure. The probable sex information could be combined with aging methods of the first rib to assess both age and sex of an unknown individual. Since few skeletal elements can both age and sex an individual, this research could have great potential for further forensic applications in adding reference information to the field.

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#### **First Rib, Sexing, Identification**

### **H99 Asymmetry of the Deltoid Tuberosity and the Possible Impact on Osteometric Sorting**

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After attending this presentation, attendees will gain an understanding of how individual asymmetry may affect the sorting of humeri in a commingled assemblage.

This presentation will impact the forensic science community by emphasizing the variability in skeletal elements and how such variability and individuating characteristics related to asymmetry need to be taken into account when using osteometric sorting to segregate individuals from a commingled assemblage.

This research investigates whether asymmetry of the humerus, specifically the deltoid tuberosity, can be great enough to cause elements to be determined an unsuitable match when utilizing osteometric sorting. Osteometric sorting is used to assist in the evaluation of associating human skeletal elements based on size.<sup>1</sup> The sorting of skeletal elements from a commingled assemblage into individuals is potentially adversely impacted by varying degrees of asymmetry in individuals. Asymmetry is the degree of variability between the contralateral sides of the body, and occurs naturally due to the exertion of various forces over time. Since these forces vary on an individual level, based on factors affecting the growth and muscularity of elements, further evaluation of the methods used to associate contralateral elements in an individual was conducted.

Osteometric sorting, a sorting method created by Byrd and Adams, was produced to provide an objective use of size to sort through commingled skeletal assemblages. This method is currently being used in the sorting of a large, commingled assemblage at the Joint POW/MIA Account Command – Central Identification Laboratory (JPAC-CIL) that involves U.S. losses in the Korean War. This assemblage is largely fragmented and has been heavily sampled for DNA, which limits the number of measurements that can be obtained. Due to the nature of the remains, measurements recommended by Byrd and Adams for use in osteometric sorting will typically comprise the overall number of measurements that can be taken. One of the humeral measurements (maximum diameter of diaphysis at the deltoid tuberosity), is often one of the only measurements that can be taken on a humerus due to survivability. This measurement is strongly influenced by individual muscularity. The measurement includes the attachment site of the deltoid muscle. As the muscle increases in size, the tuberosity changes shape and often enlarges.

The reference data utilized for osteometric sorting at the JPAC-CIL was used as the reference sample for this study, and data collected from JPAC-CIL reference skeletons was added to this dataset. The total sample size used in this analysis is 59 individuals. The measurements were added to a spreadsheet and the osteometric sorting formulae for pair matching elements were utilized.<sup>1</sup> This method, which utilizes multiple measurements on the humerus, was used to assess asymmetry by assigning as a cut-off-any *p-value* less than 0.10, and declaring any individuals below the cutoff to be asymmetrical. Of the 59 individuals, 37 had larger right side measurements. Five individuals (8%) had significant *p-values*, which rejected the element, implying they were not from the same individual due

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\* Presenting Author

to their dissimilarity in size. Note that this number falls below the expected Type I error rate of 10%. The analysis of the maximum diameter at the deltoid tuberosity combined with the maximum epicondylar breadth of the humerus resulted in 10% of the individuals having significant *p-values*. However, the analysis of the maximum diameter at the deltoid tuberosity combined with the maximum length of the humerus resulted in 21% of the individuals having significant *p-values*. In this case, 10 of the 11 individuals with significantly small *p-values* had larger right measurements. These results suggest that the asymmetry of the humerus as reflected in the deltoid tuberosity must be taken into account in order to mitigate the potential errors stemming from varying degrees of asymmetry on an individual level when sorting large commingled assemblages.

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1. Byrd JE. Models and methods for osteometric sorting. In: Adams BJ, Byrd JE, editors. *Recovery, analysis and identification of commingled human remains*, Totowa: Humana Press, 2008: 199-220.

#### Osteometric Sorting, Deltoid Tuberosity, Asymmetry

### H100 Skeletal Maturation of the Medial Clavicle and First Sacral Segment in Modern Colombians

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The goal of this presentation is to provide important information about skeletal maturation in modern Colombians as a first step in addressing the dearth of research in this area. This project is part of a larger collaboration aimed at providing Colombian forensic practitioners with population-specific standards for establishing a biological profile from skeletal remains.

This presentation will impact the forensic science community by demonstrating ages-at-transition for the medial clavicle and first sacral segment in modern Colombians. This information can be immediately applied in Colombia as one tool for young adult age estimation.

In the last two decades, the field of forensic sciences in Colombia has implemented important age estimation methods, including standards that have not been fully validated for the Colombian population. The purpose of this paper is to present preliminary results of ongoing research on age estimation of young adults, specifically focusing on the medial clavicle and first sacral segment. These two skeletal maturation indicators are particularly useful in forensic contexts, as they are highly diagnostic for age estimation in the 20-30 year old range, which comprises a large portion of forensic case work in Colombia.

The skeletal sample for this study is a subset of the Colombian Modern Skeletal Collection from the Institute of Legal Medicine and Forensic Sciences in Bogotá, Colombia (39 individuals aged 19 to 33 years). The mean age of the sample is 25 years, and 80% of the sample is comprised of males. Whereas it is important to analyze male and female fusion separately because females are typically precocious in skeletal maturation, the small sample size in this preliminary investigation does not permit a statistically sound analysis of the female sample (n=9). Consequently, the sample was analyzed in two ways: as a combined sample, and males only. Each author independently scored the two age indicators in order to facilitate inter-observer error analyses. The epiphyses were scored as unfused, fusing, or fused. Transition analysis, or probit regression, was performed using the cumulative probit option in NPHASES2 in order to determine the average age of the transition from one fusion stage to the next.

The combined and male-only analyses did not yield significantly different results, likely on account of the small female sample size. For the medial clavicular epiphysis, males transition from unfused to fusing at 20 years and from fusing to fused at 27 years. The combined sample gave

similar results: the transition from unfused to fusing was 20 years, and the transition from fusing to fused was 26 years. These results differ from those obtained by Langley-Shirley and Jantz (2010) on a modern American sample with respect to the first transition (modern American males transition from unfused to fusing at 16 years), but not with respect to the second transition. This difference in the first transition is more likely a result of the sample age distribution of the Colombian sample than actual differences in age-at-transition. Consequently, American standards may be appropriate for the Colombian population, but further investigation is necessary to ascertain this for certain.

Transition ages of the first sacral segment were similar to those of the medial clavicle. Males transition from unfused to fusing at 20 years and from fusing to fused at 27 years. The combined sample also transitioned from unfused to fusing at 20 years and from fusing to fused at 29 years.

This presentation will discuss the implications of these results to forensic practice in Colombia and evaluate epiphyseal union in the Colombian population compared to other modern populations, taking into consideration the country's political history and general socioeconomic status. More importantly, the humanitarian impact of this project will contribute to the search for the truth and the protection of the most vulnerable sectors of a population exposed to the rigors of ongoing conflict.

#### Reference:

1. Langley-Shirley N, Jantz RL. A Bayesian approach to age estimation in modern Americans from the clavicle. *J Forensic Sci* 2010;55(3):571-83.

#### Epiphyseal Fusion, Age Estimation, Transition Analysis

### H101 Evaluation of Methodologies for Stature Estimation Based on Tibiae in Colombia: Forensic Stature Versus Cadaver Stature

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The goal of this presentation is to provide insight into new research on stature estimation that has been recently conducted in Colombia. This project aims to utilize centralized stature data from national identification cards in order to generate stature equations for the tibia.

This presentation will impact the forensic science community by demonstrating how stature can be generated from the tibia of positively identified individuals. In addition, it presents two discriminant function equations that can be utilized by Colombian forensic anthropologists to estimate stature and considers the difference between self-reported stature (FSTAT) and cadaver stature (CSTAT).

The country of Colombia is fortunate to have an infrastructure that includes a government-sponsored database which maintains stature data of the entire Colombian population aged 18 years and older. These data are sourced from self-reported identification cards that all Colombian citizens are required to carry. However, studies have not been conducted to prove the reliability of this source and its usefulness for the forensic area. Stature information is frequently used to compare the forensic identification of missing individuals, even though no methods have been developed for stature estimation based on this information. This study evaluates the reliability of the first stature formulae developed on the basis of forensic stature data (FSTAT) from tibiae in Colombia.

Right and left tibiae of positively identified male individuals were analyzed (n=63). The mean age of the sample was 48 years old and stature information was recorded on each individual's identification card. Two types of stature data were available in this study: stature recorded on the identification card (FSTAT) and the cadaver stature obtained during autopsy (CSTAT). Maximum length (XLN), as defined by Buikstra and Ubelaker, was measured for each complete tibia.<sup>1</sup> Regression equations for left and right tibiae were obtained: [FSTAT = 86.0 + 2.24(XLNL) ± 3.384] and [FSTAT = 84.7 + 2.27(XLNR) ± 3.286]. These new equations were applied

to a control sample (n=22) and compared with five other stature estimation methods developed on various Hispanic populations. The figures estimated for each formula were compared to known statures of the control sample.

The results show that the stature data recorded on the identification card provide a high level of correlation (0.84 and 0.83) and standard error of the acceptable estimation (3.2% and 3.3%) in the stature equations developed for this research. Paired t-tests demonstrated that the formulae of this study were reliable, both for forensic stature estimation and cadaver stature estimation, while the other tested methods generated statistically significant differences (*p-value* < 0.05) between estimates and known statures.

On the other hand, the comparison of the Mean Square Error (MSE) showed that the equations developed in this research are more precise when estimating FSTAT than CSTAT. The results of the remaining methods evaluated do not differ significantly between FSTAT and CSTAT estimates. The analysis showed that the equations presented here are more reliable for the estimation of both FSTAT and CSTAT than other methods developed earlier in Colombia.<sup>2</sup>

To conclude, it must be noted that the database sponsored by the Colombian Vital Statistics Office is a valuable source of forensic information, not only for data comparison but for the development of stature estimation methodologies. On the other hand, a study should be undertaken in Colombia on the relation between living stature and data recorded on the ID cards, in order to establish the potential biases that may arise when these data are used. Finally, this research is expected to contribute nationally and internationally to an understanding of the importance of using forensic stature for the identification of missing persons. The data generated will also strengthen forensic services in Colombia.

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#### Forensic Stature, Cadaver Stature, Colombian Population Standards

## H102 Isotopes and the Future of Region of Origin Identification: Geochemical Boundaries, Political Boundaries, and Modeling Methods with a Mexican Sample Population

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The goal of this presentation is to discuss the latest methodologies currently in use in the creation of isotope-based databases for identification. Using examples from a Mexican population, this presentation will identify when and where each database methodology can be practically and successfully applied. This presentation will investigate identification databases consisting of “authentic known samples” and discuss the statistical success of political/state groupings versus geochemical separations. In addition, this presentation will compare databases consisting of “authentic known samples” with those based on predictive models. After attending this presentation attendees will, understand the two major types of isotope databases currently in use (authentic sample, and predictive model), and be able to identify when and where these database types will be most successful.

The use of isotopic analysis for the purposes of forensic identification has become a focus in recent years. As more isotope databases are constructed it is critical to understand the pitfalls and limitations of different types of databases and to recognize the influence that country of origin may have on successful identification. This presentation will impact the forensic

science community by opening a dialog on isotope databases to maximize the creation of successful isotopic identification and encourage the increased use of this powerful identification tool.

Isotope ratios in teeth, bones, hair and nails have been analyzed by archaeologist and forensic scientists to investigate patterns of residential mobility and region of origin. Much of the isotope work coming out of the forensic anthropology community has utilized samples of known origin for identification comparison (Juarez, Regan).<sup>1,2</sup> In 2011, a comparative isotope database documenting ( $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ , 87/86Sr) for 10 states in Mexico was completed. In its initial form, this database focused on political separations for samples of known origin as a comparative method.

Recently, the focus on isotope identifications has begun to turn towards predictive modeling (Ehleringer).<sup>3</sup> Ehleringer initiated the development of a model to predict the geographic region of origin for humans living in the United States based on the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values of scalp hair.<sup>3,4</sup> The most recent results from this research suggest that database comparison studies have limited predictive power when compared to predictive model studies (Ehleringer).<sup>3</sup> In principle, this claim has significant merit when the robustness of available models are analyzed and compared. The Ehleringer *et al.* predictive model achieved an overall 86% correct success rate between observed and predicted region of origin locations for training samples.<sup>4</sup> This is compared to a variable rate of success for the comparative model of modern Mexican populations, which achieved 46-86%. In practice, the difference in robusticity between the predictive and the comparative models and the sample regions suggests some interesting trends that merit further analysis. First, the predictive model based on U.S. populations suggests that oxygen and hydrogen isotopes alone have the power to discriminate between different regions of origin even for contiguous areas. This is in contrast to the results from the comparative Mexican data set, where  $\delta^{18}\text{O}$  values alone separate the Mexican states involved into only two major groups, with  $\delta^{18}\text{O}$  values contributing only minimally to the discriminant function. Individuals that had a close relationship to the local water mainly represented the samples in predictive model. This relationship was not naturally occurring in the Mexican population studied, and it is clearly documented that Mexican populations have the highest bottled water consumption in the world. In addition, the possible range of tap water  $\delta^{18}\text{O}$  values in the predictive model was much larger (-5 to -20‰) than the range of precipitation values in the Mexico sample (-2.9 to -10). This suggests that the sample areas themselves may not be of equal quality and that Mexico may not be an ideal location for region of origin discrimination on the basis of oxygen.

Second, the Mexican comparative model had a more significant problem with misclassifications than the predictive model. The Ehleringer model is a predictive isoscape model. Isoscapes provide expected isotope values for a given geographic coordinate. This method is useful for studying processes that lead to changes in isotope values over space. Essentially this model type can provide a sense of the extent to which distributions of isotope values from different locations are the same. This is something that does not happen with a comparative model.

This presentation will impact the forensic science community by opening a dialog on isotope databases to maximize the creation of successful isotopic identification and encourage the increased use of this powerful identification tool. As more isotope databases are constructed it is critical to understand the pitfalls and limitations of different types of databases and to recognize the influence that country of origin may have on successful identification. In certain locations like Mexico where the relationship between local food and water intake is complicated by the pervasive use of bottle water comparative models offer valuable insight. The study comparison suggests that the best models take into consideration the unique limiting factors in each given situation and push for careful analysis of a given situation and the creation of a flexible hybrid database when and where appropriate.

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#### Isotope, Database, Identification

### H103 Unveiling Ancestry in Colombia through Morphometric Analysis

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After attending this presentation, attendees will understand the complexity of accessing ancestry in Colombia given its heterogeneous population history. In addition, attendees will learn that recent analyzes of a documented modern Colombian skeletal collection have initiated a quantitative approach to understanding variation in Colombia.

This presentation will impact the forensic science community by presenting results on the first geometric morphometric ancestry study in Colombia. In addition, this presentation will contribute to recent research that has attempted to quantify population variation in Latin America.

Colombia is a multiethnic and multicultural country where various human groups of European, African, and Indigenous ancestry have intermixed and therefore shared the social, cultural, and biological characteristics of their place of origin. The concept of ancestry in Colombia is more complicated than in many other countries as a result of the particular demography, history, anthropology, and genetics of its inhabitants. Genetic research has found marked mtDNA differences among contemporary Colombians, indicating numerous geographic haplotypes, all of which have contributed to the regional division of the country's population.<sup>1</sup> To date, these differences have not yet been compared to the morphological and morphometric cranial features of the same populations.

In forensic anthropology, ancestry estimations have traditionally and generally been done on the basis of physical anthropological principles, such as craniofacial morphology. This morphology has been used to create categories of different groups of human beings, showing that there are perceptible metric and morphological differences between populations. Traditionally-used categories (European, African, and Asian) may correspond to the social categories used for ancestry in Colombia (Indigenous, African-Colombian, and Mestizo). However, ancestry analysis in the forensic context has been based primarily on foreign population studies, which have recently sparked discussions on the credibility of these studies in Colombia.

This project is the first geometric morphometric characterization to study ancestry in Colombia. Based on Buikstra and Ubelaker, the craniometric landmarks of 127 adult individuals, 48 females and 79 males, were registered with a MicroScribe GT using the modern skeletal collection curated by the National Institute of Legal Medicine and Forensic Sciences.<sup>2</sup> The individuals in the sample came from five of the six natural regions of the country, mostly from the Andean area. The data obtained were processed with the 3D-ID software package in order to compare the

population categories generated by the program with morphological features of each cranium.

Not surprisingly, results from 3D-ID indicate a diverse array of potential population groups. For example, 13% of the sample was classified as having African descent, 34% were classified as having a European origin, 10% were classified as Mesoamerican, and 33% were classified as South American. We argue that these results confirm a heterogeneous population structure and are a good first step in unveiling ancestry in Colombia. It is hoped that through increasing the sample size and additional statistical analyzes (i.e., k-means cluster analysis), we might begin to better understand population variation in Colombia and contribute to discussions surrounding population variation throughout Latin America.<sup>3</sup>

This is a useful analysis in the forensic context because it is the beginning of a series of geometric morphometric studies of the modern Colombian population. It will facilitate comparisons with other Latin American samples as programs such as 3D-ID and FORDISC begin to incorporate these new data. It is our hope that in the Colombian forensic context, the human identification process and the estimation of ancestry will contribute to the accuracy of presumptive and/or positive identifications.

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#### Colombian Ancestry, Geometric Morphometrics, Population Variation

### H104 Univariate Sex Discrimination from the Postcranial Skeleton for a Colombian Population

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The goal of this presentation is to present a discriminant function analysis of the postcranial elements from a new human skeletal collection of known individuals in Colombia.

This research will impact the forensic science community in the development of population standards for sex discrimination in Colombia and South America. This research may help pave the way for future courtroom validation methods.

This research explores the best univariate indicators for sex estimation using measurements of the postcranial elements. In a recent study of African Americans and European Americans from the Forensic Database, the postcranial elements that demonstrated the most effective sex discrimination were the femur, tibia, humerus and scapula (Spradley et al. 2011). The hypothesis for the current study is that the population from Bogotá, Colombia will follow a similar pattern with the femur and tibia at the knee demonstrating the highest classification rates.

The sample consists of 134 individuals (50 females, 84 males) between the ages of 19 and 93 with a mean age of 47 years. The sample is part of a new collection, the Modern Colombian skeletal collection of Colombia, from cemeteries in Bogotá, Colombia. These skeletons are curated at the National Institute of Legal Medicine and Forensic Sciences (INML y CF). The collection of known individuals includes data on age, sex, stature (as reported on identification cards) and place of birth. The

collection represents remains that were either unclaimed after a four-year burial period and were collected before becoming comingled in a common grave. All individuals died in 2005. The methods include discriminant function analysis and univariate ANOVA using SPSS. Only the bones of the left side of the body were included in the analysis in order to lessen the effect of handedness and potential occupational markers, especially in the upper limb.

The results for this Colombian population indicate the same general pattern of classification effectiveness as seen in the North American sample using the univariate ANOVA and cross-validated discriminant function analysis. The humerus performs slightly better than the distal femur and proximal tibia as demonstrated in the North American sample. The percent correct classification for the postcranial elements ranges from 72.9% to 92.1%, with all of the humeral measurements correctly discriminating sex more than ninety percent of the time ( $p$ -value < 0.00). The highest classification rate was for the humeral head diameter (92.1%), the humeral epicondylar breadth (91.4%) and the humeral maximum length (90.7%). Only one femoral measure achieved just at 90.0% correct classification, which was the femoral midshaft transverse diameter.

In conclusion, these univariate results for the femur and tibia are very similar to the previous study by Spradley et al. (2011), but the higher discrimination in the humerus approaches some of the higher multivariate success rates achieved in the same study. This could be extremely useful for identification, as the humerus is more resistant to taphonomic properties than many of the bones. Furthermore, the ability to achieve such a high degree of success from a single bone is more efficient and thus preferable for the fast paced forensic laboratories in Colombia that see hundreds to thousands of forensic anthropology cases each year. This research will potentially play an important role in the development of population standards in Colombia and South America. This research hopes to help pave the way for courtroom validation methods not currently required in the Colombian medicolegal system, but validation methods will likely play an important role in the near future.

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**Sex Estimation, Postcranial Skeleton, Colombia**

## **H105 Sexual Dimorphism in Argentinean Populations: A New Approach**

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After attending this presentation, attendees will gain a better understanding of the expression of sexual dimorphism in Argentinean populations, as well as to explore a new methodological approach for the examination of inter-group differences

This presentation will impact the forensic science community by contributing to knowledge of the nature and magnitude of sex variation in Argentinian populations. These results will affect the way in which anthropologists interpret sex differences from the skeleton.

The assessment of sex is the first analysis performed on an unknown skeleton. Due to different environmental and genetic factors, the osteometric proportions of a skeleton vary significantly within and between populations. Thus, it is not possible to devise universal formulae and standards for evaluating sexual dimorphism. Population specific standards need to be constructed for each group, not only for improving forensic analysis, but also for establishing a better understanding of the patterns of sexual dimorphism. Generally, males are larger and more robust than females. However, researchers have yet to determine which suite of measurements express the greatest amount of sexual dimorphism in the human skeleton.

The purpose of this research is two-fold. Firstly, to obtain population specific standards for an Argentinean population using discriminant

function analysis (DFA). Secondly, to explore the idea that if allometry is statistically detected and describes the shape variable which covaries with size, then explanations may be found in terms of growth and biomechanics. Once these patterns are found in one population, several comparisons of sexual dimorphism can be made between populations. From a methodological perspective, differences between sexes can be analyzed through DFA, and as shown in this research, by principal component analysis (PCA).

The sample is comprised of 124 unidentified adult skeletons (64 female and 60 males) which were exhumed from cemeteries in the Buenos Aires area by the Argentinean Forensic Anthropology Team (EAAF). Sex was ascertained through DNA, though positive identifications are still pending. The skeletons were buried between the years of 1976 and 1979.

Standard anthropometric post-cranial measurements were taken and collected in a database. Variables selected through DFA were examined to explain their contribution to sexual dimorphism (females/males). In particular, whether they were expressing merely sex size dimorphism (SSD) or true sex differences in developmental patterns.

Univariate discriminant functions were constructed using direct methods for each of the metric measurements. Multivariate discriminant functions were developed for each bone using a stepwise procedure for all the dimensions.

When using univariate discriminant function, the breadth measurements from all the bones had the highest percentages of correct classifications. The humerus yielded four measurements with correct classification higher than 90%. When using multivariate discriminant functions, all elements within the study show correct classifications higher than 94%.

On the other hand, when pooled groups were analyzed through PCA, only one component was obtained. The traditional interpretation of PCs indicates that the main differences between sexes are size differences (SSD); this result is also supported by DFA. When eliminating the differences between groups, the differences within groups are expressed. Both DFA and PCA by group show that within-group allometric patterns are masked when using pooled groups. Group size differences often result in spurious correlations when multiple variables are considered. Therefore, the more size dimorphic variables enter both the PC1 and DF. This research shows that PC1 is including size, allometric shape, and also size free shape. From a geometric point of view, PCA and DFA are doing very similar things.

Among the Argentinean sample, DFA detected overall size disparity between the sexes rather than differences in allometric patterns, even though the latter do exist. When comparing populations, it is necessary to assess whether the group differences are related to allometric patterns or solely raw size.

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**Discriminant Function Formula, Principal Components Analysis, Allometry**

## **H106 A Radiographic Study on the Utility of Cranial Vault Outlines for Positive Identifications**

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The goal of this presentation is to explore the applicability of radiographs in medicolegal investigations.

This presentation will impact the forensic science community by highlighting the importance of biological variation and the utilization of radiographs in unidentified remains.

The visual and statistical assessment of the utility of radiographs for positive identification was examined in accordance with the *Daubert* and

the 2009 National Academy of Sciences Report, which calls for more testable and reliable scientific research. Currently, there has been a surge in research pertaining to morphological variation in the crania and post-crania for positive identification. The areas most explored include the frontal sinus, chest, and vertebrae. The availability of antemortem radiographs of particular areas of the body can be used for positive identifications in cases where other measures of identification cannot be employed. The utility of radiographs for medicolegal purposes is shown by the uniqueness of certain features of the skeleton in previous research; however, there is a need to quantify their uniqueness to appease the *Daubert* ruling and National Academy of Sciences Report. The purpose of this research is twofold: (1) to test the visual accuracy of positive identification in antemortem and postmortem radiographs of the lateral cranial vault outline among practitioners with different levels of forensic experience; and, (2) to use shape analysis (elliptic Fourier analysis) to evaluate the uniqueness of vault outlines and its applicability to positive identifications.

A sample of 90 individuals with varying levels of education participated in a visual accuracy test, which included: Ph.D. (n=34), M.D. (n=6), M.A. or M.S. (n=39), and B.A. or B.S. (n=11). Along with education, forensic case experience was also recorded, which included: none (n=13 or 14%), 1-10 cases (n=23 or 25%), <50 cases (n=25 or 27%), and >50 cases (n=31 or 34%). The visual test was comprised of left lateral radiographs taken from 14 crania (labeled A-O) representing the “antemortem” radiographs, and the “postmortem” radiographs were comprised of five randomly chosen crania from the same sample of 14 crania comprising the “antemortem” set that were radiographed a second time. Participants were asked to match the “postmortem” radiographs with the “antemortem” radiographs. Out of the 90 individuals, 38 (or 42%) correctly assigned all of the radiographs, with accuracy rates ranging from 70-93% for each of the five radiographic comparisons. Individuals with the highest level of education (PhD) and the lowest level (BA or BS) also had the most correct responses (45% correct) along with individuals with the highest forensic case experience (50% correct). Participants were also asked to list the skeletal markers that aided in their assessments and theinion hook (22%) and overall vault shape (21%) were listed as the most useful for identification.

Vault shape was compared among the 14 antemortem cranial vault outlines and the five postmortem vault outlines with geometric morphometric methods of contour shapes using the statistical program SHAPE 1.3, which performs an elliptic Fourier analysis. A principle component analysis was run on 30 harmonics to examine the variation in the morphological features of the cranial vaults. Paired t-tests were computed on the effective principle components to assess significant differences between each of the five antemortem and five postmortem radiographs and a two tailed t-test was computed between all of the antemortem and postmortem radiographs. The results indicate that there were no significant differences between any of the radiograph comparisons.

The visual comparison test and shape analysis was performed to evaluate the uniqueness of cranial vault outlines in radiographs, accuracy rates, reliability, and their utility in positive identifications. The visual accuracy test shows that the visual assessment of radiographs is not useful for positive identifications under the *Daubert* criteria due to the low accuracy rates (only one comparison had over 90% accuracy). However, education and experience did appear to affect the ability to correctly assign the radiographs. The shape analysis also indicates that vault outlines are not useful for positive identifications alone, although they could be used in conjunction with other features (such as the frontal sinus) for positive identifications.

#### **Cranial Vault Radiographs, Elliptic Fourier Analysis, Positive Identification**

## **H107 Identification of a United States Airman Using Stable Isotopes**

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After attending this presentation, attendees will become familiar with a recent identification of an individual using stable isotopic analysis of his dental remains.

This presentation will impact the forensic science community by raising awareness of the possibility of employing isotopic analysis in the identification of human skeletal remains.

In February 1969 a U.S. Air Force pilot was attacking an anti-aircraft artillery position in Savannakhet Province, Laos, when his F-100D Super Sabre was struck by enemy fire and crashed. Crewmen of three other American aircraft involved in the mission reported seeing no parachute or any other indicator that the pilot of the F-100 had successfully exited the aircraft or survived the impact. He initially was placed in the status of Missing In Action; however, a military review board subsequently amended his status to Killed In Action.

In 1991, 1992, 2005, and 2009 joint U.S./Lao People's Democratic Republic teams located and excavated the 1969 crash site, which was located within 100 meters of the reported historic map coordinates for the incident. Over 450 m<sup>2</sup> was excavated using standard archaeological procedures and the site yielded human remains, pilot related material evidence, and aircraft wreckage. The U.S. Air Force Life Sciences Equipment Laboratory (LSEL) confirmed that the life-support equipment found at the site was consistent with the pilot being on board at the time of impact, and in the LSEL's opinion, the crash was non-survivable. The remains consisted of a single crown fragment from a human left maxillary canine (tooth #11). The tooth was unrestored and lacked any morphological characteristics that would allow for individualization.

Analysis of stable isotopes in the enamel from the tooth fragment—specifically, carbon and oxygen—revealed typical Western values consistent with those seen in individuals raised in the United States. The permanent maxillary canine completes its crown formation by approximately age six; thus, isotopes present in the enamel reflect exposure (through diet) to these isotopic ratios during this early period of growth. Conversely, the isotopic values differed significantly from those seen in indigenous Southeast Asians. Based on this analysis, it was inferred that the human tooth fragment was that of a Westerner (e.g., American) and did not derive from an indigenous native to Southeast Asia.

In sum, the pilots of other U.S. aircraft involved in the same February 1969 mission witnessed the F-100D aircraft crash into a mountainside in Savannakhet Province, Laos. No parachute was observed and they believed the crash was not survivable. An aircraft crash site that was located within 100 meters of the incident map coordinates was located and excavated from 1991 to 2009. The site yielded wreckage exclusive to that of an F-100D aircraft; historical records indicate that there is only one F-100 that crashed within 40 kilometers of that location. Identification media purportedly found at the site by Laotian nationals living nearby consisted of two military identification tags that correlated to the pilot by name, service number, blood type, and religious preference. Based on aircraft type and location, the site was correlated to the 1969 loss of a specific F-100D aircraft to the exclusion of all other reasonable possibilities. Analysis of the tooth fragment found amid the wreckage field revealed it to be human. Isotopic analysis of the enamel excluded the reasonable supposition that the tooth was that of an indigenous Southeast Asian and thus precluded the probability that the tooth fragment was a site contaminant. Given the totality of the evidence, including the exclusion of all reasonable alternatives, the human remains found at the crash site were identified as

those of a U.S. serviceman who died in 1969 when his aircraft was shot down over Laos.

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**Forensic Science, Stable Isotopes, U.S. Military Identification**

## **H108 Ongoing Development of the Novel Computer-Assisted Radiograph Identification Method**

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After attending this presentation, attendees will receive a brief description of the ongoing development of the enhanced version of QMA<sup>®</sup> and fine-tuning of this quantitative approach to identification basis for the computer-assisted radiograph comparison method and the proposed practical application of the method in medical examiner/coroner offices. Milestones achieved in the development of the method in the previous 12 months will be discussed and plans for an imminent large-scale validation study will be outlined.

The presentation will impact the forensic science community by describing successes in the development and validation progress of a new practical identification method responsive to the National Academy of Science Report recommendations and post-*Daubert* evidence admissibility standards.

A statistically validated, time-sensitive and relatively inexpensive scientific identification method is under development for routine use in the medical examiner/coroner setting. The method is based on an enhanced version of “Quantitative Motion Analysis” software. QMA<sup>®</sup> has been validated in multiple clinically based peer-reviewed studies of spinal biomechanics and spinal treatments. The software allows for computer-assisted matching of specific skeletal elements, such as vertebral bodies, by tracking them through multiple radiographic images.

Forensic identification of unidentified or tentatively identified remains through comparison of antemortem and postmortem radiographs currently relies on a visual assessment by a forensic anthropologist. The accuracy of the method is dependent on the experience level of the anthropologist and the presence or absence of features traditionally believed to vary significantly among individuals. To objectify this process, QMA<sup>®</sup> was developed to quantify how well specific anatomic features “match” in a set of radiographs. The ultimate goal of the project is to validate an objective image matching system for forensic radiograph-based identification. The pilot study of this project began with the development of a processing algorithm that provided QMA<sup>®</sup> with the ability to successfully calculate the required quantification of the match, the “match score.”

Several different algorithms have been developed over the course of this project that can be used successfully to quantify a match score. There are also multiple ways to arrive at a composite match by combining match scores for multiple features in one set of radiographs. The final match score reported by the software must be scientifically validated and this requires a systematic method to find the optimal protocol out of the many available options.

The strategy of this study to identify the optimal protocol is to apply the various permutations of the available options to a large collection of radiographs and assess the resulting data based on the sensitivity and specificity for detecting a correct match. As a test, multiple options for preprocessing images and the various successful match score algorithms were applied to a collection of paired radiographs, some of which were correctly matched and others incorrectly matched. A spatial registration process was applied to the images prior to preprocessing to minimize

variability caused by the relative position of anatomic features within the radiographs. The preprocessing filters that were tested included histogram equalization, unsharp masking, and shadow enhancement filters. Testing of the match scores included mutual information, image correlation, Dice similarity, and the Jaccard coefficient methods. The resulting data were analyzed to identify the algorithms with the highest sensitivity and specificity for identifying correctly matched images. As expected, the type of preprocessing filter applied and the match score algorithm were significantly associated with the resulting match score (Correlation: sensitivity 71.43, specificity 77.78, *p-value* < 0.001). The sensitivity and specificity tended to be higher using the Dice (sensitivity 85.71, specificity 88.89) and Jaccard (sensitivity 92.86, specificity 88.89) match score algorithms.

The large number of potential preprocessing protocols available and the multiple options for calculating match scores in QMA<sup>®</sup> require an optimization scheme to identify and validate the optimal protocol for the computer-assisted identification method. The results of this study demonstrate that the optimization process can be completed using sensitivity and specificity data as the primary outcome measures, and that sensitivity and specificity are highly dependent on the combination of preprocessing steps and match score algorithm used. Testing of the optimization process is underway using large sets of example imaging until the sensitivity and specificity data converge to steady values in the optimal protocol for the matching process.

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**Radiograph Identification, Forensic Anthropology, QMA<sup>®</sup>**

## **H109 Virtual Anthropology: A Comparative Study of Real Bone vs. Virtual Bone Surface**

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After attending this presentation, attendees will understand the advantages and limits in applying anthropological methods on virtual bones.

This presentation will impact the forensic science community by demonstrating the future role of cross-sectional techniques for anthropological studies

In recent years, modern cross-sectional imaging techniques such as multi-detector computed tomography (MDCT) have pioneered applications in various postmortem investigations. Since 2008, MDCT is used in the routine investigation of all bodies examined by the University Center of Legal Medicine in Lausanne. This data pool is opening new opportunities in several research fields. Concerning forensic anthropology, problems, such as insufficient sample composition and lack of individual data, could be solved, if those data can be made available. For this reason, we started to create the anthropological data base of virtual skeletons of Lausanne. Consequently, basic research should focus on data comparability in order to understand limitations of applying conventional anthropological methods on virtual bones.

Preliminary studies in the field of virtual anthropology have shown that anthropological investigations can only be performed if the radiological data are of highest quality. Accordingly, special scanning protocols are important to gain sufficiently high resolution to investigate details. The

purpose of this study was to evaluate the application of anthropological-morphological methods of age estimation to 3D reconstructions of virtual bones and to develop appropriate scanning parameters.

Therefore, the reliability of the applied methods and their validity for MDCT data were tested. Eleven skulls and 21 hip bones, which originate from archaeological, anatomical and medicolegal collections, were examined by two experienced and four inexperienced observers. First, each bone was classified by conventional anthropological methods. In a second run, which was performed within an appropriate time lag, the virtual bones have been investigated using the same scoring system. The method applied on the hip bone is based on four traits of the sacro-iliac joint and the aspect of the iliac tuberosity. The method used for the skull is based on the 16 sections and five scoring stages of the ecto- and endo-cranial sutures. The bones were scanned on an eight-row MDCT using a high resolution (slice thickness of 0.625 mm). The 3D reconstructions were made on the CT-workstation.

Our study shows that, in general, estimating age of virtual bones shows a higher risk of false classification. While correlations between real and virtual bone-age estimations are quite sufficient in case of ecto-cranial sutures, the endo-cranial sutures and the sacro-iliac area are showing very low correlations. Two problems that interfere with the age estimation of virtual bones could be observed: The first is caused by areas of low bone density, which are not recognized by the software program of the workstation and which are therefore not calculated for the 3D image of the bone. This leads to a loss of substance during the 3D reconstruction that renders impossible its interpretation. Secondly, an automatic smoothing of the surface occurred during the reconstruction process. Thus, the examination of fine structures became infeasible and it affected the scoring of higher stages.

Besides, significant differences could be observed between the raters for estimations performed on virtual as well as on real bones. The reliability of estimates is increased by experience.

These results indicate firstly, that MDCT does not permit a sufficiently precise 3D reconstruction of the surface of dry bones in order to apply conventional anthropological methods. Consequently, the development of a special scanning protocol will be inevitable. Secondly, modifications of classical anthropological-morphological methods will be necessary for using the reconstructed surface of virtual bones.

**Virtual Anthropology, Bone Imaging, MDCT**

## **H110 Multidisciplinary Combination of Traditional and New Techniques in the Determination of Identity: The Utility of the Sphenoid Sinus Rediscovered**

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After attending this presentation, attendees will learn of factors limiting the usefulness of the frontal sinuses in identification of unknown decedents by radiological comparison. A model of multidisciplinary cooperation will demonstrate the utility of the sphenoid sinus for that purpose.

This presentation will impact the forensic science community by demonstrating how successful comparison of the sphenoid sinus or other structures difficult to image by conventional x-ray examination can be

achieved with CT equipment available in almost any community hospital or diagnostic center.

For 85 years the frontal sinuses have enjoyed the enthusiastic focus of forensic investigators as a prime site for identification by radiological comparison. The usefulness of other paranasal sinuses, particularly the sphenoid sinuses, has been largely ignored. Increasing acceptance and availability of CT as the primary method of imaging the skull promotes utilization of the more protected and, hence, most imperishable of the paranasal sinuses for the same purpose.

Boaters discovered a human skull on the tidal flats of the Delaware River just 300 yards south of the Port of Wilmington. The skull was removed from its original site to higher ground and the police were notified. The skull was discolored, covered and impregnated with gravely mud from the waterway. A portion of the skull had been damaged, and the mandible was missing. A single maxillary tooth (#2) was in place and contained an occlusal restoration. Photographs of the skull were sent to a physical anthropologist who provisionally reported them as an adult male, probably under 50, with possible Asian or Native American morphology.

There were several persons missing from aquatic accidents or suicides in the area. This description best fit a 43-year-old male who had fallen off a boat into a tributary of the Delaware River 38 months earlier—but he was listed as Black.

The skull was sent to a forensic odontologist along with dental radiographs of the possible decedent. The single tooth in the skull (#2) was compared with the antemortem radiographs. The occlusal amalgam restoration in that tooth had a similar radiographic outline consistent with a match but not sufficient to reach the level of positive dental identification.

The skull was then sent to the anthropologist who confirmed the initial assessment from the photographs as to age and sex, refining the age estimate to 35-46, and suggesting a postmortem interval less than five years. She reiterated that the cranium exhibited primarily mongoloid characteristics, supported with a FORDISC 3.0 osteometric assessment, although with very few (non-metric) African-American traits. In view of the racial ambiguity, she requested further interrogation of the presumed victim's family. They readily revealed that there was Native American ancestry in both maternal and paternal lines, and added that their missing relative had a CT examination of the head a year prior to his disappearance. This examination (actually a CT of the paranasal sinuses in the coronal plane) was compared with a frontal x-ray of his skull obtained by the anthropologist, who could not rule out the match. She suggested the case be referred for forensic radiology for possible identification.

The postmortem x-ray and the antemortem CT were examined by forensic radiologists who confirmed some similarity, but successfully argued the necessity of a postmortem CT of the skull. Meticulous positioning of the skull enabled reconstruction of images to almost exactly replicate the antemortem CT, which produced many compelling, matching features, particularly in the sphenoid sinuses and, finally, an unequivocal positive identification.

This case is presented as a model of multidisciplinary cooperation and tenacity. We believe it is the first successful identification of an unknown body by comparison of the sphenoid sinuses on CT images of a skull when visualization of frontal sinus by conventional radiology was compromised.  
**Identification, Computed Tomography, Sphenoid Sinuses**

## **H111 Forensic Anthropology and the Art of DNA Sampling**

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The goals of this presentation are to outline the best skeletal sampling strategies for forensic DNA analysis and the importance of basic genetic

knowledge when working within a field that strives for the positive identification of human remains.

This presentation will impact the forensic science community by discussing the benefits and advantages of various ways to sample the human skeleton for DNA analysis, the elements of the skeleton most likely to yield subsequent DNA profiles and how depositional environment or alteration of the remains may impact the ability to produce a DNA profile for positive identification.

The use of DNA technology for the positive identification of skeletonized and decomposed remains has become increasingly relied upon within the forensic community. While the forensic anthropologist plays an essential role in estimating descendant characteristics from skeletonized remains, such as age-at-death, biological sex and ancestry, as well as discerning peri-mortem trauma, estimating the postmortem interval and taphonomy, the actual positive identification is often done by the forensic DNA analyst. Therefore, the positive identification of human remains is most often a collaborative process incorporating multiple specialties within forensic sciences.

The process of obtaining analyzable DNA from bone is a destructive, timely and costly process. As experts in the human skeleton, forensic anthropologists are often responsible for the selection of skeletal elements for DNA analysis or consulting with coroners and medical examiners on appropriate samples for successful DNA profiling. Choosing the appropriate area of the skeleton to sample or amount of bone to be submitted for DNA typing can influence the success of subsequent examinations. It is important for the consulting anthropologist and DNA analyst to recognize how different sampling strategies influence subsequent osteological analysis, as some strategies involve cutting and removing large portions of the bone or sending entire skeletal elements for genetic analysis and therefore losing landmarks for discrete or metric investigations. In addition, sampling strategies can influence subsequent DNA analyzes as some skeletal elements can be more or less likely to produce genetic profiles based on intrinsic factors of the bone itself. Lastly, environmental factors such as heat and moisture play a large role in DNA preservation and having a basic working knowledge of those factors that are detrimental to DNA preservation may save time and money on fruitless genetic analyzes in situations where no analyzable DNA is likely to remain within the bone.

The goal of this presentation is to explore differences in basic DNA knowledge between forensic anthropologists working within different institutional contexts, such as government versus academic organizations and highlight the frequency at which forensic anthropologists are sending skeletal samples for genetic analysis. The different sampling strategies performed by practicing forensic anthropologists will be presented along with a discussion of the benefits and disadvantages of each technique. The intrinsic and extrinsic factors influencing molecular taphonomy will be discussed within the context of recent research and case studies to highlight the importance of a working knowledge of DNA and genetic technologies for a practicing forensic anthropologist.

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**Forensic Anthropology, DNA Sampling Strategies, Genetic Profile**

## **H112 Anthropological Facial Approximation in 3D (AFA3D) – Computer-Assisted Estimation of Facial Morphology Using Geometric Morphometrics**

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The goal of this presentation is to describe a new method that produces three-dimensional (3D) facial approximations using geometric morphometrics.

This presentation will impact the forensic science community by proposing an objective tool for facial approximation based on statistics and craniometrics.

According to the Scientific Working Group for Forensic Anthropology (SWGANTH), the aim of facial approximation is to estimate the antemortem facial appearance, suggest the identity of the person, and capture public attention. This study seeks to improve accuracy and reliability of the facial shape estimation process. Quantitative evaluation of the morphology of both facial features and soft tissue depths (STD) was performed through 3D measurements of Computed Tomography (CT) exams.

Five hundred CT-scans of French adult subjects (known age and sex) have been collected in medical centers after approval of ethical committees (265 males, 235 females; age range: 18-96 years; mean=52; sd=20). DICOM files were treated with TIVMI (Treatment and Increased Vision for Medical Imaging, developed by Bruno Dutailly, UMR 5199). This software (freely downloadable at <http://www.pacea.u-bordeaux1.fr/TIVMI/>) allows for the 3D surface reconstruction of both osseous and cutaneous faces of the patients, using the Half-Maximum Height algorithm. Cranial and facial landmarks (n=178) were collected using reference planes based on the Frankfurt Horizontal in order to enhance their repeatability and reproducibility.

Because the sample contained several partial exams (approximately 2/3 of superior and inferior faces for 1/3 of complete exams), eyes, nose, mouth and ears regions were studied independently. Geometric morphometrics offered objective form quantification in 3D through Procrustes superimpositions; asymmetry, influence of age, sexual dimorphism, and allometry were evaluated. Partial Least Square (PLS) analysis was used to evaluate the covariation between bone and skin matrices (landmarks configurations). Results allowed for the definition of four bony matrices optimally correlated with the facial organs matrices (eyes, nose, mouth and ears). Soft tissue depths were explored in parallel, which enabled the estimation of the corpulence of the 500 individuals, based on the literature. Regression formulas were elaborated using craniometrics, age, sex and corpulence, in order to predict subject-specific STD at 59 landmarks (right and left included).

The module AFA3D has been integrated to TIVMI. It requires the 3D coordinates of 78 skull landmarks that can be positioned directly in the module (on a surface reconstructed from CT or laser scanner). The landmarks may also be imported in AFA3D, if previously digitized. The user can specify sex, age (under or over 40 years) and corpulence (normal or overweight) if such factors are known. A geometric morphometric routine superimposes each facial region of the referential on the unknown subject, and computes Principal Component Analyzes on both osseous and cutaneous parts. Principal Component (PC) scores of the skull configurations of the unknown, along with the biological factors, are used to estimate the PC scores of the facial organs. PC scores are thus transformed into 3D coordinates with the PC coefficients to render the shape of the eyes, nose, mouth and ears independently. Once the STD predicted with the formula, AFA3D produces a total of 100 skin landmarks. A neutral synthetic face is then used (for more objectivity), and distorted according to the target landmarks. The distortion (developed in collaboration with the LaBRI, UMR 5800) allows for a smooth warping of the global face, and a more precise deformation at the anatomical landmarks around the facial organs.

Mathematical validation on 17 subjects is proposed, using a leave-one-out resampling. Each individual is thus treated after being excluded from the reference sample. A comparison of the standard error of the estimate induced by traditional facial reconstruction guidelines indicates that the approximation performed by AFA3D enhances the accuracy of the technique. Theoretical reliability is also assessed, if the cranial morphology of the subject falls into the variability of the French population.

AFA3D produces an estimation of the face shape, which is an objective basis for facial approximation. The next step, suggesting identity of the person, may be performed with computer-assisted forensic art. The 3D surface can be exported to other software, refined, and individualized according to the case investigated, before public diffusion. Further tests will have to be performed in order to validate the resemblance and the recognition potential of faces approximated with AFA3D.

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**Facial Reconstruction, Facial Reproduction, TIVMI**



ATLANTA 2012

# PSYCHIATRY & BEHAVIORAL SCIENCE



ATLANTA 2012

## II The Cowl Does Not Make the Monk: A Case of Sexual Abuse Committed by a “Bogus Priest” on a Group of Minors

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After attending this presentation, attendees will have a better understanding how psychological damage to the developmental processes in minors is much more severe when the abuser is a significant figure who is emotionally tied to the victim, especially when the abusers are parents. In this particular instance, the abuser was a bogus priest who, in a sense, was a parental figure. The fact that he was not even a genuine priest served to further amplify the victims’ feelings of confusion and betrayal.

This presentation will impact the forensic science community by highlighting the concept that sexual abuse committed by a priest, albeit a bogus one in this case, is just as psychologically damaging to a child as sexual abuse perpetrated by a close family member.

**Introduction:** It seems paradoxical, but the more rational a society becomes, the more its need for spirituality grows. The phenomenon of magical religiosity is not associated with any particular social class, but may be found in all social strata of the population. People often turn in this direction at times when they are unable to face negative life events. Unscrupulous criminals, who take advantage of such weaknesses and problems of others, exploit these characteristics of fragility. The case presented demonstrates this concept.

**The Case:** The subject is a 53-year-old man who is legally declared as blind, and who has various previous convictions for fraud and sexual abuse on minors. He would convince people that he was a Catholic clergyman and organized masses and personal appearances in which messages from God would supposedly come through him. In addition to overseeing two religious centers where he would gather groups of the “faithful” who believed in his visions, he would also make visits to people’s homes in order to pray and perform religious rites, as well as to offer his assistance in order to help them with their various problems.

The case of this “bogus priest” came to attention following new allegations of sexual abuse of five juvenile males; four of them belonging to one family (ages 10, 13, 14, and 17), and the other, their 14-year-old-cousin. The minors belonged to families with a multitude of problems resulting from economic hardship and relational difficulties. They had come to know the “bogus priest” during prayer meetings. When the imposter had learned of the two families’ problems, he began to make “pastoral visits” where he would offer to host the boys in his sanctuary homes during school breaks and the summer holidays. He eventually requested that custody of the boys be given over to him. He reported the families’ difficulties to social services in a manipulative way. He also stated that the boys had been sexually abused and neglected, but some of the investigations into this alleged abuse had brought his true identity to light.

Judicial investigations revealed that the boys had been the objects of sexual abuse at the “priest’s” hands over a period of time. It was found that these episodes had occurred during prayer, at confession, and when receiving spiritual guidance. The victims recounted stories of a well-planned strategy by the abuser. In addition to isolating them from their families and their home environments, the “bogus priest” touched them on their genitals, asked them to perform sexual acts on each other, tried to engage in oral sex with them, masturbated them, showed them

pornographic films, and asked them very intimate questions during “phony confessions.” The fake clergyman also gave the boys money in order that they not reveal what had happened. Moreover, he threatened them, saying that if they divulged what had transpired; they would be institutionalized and would lose all contact with their families. In order to prevent the parents from speaking to each other, or with social service representatives, he spoke badly to each one about the others, thereby creating isolation and conflict between the families. After being exposed, the “bogus priest” was arrested and found guilty.

**Conclusions:** The literature, as well as clinical and rehabilitation experience on juvenile victims of sexual abuse, all demonstrate that the psychological damage to developmental processes on minors is much more severe when the abuser is a significant figure who is emotionally tied to the victim, particularly mothers and fathers. For this reason, intrafamilial sexual abuse is particularly serious and harmful to a child. It can be hypothesized that a priest is perceived as a father inasmuch as children have fewer instruments to separate the concept of God the father from a concrete figure who represents him. Therefore, it is probable, that abuse by a priest would result in wounds just as deep as those caused by a family member. In this case; however, the damage is double because the perpetrator in question was not even a real priest. For a long period of time he presented himself as genuine to the children. For this reason it is concluded that beyond the psychic damage caused, the associated feelings of confusion and betrayal are amplified.

### **Intrafamilial Sexual Abuse, Bogus Priest, Psychological Damage**

## II Female Sexual Offenders: Five Italian Case-Studies

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After attending this presentation, attendees will understand how little the subject of sexually abusive mothers is studied and that a more comprehensive exploration of this offender population is needed in order to more appropriately understand and classify female child sex offenders.

This presentation will impact the forensic science community by helping to increase sensitivity and awareness of the phenomenon of female sexual abusers.

Historically, there has been an assumption that women do not commonly perpetrate acts of sexual abuse against children (Wakefield & Underwager, 1991). Recent interest in women offenders has brought attention to the issue, and challenges the perceptions regarding women who abuse children. The growing interest in the topic of female-perpetrated incidents of sexual victimization has produced some empirical research on the subject. A brief search of the current literature also yields a few published case studies, consisting primarily of reports on personality characteristics and developmental information from female perpetrators.

Although this “provides a necessary starting point in understanding female sexual abuse perpetrators” (Wakefield & Underwager, 1991, p. 56), the findings from this data should be seen as preliminary, and may not describe the full range of women involved in the perpetration of sexual abuse (Grayston & De Luca, 1999).

Women who sexually abuse minors are rare and case histories are scarce; even less is known about sexually abusive mothers. Current data suggests that females are responsible for only a small percentage of sexual offenses against children in the general population, and men remain the most common perpetrators of child sexual abuse. Unlike male offenders, however, females can often disguise sexual offending by performing normal daily activities associated with childcare (i.e., affection, bathing, and dressing). Their behavior may appear to be nothing more than excessively protective mothering. Moreover, mothers may also commit more overt and highly eroticized, seductive behaviors, even going so far as to bestow the role of “lover” upon the child. Due to the low number of cases in which women sexually abuse minors, more in-depth studies on this subject are needed. The clinical files of five women who are currently serving time in Italian prisons for the sexual abuse of minors are presented here. The cases involved varying degrees of participation in the abuse by the mothers, and ranged from active sexual engagement to allowing others to abuse their children. Records of the abuse cases and other records were utilized. In three cases, the women revealed an antisocial personality disorder (ASPD), while in two cases a borderline personality disorder (BPD) was diagnosed according with DSM-IV criteria.

While existing studies of female child sex offenders provided a range of insight and knowledge regarding women who sexually abuse, the current literature does not adequately represent the full spectrum of female-perpetrated child victimization (Grayston & De Luca, 1999). Considerably more well-documented empirical research is required to guide law enforcement and clinical professionals in their understanding of female sex offenders. In recent years, several preliminary typologies of female sex offenders have begun to emerge in an effort to more clearly specify characteristics, dynamics, and offense patterns of female sexual perpetrators. However, the current review has shown that most female sex offenders fall into several suggested typology models. Therefore, the existing typologies used to describe these women are insufficient. It is recommended that more comprehensive exploration of this offender population continue in order to more appropriately understand and classify female child sex offenders.

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#### **Female Sexual Offenders, Sexually Abusive Mothers, Cycle of Abuse**

### **13 Violence and Intimacy-Seeking in a Female Adolescent Stalker**

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After attending this presentation, attendees will recognize characteristics and nature of stalking by juveniles.

This presentation will impact the forensic science community the role of family and psychosocial factors in the etiology of stalking among juveniles.

The case presented here is of an adolescent female stalker who came to attention when Juvenile Court of Justice requested an expert opinion. This case led to reflection on the characteristics and peculiarities of juvenile stalking as compared to the adult phenomenon. As is well known, Mullen (2000) classified adult stalkers as intimacy-seekers, rejected, incompetent, resentful, and predatory stalkers. The only systematic study in a large sample of adolescent stalkers (Purcell et al. 2009) introduced the

classification of this stalking as an extension of bullying, retaliating stalkers, rejected stalkers, disorganized and disturbed stalkers, predatory stalkers, and intimacy-seeking stalkers. According to this study there are substantial differences between adult and adolescent stalkers. Firstly, there is a greater prevalence of female stalkers than in the adult population. Again unlike the situation in adult stalkers, in which the “rejected” stalker seeking revenge is the most common, among adolescents stalking as an extension of bullying seems to be the most prevalent, the only motive seeming to be the desire to intimidate and torment the victim. Moreover, whereas among adult stalkers, the greater the prior intimacy between the stalker and his/her victim the greater the risk of violence, among adolescents the category of disorganized and disturbed stalkers seems to show the greatest degree of violence. These youngsters are unhappy, angry adolescents at war with the whole world. Various studies (McCann, 1998; Vaidya et al. 2005; Purcell et al., 2009; Evans & Meloy, 2011) have reported specific features of adolescent stalking, also based on case reports. The victim is most commonly a peer, although Purcell (2009) stated that for adolescent disorganized and disturbed stalkers, the victim is generally an adult. Another important point made in the same study is that among adolescent stalkers, females are statistically significantly more prone to involve accomplices in their intimidatory behavior. In literature, the data were not sufficient to distinguish a single category in which to classify the case observed, which led to further exploration and examination of the correlation between stalking and pathological attachment. In this regard, various authors (Devis et al., 2000; MacKenzie et al., 2008; Kienlen et al., 1997; Langhinrichsen-Rohling et al., 2000; Meloy, 1996) have shown that insecure attachment is highly correlated with persecutory behavior. In fact, often in the infancy of stalkers the reference attachment figure was lost or parental negligence was present for various reasons: death, a jail sentence, drug addiction, or mental disease of a parent, so the child was handed over to other family members or community facilities (Meloy, 1996). In this sense, it can be claimed that, the stalking could be interpreted as an exaggerated form of protest against the loss of the attachment figure. Another possible explanation is seeking approval from the most important attachment figure, to reinforce the subject’s lack of self-esteem (Dutton, 1995).

In this case, a 16-year-old girl in the second year of high school came in contact with the new math teacher. From March 2010 until she was taken in custody in February 2011, she perpetrated a series of intimidatory and violent actions against the man and his family (unwelcome phone calls, insults, threats, molesting behavior, lurking, burning the car, damaging property, and slander, to include social networking sites). During this period, despite the intervention of the police and warnings from the headmaster, she continued the persecution which became progressively more violent as the victim was seen to be “indifferent” to her advances. It should be noted that these actions were often carried out in the presence of peers. The stalker was diagnosed with a borderline intelligence and came from a multi-problem family assisted by social services. Her mother was diagnosed with a delusional disorder, her father, an ex drug addict with a criminal record, was out of stable work. Related to the precarious family situation, the young girl had been living together with the two youngest of the five siblings at a community home for minors since the age of 11. The stalking began while she was living in this community.

In this case, in agreement with the literature, accomplices were involved in this case of adolescent harassment but the choice of victim was less common, being an adult. In literature, violent stalking is largely reported in adolescent disorganized and disturbed stalkers. In this case, instead, the young girl’s primary motivation was a desire to establish a relationship with the victim (an “intimacy-seeking stalker”). To gain a better understanding of the complex variables involved in juvenile stalking it is important to explore the psychological, family, and social factors that play so important a role at this age.

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#### **Juvenile Stalking, Violence, Family and Psychosocial Factors**

## 14 Predictors of E-Therapy and Face-to-Face Therapy Use for Internet Users: Personality, Attitude, Gender, and Ethnicity

Janise M. Pratt, PhD\*, Janise M. Pratt, PhD Forensic & Media Psychology, PO Box 9373, Laguna Beach, CA 92652

After attending this presentation, attendees will be provided information about how personality and dysfunctional thinking relate to an individual's attitude about seeking assistance for psychological distress. Further, the presentation will provide new knowledge about what types of individuals would be more inclined to seek professional assistance online versus those who prefer traditional face-to-face therapy arrangements. The study's findings hold important implications in terms of addressing the problem of underutilization of mental health services among neurotic individuals despite there being an increase in the provision of these services. Exacerbation of symptoms as a result of not receiving professional help can result in suicide attempts and violence directed at others. This presentation will help to inform professionals about how e-therapy services are perceived and the types of clients that may be well suited for e-therapy services.

This presentation will impact the forensic science community by showing how the findings potentially provide prospective research that focuses on the wide-spread problem of underutilization of mental health services by those who need help the most and how personality and attitude variables play a role in this problem. E-therapy is proposed to offer a viable alternative for individuals who find face-to-face therapy arrangements too uncomfortable due to the symptoms of their mental disorder and may provide a way to reduce suicide attempts as well as violence toward others among those who are psychologically distressed.

**Hypothesis:** This study explored whether attitudes toward seeking professional help in face-to-face therapy (Attitude Toward Seeking Professional Psychological Help; ATSPPH, Face-to-Face Counseling Attitudes Scale; FCAS) and e-therapy formats (Online Counseling Attitudes Scale; OCAS, Pratt Survey Instrument) could be predicted by personality traits (NEO Personality Inventory-Revised; NEO PI-R), dysfunctional attitudes (Dysfunctional Attitude Scale; DAS), gender, and race.

**Methods:** This study utilized a non-experimental study design to examine attitudes toward seeking professional help in traditional face-to-face format and with various forms of e-therapy. Personality and dysfunctional attitudes were also measured. The design employed self-report measures to assess these areas of interest. The participants for this study were recruited exclusively from an online research panel maintained by United Sample, Inc. drawn from a pool of adult internet users who were recruited using a controlled by invitation only method to comprise a sample that closely parallels the U.S. population proportions on all key profile characteristics ( $n = 199$ ).

**Results:** Data were analyzed utilizing hierarchical multiple regression. Results revealed that gender (male) and the dysfunctional attitude, cognitive imperatives (DAS) were inversely related to recognition of need for professional help (ATSPPH). Analysis of the data also revealed that race (Latino), the dysfunctional attitude, avoidance of appearing weak (DAS) were inversely related, and the personality trait, openness to experience (NEO PI-R) was positively related to interpersonal openness regarding one's problems (ATSPPH). Further, results showed that openness to experience and self-consciousness (NEO PI-R) were positively related to online counseling attitude (OCAS) and extraversion (NEO PI-R) was positively related to face-to-face counseling attitude (FCAS). Standard discriminate analysis revealed that those who prefer some form of e-therapy are likely to be low in extraversion and high in openness to experience and self-consciousness.

**Conclusion:** This study adds clarification about what personality and dysfunctional attitude variables relate to attitudes toward seeking

professional help in e-therapy and face-to-face formats. This study sets forth a direction for future research to parallel the explosion of the online universe in a necessary effort to reach countless individuals in need of professional assistance.

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**Help-Seeking Attitudes, E-Therapy, Online Psychotherapy**

## 15 Cybertherapy: Fad or Future of Mental Health Care?

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The goals of this presentation are: (1) to present and discuss the technology of cybertherapy and other technological means of communication that are being used more and more in mental health care; (2) to understand the uses of these technologies and the ethical issues involved in them; and (3) to discuss the responsibility of forensic psychiatrists and other health professionals in providing standards for this technology that seems here to stay.

This presentation will impact the forensic science community by underscoring the ethical issues and the impact of cybertherapy on mental health care today.

Cybertherapy is a technology that has been around for over a decade and is becoming increasingly more mainstream, as evidenced by a new Showtime show called "*Web Therapy*." It is important that clinicians as well as forensic scientists understand this technology because it is being used more commonly around the country and the world. This presentation will discuss the literature on cybertherapy including the treatment outcomes, and the ethical considerations involved with using this remote form of therapy as a treatment for mental health issues. The ethics of cybertherapy have been addressed as early as 1996 in an article in *Ethics and Behavior* called "*Case vignette: Cybertherapy*" where a case is described in which someone across the country from the web therapy doctor threatens suicide and hangs up.<sup>1</sup> Ethical issues also include going beyond one's area of expertise, and issues of confidentiality. The ethical and boundary issues presented by cybertherapy are important concerns and need to be constantly considered as the technology is growing and taking over some parts of the U.S and the world. Therapy over the internet may become more cost effective during economic crisis, and these considerations for some may potentially outweigh ethical issues and treatment considerations.

For over a decade, clinicians have been relying more and more heavily on other means of communication besides traditional face-to-face and telephone contact. These include email, texting, videophone, and the use of cybertherapy. These tools have both benefits as well as possible detriments to the clinician-patient interaction. It is important for clinicians, researchers, and forensic scientists to understand the technology and its impact on the psychiatry and psychology as it is practiced today. An Italian study entitled "New tools in Cybertherapy: the VEPSY web site" discusses this concept and provides a framework for the integration of old and new technology in mental health care.<sup>2</sup> When reviewing the literature, it is clear that there is a lack of standardization and professional leadership in the field of cybertherapy and the use of technology in medicine, particularly in mental health care.

Experiences in the United States and in Italy will be compared. Arguments for and against the technology will be addressed and discussion among the audience will be encouraged. Because the technology is constantly in flux, it is important for forensic psychiatrists and other health care professionals to have knowledge and a voice in the direction that cybertherapy as well as other forms of communication through technology is taking. The ethical issues of using technology in psychotherapy and other mental health treatments will be presented and discussed. Research possibilities and future areas of interest in this field will also be addressed.

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## Cybertherapy, Technology, Ethics

## 16 Update on Forensic Considerations for Gender Non-Conforming Individuals

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After attending this presentation, attendees will be familiar with terms and definitions associated with gender non-conforming, transgender, and intersexed individuals. This presentation will also provide understanding of the psychosocial stressors specific to this population by identifying the intersections of this population with the criminal justice system and understanding the unique dilemmas facing gender non-conforming individuals in confinement.

This presentation will impact the forensic science community by raising awareness about a particularly vulnerable, marginalized, and victimized inmate population within the corrections system. By raising awareness in the forensic community, efforts can be mobilized to develop empirically driven studies to better understand the experience of gender non-conforming individuals in confinement, which can then result in thoughtful and humane policy change.

Homosexual and bisexual individuals have moved over the last four decades from marginalization in society into mainstream American society. Same sex marriage is legal in six states and many states have non-discrimination laws. Psychiatry has also changed its assessment of gays and lesbians with the removal of the former diagnosis of homosexuality as being pathological. Many NATO countries permit gays and lesbians to be open members of their armed forces and the United States has taken steps to make this transition too. Gays and lesbians still experience discrimination and victimization in the correctional settings, but even here there is progress. Gender non-conforming individuals; however, have not experienced this progressive acceptance in society and certainly not in the correctional setting.

Gender non-conforming individuals merit special consideration in correctional facilities to preserve human dignity and equal protection under the law. There is very little empirical data on the experience of gender non-conforming individuals in confinement, in large part because correction systems do not collect data on the number of gender non-conforming individuals in their institutions or the experiences of these individuals while in confinement. However, there have been legal cases that have documented the targeted violence perpetrated against this population, as well as gender-related medical discrimination while in confinement. Large, empirically driven studies are needed to better characterize the experience of the confined gender variant individual.

In the community, gender non-conforming individuals are a marginalized population. They face discrimination in housing, employment, education, and have decreased access to healthcare. Because of this pervasive discrimination, these individuals are also disproportionately poor, homeless, criminalized, and imprisoned. Moreover, they are at increased risk for depression, anxiety, and substance abuse. The most up-to-date epidemiological findings regarding the psychosocial stressors of gender-non-conforming individuals will be reviewed. While incarcerated, discrimination and distress related to their gender variance is exacerbated.

Correctional facilities are sex segregated according to a prisoner's birth sex and/or genitalia. As such, gender non-conforming individuals are placed in facilities where their gender identification is not recognized and where they are visible and frequent targets of violence and discrimination by other prisoners and correctional officers. These individuals have unique medical needs to maintain their gender identity, which may include hormone treatment and sex re-assignment surgery. These treatments may not be readily available in the correctional setting, which may result in severe and profound psychological suffering on the part of gender non-conforming individuals. This presentation will review the current case law regarding the gender non-conforming individual's right to protection from violence, as well as their right to medically necessary treatment.

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## Transgender, Gender, Incarceration

## 17 Minimizing Malpractice Exposure in Plastic Cosmetic Surgery

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The goals of this presentation are to understand psychiatric issues in cosmetic plastic surgery, recognize patient groups that tend to be the most satisfied with cosmetic plastic surgery procedures and their motivations to seek surgery. This presentation will also recognize psychiatric conditions that prevent patients from achieving satisfaction with their cosmetic plastic surgery and recognize patient groups that tend to be the most litigious.

This presentation will impact the forensic science community by identifying and understanding the psychiatric issues in plastic surgery which will help surgeons identify patients that are suffering from or are likely to suffer from Body Dysmorphic Disorder/minimal defects, personal crises, multiple revisions, and loss of identity. These patients may be unable to achieve satisfaction with the result of their cosmetic plastic surgery procedures. Limiting exposure to these patients will minimize liabilities a cosmetic plastic surgery practice is exposed to.

The most common reason for litigation in cosmetic plastic surgery is patient dissatisfaction. Therefore, a physician must strive to accept only patients that are capable of being satisfied with a cosmetic plastic surgery procedure in order to limit the liability of a cosmetic plastic surgery practice.

The objective of cosmetic surgery is increased patient self-esteem and confidence. Most patients undergoing a procedure report these results post-operatively. With these goals in mind, the success of any procedure is measured in patient satisfaction. In order to optimize patient satisfaction, psychiatric literature suggests careful pre-operative patient preparation including a discussion of the risks, benefits, limitations, and expected results for each procedure undertaken. As a general rule, the patients that are motivated to surgery by a desire to align their outward appearance to their body-image tend to be the most satisfied. Patients that are not motivated by self-improvement tend to be less satisfied.

There are some psychiatric conditions that can prevent a patient from being satisfied without regard to aesthetic success. The most common examples found in psychiatric literature are Body Dysmorphic Disorder minimal defect, the patient in crisis, the multiple revision patient, and loss of identity. Patients that fall into these categories also cannot make fully reasoned decisions regarding cosmetic plastic surgery. This presentation will familiarize the audience with these conditions, symptoms, and related illnesses. Case examples from clinical practice are described and then explored in terms of the conditions presented. A discussion of the patient's motivation for surgery, goals pertaining to specific attributes, as well as an evaluation of the patient's understanding of the risks, benefits, and limitations of the procedure can help the physician determine if a patient is

capable of being satisfied with a cosmetic plastic surgery procedure. If each potential patient is screened, then the practice limits its exposure to a class of patients that tend to be the most litigious.

Plastic surgeons can screen patients suffering from these conditions relatively easily, as psychiatry is an integral part of medical school education. If a psychiatric referral is required, then the psychiatrist needs to be aware of the nuances of each of these conditions.

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**Plastic Surgery, Psychiatry, Malpractice**

## 18 Psychiatric Evaluations in Immigration Cases in the United States

*Vivian Shnaidman, MD\*, Jersey Forensic Consulting LLC, 812 State Road, Suite 220, Princeton, NJ 08540*

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After attending this presentation, attendees will be familiar with the types of immigration cases in which psychiatric opinion and testimony is utilized, and will learn how to apply their knowledge of psychiatry and forensic psychiatry in this growing niche field.

This presentation will impact the forensic science community by addressing one of the big issues in the United States and across the world today — immigration. This year's meeting theme, *Global Research: The Forensic Sciences Edge*, illustrates how small the world has become. The desire of individuals to move from one country to another for a variety of reasons makes this area of psychiatric expertise very current and important, and psychiatrists and other mental health practitioners should be aware of their potential roles in assisting with this process in a responsible and ethical manner.

In this presentation, the legal basis for utilizing psychiatric expert testimony in the immigration courts will be presented. The various legal applications will be discussed, in addition to relevant case law. The topics of removal and cancellation of removal, as well as the concept of asylum will be detailed in both legal language and plain English. The various situations that can lead to removal will be identified, described, and differentiated. The fact that the laws are actually written to protect American citizens or permanent residents, and not the alien subject to removal will be explained. Certain recent cases that have been in the news will be shared with the participants in order to illustrate the importance of this work.

Specific topics will include:

- General classes of aliens ineligible to receive visas and ineligible for admission; Waivers of inadmissibility and exceptions
- Cancellation of removal – adjustment of status
- The concept of extreme and unusual hardship/exceptional and extremely unusual hardship
- Special rules for battered spouses or children
- Other examples of automatic or potential asylum

The presentation will then focus on how to formulate the psychiatric-legal question, as the statutes are non-specific in this regard. Understanding an immigration evaluation as a risk assessment is critical and is the crux of why these evaluations are best performed by psychiatrists/psychologists with forensic training.

Specific topics will include:

- Who is eligible for evaluation/petition?
- How to work with an attorney in an immigration case
- How do you write your report?
- What sources of information should be used?
- How to get this type of work!

Finally, specific cases will be presented and attendees will engage in a see-one-do-one discussion of how to approach these cases within the framework of the statutes. Cases will be presented illustrating a variety of possibilities, including risk assessment based on existing psychiatric disorder (the easiest type), asylum (more difficult because of documentation), and possible future psychiatric or medical issues (most

difficult especially when postulating future psychiatric problems). Hopefully, a spirited discussion will ensue and international participants will bring forward parallels from their own countries.

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**Immigration, Deportation, Removal**

## 19 Mental Illness and Legal Fitness (Competence) to Stand Trial in New York State

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After attending this presentation, attendees will be able to describe recent scientific data on the association between mental illness and fitness to stand trial. Specifically, attendees will be able to describe a statistically significant association between findings of mental illness and forensic opinions on fitness to stand trial. In addition, attendees will be able to describe further, in-depth analysis of the "Not Fit" cases, including the statistical analysis of the significance or lack of significance of the BPRS scales. This analysis includes both individual symptom constructs and also the clusters of symptom scales as informed by the literature.

This presentation will impact the forensic science community by emphasizing research methodology and statistical analysis in achieving its educational objectives.

The proposed presentation is a report on the outcome of a research study conducted under the auspices of the New York University (NYU) School of Medicine Forensic Psychiatry Training Program. It was approved by the Institutional Review Boards of both NYU (R#11-00503) and the New York City Health and Hospitals Corporation (Bellevue Hospital #030711-Bel-S0069). The study was a case-controlled review of charts at the Forensic Psychiatry Clinic of Bellevue Hospital, a large, urban general and specialty care public hospital in New York City. This clinic provides evaluations of Fitness to Stand Trial for criminal defendants referred under New York State Criminal Procedure Law 730.

"Fitness" to stand trial is an important issue in the adjudication of criminal defendants with known or suspected mental illness. When adjudicating a criminal defendant, the assessment of the defendant's fitness to stand trial can be a crucial decision point in legal disposition. Most defendants are presumed to be fit (competent) to stand trial. When a defendant's competence is called into question, lawyers and judges consult psychiatrists and psychologists for forensic opinions on the defendant's capacity to stand trial. "Competence" to stand trial is the legal term for fitness, as designated by a judge. "Capacity" is a finding of competence as opined by the forensic clinician.

In 1960, *Dusky v. U.S.* (362 U.S. 402), a landmark case, established what is usually taken to be the minimal constitutional standard for adjudicative fitness in the United States. In this case, the U.S. Supreme Court stated that the test for competence to stand trial was "whether [Dusky had] sufficient present ability to consult with his lawyer with a reasonable degree of rational understanding—and whether he [had] a rational as well as factual understanding of the proceedings against him."<sup>1</sup> Psychiatrists and psychologists generally rely on *Dusky* as the standard when evaluating defendants' fitness to stand trial. New York State's Criminal Penal Code defines Incapacitated Persons as those defendants who, as a result of mental disease or defect, lack either the capacity to understand the proceedings against them, or lack the capacity to assist in their own defense.<sup>2</sup> Incapacitated persons are then court-ordered for interventions to restore fitness.<sup>3</sup>

Many clinical instruments have demonstrated validity and reliability in quantifying the degree of functional incapacity resulting from mental disorders. The Brief Psychiatric Rating Scale (BPRS) is an instrument developed to quantify psychiatric symptomatology.<sup>4</sup> The BPRS is a very

widely used and relatively brief scale that measures major psychotic and non-psychotic symptoms in individuals with major psychiatric disorders, particularly schizophrenia. The BPRS rating is based upon observations made by clinicians during clinical evaluations, and is generally accepted as appropriate for evaluating baseline psychopathology in outpatient as well as inpatient populations.<sup>5</sup> Numerous studies utilize the BPRS as a highly efficient and rapid evaluation procedure to yield a rather comprehensive description of major psychiatric symptom characteristics.<sup>6,7</sup> Sixteen symptom constructs were originally listed for rating on a seven-point scale, which document the intensity of symptoms in relatively independent areas. The sixteen original items were: conceptual disorganization; unusual thought content; anxiety; guilt feelings; grandiosity; depressed mood; hostility; somatic concern; hallucinatory behavior; suspiciousness; blunted affect; tension; emotional withdrawal; mannerisms and posturing; motor retardation; and, uncooperativeness. Subsequent additions to the scale were two additional items of excitement and disorientation. The 18-item BPRS is perhaps the most researched instrument in psychiatry.<sup>5</sup> A more recent publication highlighted the need for replicating BPRS factor analysis in forensic samples.<sup>8</sup>

The study's research methodology and resulting data structures have important implications for the reliability and validity of its interpretations. Inter-rater reliability, test-retest reliability and inter-method reliability, all apply to both the scales of the BPRS and the findings of Fitness/Unfit. In addition, the BPRS is being explored as possibly to become an initial screening tool, to aid New York lawyers and judges in deciding which criminal defendants to refer to the clinic for Fitness assessments. Thus, the degree of validity of the BPRS as a screening tool for Fitness will be discussed.

The study's limitations will also be discussed, including the reliability of the clinic's slightly-modified version of the BPRS. Separately, the evaluators' training in using the BPRS instrument will be discussed as well.

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#### Forensic Psychiatry, Mental Illness, Fitness (Competence) to Stand Trial

## 110 One Academy, Eleven Sections, How Many Ethics Codes?

John L. Young, MD\*, Yale University, 203 Maple Street, New Haven, CT 06511-4048

After attending this presentation, attendees will grasp the major complexities involved in the creation, evaluation, and application of ethics rules for their forensic disciplines.

This presentation will impact the forensic science community by deepening awareness of critical ethics issues shared across disciplines and enabling informed responsible dialog that will result in progress towards the goals of the 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*.

The 2009 call from the National Academy of Sciences (NAS) for the establishment of a regulatory body to be known as the National Institute of Forensic Science has been stirring up a vigorous conversation across AAFS membership. Upon taking office in 2010, President Bono required an *Academy News* newsletter column from each section providing an account of its responses to the NAS document. This was carried out over the succeeding year and the subsequent annual scientific meeting program included a variety of sessions devoted to aspects of quality improvement in forensic work. Building on that foundation, this presentation addresses critical issues that inevitably require further discussion in order to achieve success in our efforts to codify quality concerns into ethics guidelines.

Those who would attempt to develop any set of professional ethics codes immediately face two obstacles. First, the past generation has experienced an explosion of access to information, with no corresponding means for judging its quality and thereby its potential ethical weight. Second, professions have not appeared eager to embrace ethics codes, economists being an interesting example.

In addition, the sheer volume of material involved is formidable. Hundreds of ethics codes are already in existence, readily available on the internet along with detailed instructions for composing more of them, including potential examples. Although it runs to 4 ½ pages, one model code presented at the Chicago AAFS meeting is by design incomplete.

Certification issues are crucial. Some have boldly proposed that certification be required in order to testify in court. There are forensic disciplines with their own recognized certification systems unrelated to AAFS. For psychologists certification is highly demanding, and earning a license is normally considered a sufficient practice qualification. Also certifying boards are costly to establish and operate.

In order to be effective an ethics code must address the issue of enforcement provisions, with due process including qualifications for its administrators. The experience of AAFS indicates that lawsuits are to be expected. Enforcement help from civil law seems meager at best. Once censured, there may be no legal bar to keep an expert from continuing to testify.

Any set of ethics rules, codes, or guidelines must be a living document, a practical representation of professionals' daily experience. By their nature such guidelines reflect a current and substantial consensus, a willingness to support a general expectation of adherence. Over time such agreement can be expected to evolve, requiring a mechanism for revision. It is highly useful to provide a means for practitioners to submit queries or consultation requests to their organizations' ethics leadership. This encourages membership's striving to practice ethically and provides leadership an opportunity to anticipate emerging issues.

The existing ethics provisions of AAFS (Bylaws, Article II, appended to the annual *Directory of Members and Affiliates*) already provide a solid foundation for responding to the challenge from NAS. Whatever the legislative outcome of its proposal, it is clear that the public is expecting more from forensic science professionals. We need to work together to respond to these expectations where they are valid and correct them where they are not.

#### Ethics, Regulation, Quality

## 111 Beyond Medea: A Sacrificing Father

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After attending this presentation, attendees will more fully comprehend the psychodynamics involved in a case of filicide perpetrated by a father on his two-year-old son – “reverse” Medea Complex as it were.

This presentation will impact the forensic science community by demonstrating the risks that seriously dysfunctional couples pose to the well-being of their children.

Either a father or mother may commit filicide. According to the data in the literature, mothers most often carry out neonaticides and infanticides. Fathers, on the other hand, more often kill their children when the children are older. In one study of 3,459 cases of filicide, based on data obtained from the FBI, 95% of mothers who committed these crimes did so during the first week of the newborn’s life, whereas fathers who kill their children do so when they are between the ages of 13 and 14-years-old, and even more so when they are between the ages of 16- and 18-years-old (Kunz & Bahr, 1996).

Filicide is a crime that may be perpetrated by either parent, or both for that matter, on their child who is between the ages of 0 and 18 years. Although it is not often described in the literature, there are data that show that there appears to be a “Male version of the Medea Complex” in which the fathers take the lives of their own children as a retaliatory measure against the mother. Frequently, the father uses a sharp weapon, a firearm, or strangulation with his bare hands. Some of the elements that might fuel such behavior may include a sense of revenge or omnipotence; a highly attached and/or ill-defined relationship with his child; the inability to respect him or her as a person; or simply thinking about him or her as a “weapon” against his partner. At times, such acts are followed by suicide, indicating the following: the importance placed on the ties to the victim; their symbiotic relationship; the inability to see the child as an individual, but rather as an extension of his own persona, projecting his own experiences and emotions. In the eyes of the perpetrator, murdering one’s own child can represent an act of love toward one’s offspring in an attempt to eliminate all current and future suffering caused by difficulties between the parents.

This case concerns a child who died at the hands of his 26-year-old father who, in turn, unsuccessfully attempted suicide. The autopsy ascribed the child’s death to asphyxiation and hemorrhage. The child had been hanged from a door by a cord that was tied around his neck. Cuts to the musculature of the left side of the neck and left jugular vein, from top to bottom, were identified. Because cardiovascular activity was still present in the child, his father placed him on a bed and cut the victim’s left wrist from left to right. Following this, in an attempt to end his own life, the perpetrator proceeded to cut his own wrists, tried to set himself on fire, and swallowed caustic substances. Court ordered forensic psychiatric evaluations were subsequently requested. No significant evidence emerged which indicated the presence of psychopathology or mental illness at the time of the crime. Blood and urine analysis showed no traces of narcotics or prescription drugs such as barbiturates or benzodiazepine. The perpetrator was found to be completely culpable. Investigations revealed that the crime might have been an act of revenge against his partner, a 21-year-old woman, as a way to punish her for the problems regarding their relationship. Their son was the product of an unexpected pregnancy that occurred after the couple had been together for only one month. As a result, the young couple did not have the chance to get to know each other very well before beginning a life together. Due to his immaturity, he was unable

to accept his partner and his son. In addition, there were strong feelings of jealousy. All of these factors led the couple into a state of crisis.

This story concerns a relationship rife with distortions, misunderstandings, and resentment; a dominant woman with a highly individualistic nature, along with the perpetrator’s sexual dysfunction (premature ejaculation and sporadic erectile dysfunction), which was often pointed out by the woman to friends, relatives, and to her ex-boyfriend. Adding to his misery, she had encouraged him to go out with other women. She was not attracted to him and wanted to leave the relationship. An ill-defined relationship with the child, who the father saw more as “son” than “person,” and a relationship with a partner who was resentful of him led the perpetrator to feeling “frustrated” about seeing his “family plans” fall to pieces. He was humiliated, misunderstood by his partner, and felt inadequate in his role as companion. When the man perceived his wife’s intentions to psychologically divorce him, he threatened to kill himself. He planned his suicide (a suicide note was found, which included his motives, confirmed this), he declared his intentions, and made the attempt to do so, after having killed his own child.

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**Filicide, Medea Complex, Murder Suicide**

## 112 The “Beasts of Satan” Murders

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After attending this presentation, attendees will recognize some features of group murder and understand principles of forensic psychiatric assessment of members of satanic sects.

The presentation will impact the forensic science community by exploring the role of satanic sects in the etiology of this type of crime.

In January 2004, the body of a young woman was found in the garden of a small house in the woods in the province of Varese (a rich city in northern Italy, about 50 km from Milan). Forensic investigations showed that the woman had been shot in the face, buried alive, and finally killed by repeated beating on the head with a spade.

A few months later, not far from where the woman’s body had been found, the remains of two young men were discovered. They had been killed on the January 17, 1998 (“a night of the black moon”), by repeated beating with rods, causing many bone fractures, as well as by knife wounds distributed all over their bodies.

These discoveries hit the national headlines and brought to light the actions of a sect calling themselves the “Beasts of Satan.” Although the investigations of the members of this group proceeded smoothly and those responsible for the homicides were easily identified, the news about “satanic” activities occurring in a quiet, hard-working area in the province of Varese was a great shock and provoked turmoil in local society. In the following years, any violent event affecting a young person was inevitably attributed in the public mind to the activities of this sect. In actuality, the activities were much less “occult” than was commonly imagined, as demonstrated by the investigations that easily identified the criminals as nine young factory workers with no criminal record.

The shocking violence of the murders led the judge to request a forensic psychiatric assessment of one of the members (the only one with history of mental disease), but widening the investigations to study the group dynamics and lifestyle of all the members. The assessment revealed that all the young men had a fragile, immature personality, a very low level of education, and were socially disadvantaged. They had built up a crude practice they called “satanism” that was for them an exciting escape from

their humdrum reality, and a uniting element. They carried out improbable rites with a liberal use of satanic words and rites they knew nothing about. Each had a rigidly defined role in the sect, that had a hierarchical structure but no recognized leader.

The historical-cultural phenomenon of satanism, as also of spiritism and other esoteric beliefs, has nothing to do with the sub-cultural climate of violence and terror created by this group. The members of the “Beasts of Satan” were clumsily trying to mask the cultural poverty of their lives by carrying out cruel, violent actions, and identifying with a name that strikes superstitious terror (“Beasts of Satan”). The violence was aimed particularly at members who tried to renege on the group.

Although judges generally request a forensic psychiatric opinion in particularly shocking cases of murder for no apparent reason (except for mafia crimes), on the suspicion of a relationship between insanity and murder, in this case the experts did not find any trace of such a relation. The trial of the members of the “Beasts of Satan” sect was concluded with the verdict of deliberate murder by all the members, who were all given long jail sentences.

A striking point in this tale was the suicide of one of the members, before other members of the group were identified, who drove into a stone wall. The dynamics and possible responsibilities for this death remained obscure.

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#### **Satanic Sects, Psychiatric-Forensic Assessment, Group Violence**

### **I13 Utility of a Grief Services Program for Medical Examiners’ Offices**

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After attending this presentation, attendees will understand the range of services provided by a grief services program at a centralized, statewide medical examiner’s office, the frequency of utilization of these services by bereaved individuals, and a framework for the implementation or improvement of a grief services program at a medical examiner or coroner’s office.

This presentation will impact the forensic science community by presenting the first study, to the best of our knowledge, which explores the utilization of a grief services program at a centralized, statewide medical examiner/coroner’s office, and will initiate discussion of future work in this area of forensic science.

Medical Examiner/Coroner’s (ME/C) offices investigate deaths that are often sudden, violent, and unexpected, leaving family members and those close to the deceased suffering a traumatic loss with little in terms of support and counseling. Since its inception in 1976, the Grief Services Program (GSP) at the New Mexico Office of the Medical Investigator (OMI), the statewide medical examiner’s office, has assisted families and communities across New Mexico with education, crisis intervention, counseling, and psychotherapy, regardless of the cause or manner of death. The utility of a grief services program at statewide medical examiner’s office was investigated in order to better understand the needs of bereaved individuals, demonstrate the scope of services provided, and propose the findings as a model for other medical examiner/coroners offices. The OMI investigated 5,120 deaths in 2009, during which 1,085 grief services contacts were recorded. The majority of these visits occurred on-site at OMI (60.5%) by individuals residing in the same county (Bernalillo County, home to 1/3 of the state’s residents) as OMI (62%). Telephone sessions (23.1%) and off-site visits (15.7%) were the next most prevalent type of contact. The number of individuals present at each session held at OMI ranged from 1 to 22 (mean 1.6) and the range of attendees for sessions held off-site was 1 to 130 (mean 2.5). Off-site sessions included memorial

services, group sessions, and school presentations. The highest frequency of visits involved one-on-one sessions (78.6%) followed by sessions with two clients (9.1%). People seeking the services of the GSP for the first time consisted of 28.4% of the contacts, with the remaining 71.6% returning to the GSP for follow-up appointments. Ninety-one percent of the people who sought services offered by the GSP were immediate family, followed by extended family (5%), friends (1.9%), and community group (0.8%). Support was primarily provided to those suffering a loss due to homicide (28.8%), followed closely by suicide (26.1%), natural causes (20.0%), and accident (13.9%). It is believed, this is the first report looking at the utilization of a grief services program at a ME/C office. The service most frequently used by clients of the GSP was counseling related to the loss of a family member or loved one. However, grief counselors provided many additional types of assistance to grieving families, including discussions of autopsy results in conjunction with a staff pathologist, sharing information with family members, escorting families for viewings, showing family members photos of the decedent or scene, and assisting families who plan to transport the decedent. In this era of reduced resources GSPs may not be a priority for ME/C offices. Given the large number of people utilizing OMI’s GSP; however, and the diverse reasons for their visits, it is apparent there is a need for GSPs at ME/C offices, particularly given the traumatic nature of deaths investigated by ME/Cs. The goal is that this work will prompt discussion and future work in this important yet seldom published area of forensic science.

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#### **Grief Counseling, Bereavement, Medical Examiner**

### **I14 Violent Fantasies in Psychotherapy: Risks of Precipitating Violent Behavior**

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After attending this presentation, attendees will become familiar with the theory and practice of psychotherapy techniques that focus on violent fantasies. Readers will also gain an understanding of the net risks involved in the use of violent fantasies in psychotherapy and important measures for the psychotherapist to undertake before, while and after a patient offers a spontaneous or a prompted report of violent fantasies. This presentation will also consider the tradeoffs involved in such measures, including compromises in confidentiality, chilling effects on psychotherapy, and disruptions to the therapeutic alliance.

This presentation will impact the forensic science community by focusing on both violence risks associated with fantasy in psychotherapy as well as affirmative approaches to address such risk. This presentation offers important recommendations to forensic practitioners, psychotherapy providers, and the community in general.

In the context of psychotherapy, the exploration of violent or aggressive fantasies often serves the important function of providing insight into sources of attachment trauma, which in turn facilitates eventual mastery over said trauma and resolution of certain maladaptive defenses and psychological symptoms. Indeed, in some methods of psychotherapy, murderous fantasies are specifically elicited by the psychotherapist, who then assists the patient to experience and elaborate on the feelings of rage as well as relief upon imagining the acting out of those violent fantasies.

The question thus arises as to whether the use of fantasy in therapy affects the risk that the patient will act out on the violent thoughts in reality. More specifically, to what extent must therapists take into account the actual risk of violent behavior in patients who are deliberately asked to develop and articulate their violent fantasies and then encouraged to experience the catharsis of enacting their aggressive urges?

This presentation reviews the current research on the violence risk associated with patient reports of violent fantasies. Though violent fantasies are common and in fact normal in healthy populations, in certain groups, they constitute a risk factor for violent behaviors. In particular, risks may increase in persons with deficits in domains such as judgment,

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\* Presenting Author

impulse control, or reality testing, including those with borderline personality or substance use disorders and those with criminal backgrounds. The content of the reported fantasy may also be correlated to the risk of violent behavior; fantasies of rape, beating, or torture may indicate increased risk compared to fantasies of mere murder. Additionally, concern for violent acts increases when therapy patients exhibit preoccupation with or sexual excitement from violent fantasies. Taking these risks into account, the articulation of specific violent fantasies by certain patients against identified individuals may in select circumstances constitute a threat that invokes duties to warn and/or protect under Tarasoff laws.

Accordingly, psychiatrists should be aware of their duties and implement safeguards to protect their patients and the community, to ameliorate risks of both violent behavior and of liability exposure. Appropriate safeguards include proper screening and violence risk assessment to determine if specific patients can appropriately handle the experience of fantasy exploration in therapy; containment of patients who demonstrate or develop a breakdown in ability to control urges to act on violent fantasies; and reporting of patients to law enforcement agencies where identified individuals may be at risk of harm from the patient.

**Violence Risk Assessment, Tarasoff, Psychotherapy**

## **I15 Impulsive Aggression: Nature and Pharmacotherapy**

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After attending this presentation, attendees will be able to define impulsive aggression, diagnose impulsive aggression, and make evidence-based decisions in prescribing medication to control impulsive aggression.

This presentation will impact the forensic science community by showing how the ability to define, diagnose, and apply the medication algorithm will assist attendees in gaining improved knowledge and competence in effectively treating impulsive aggression in both forensic and civil patient populations.

Impulsive aggression is defined as, “a “hair-triggered” response to a stimulus which results in a sudden agitated state that lasts a few minutes to several hours (Dodge, 1991), the agitation builds to a crescendo and culminates in an aggressive act. During this state interpersonal communication appears inefficient and recall of events may be poor...Impulsive aggression [IA]...is spontaneous, unplanned, and lacking in self control. Outbursts are explosive and driven more by impulse or erupting affect than by acquisitive or self-promoting goals” (Felthous and Barratt, 2003).

A variety of psychotropic agents representing selective serotonin reuptake inhibitors (SSRIs), anticonvulsants, mood stabilizers, beta blockers, psychostimulants, and antipsychotics have been prescribed for intermittent explosive disorder and IA, either in “pure form” or co-occurring with other psychopathology. The efficacy of anti-impulsive aggression agents is directly related to accurate diagnosis of IA and mental disorder of which impulsive aggression can be secondary (e.g., bipolar disorder, manic). Scientific literature is not consistent in defining and diagnosing IA; therefore, this presentation begins with a description of the nature of IA and an evidence-based explanation for its diagnosis.

Although algorithms for the pharmacotherapy of clinical aggression (e.g., Moeller and Swann, 2007) have been proposed, a medical standard of care for the pharmacotherapy of IA has not gained general acceptance. Based upon a review of the literature, the presenters offer an evidence-based algorithm for treating IA with medication. This algorithm requires accurate diagnosis of IA and co-occurring mental disorder as well as assessment of the severity of IA in terms of intensity and frequency of episodes. Not to be overlooked is a careful history of prior psychotropic medications and their effect on aggressive episodes. Substance abuse and

sedative/stimulant seeking behavior can complicate the assessment and treatment of IA.

The suggested sequence in which the medications could be used to treat IA takes into account their efficacy, side effect profile, the ease of administration and the recommended monitoring. With all these in mind, an SSRI such as fluoxetine, sertraline, paroxetine, citalopram, or escitalopram is a reasonable first line medication, particularly if the IA is not severe. There is enough evidence based literature to support their efficacy in these scenarios. They have minimal side effects and are generally well tolerated. If the IA is severe or the SSRI is ineffective, a mood stabilizing anticonvulsant is a prudent selection. Most of the anticonvulsants used in clinical practice have shown to be efficacious, with extensive studies done with carbamazepine, valproic acid, oxcarbazepine, and phenytoin. The drawbacks of anticonvulsants are their side effects and the need for regular blood testing. It is also important to consider the possibility of drug-drug interactions with other medications that the person might be on, before starting them. Another class of drugs which helps control IA are the antipsychotics. Drugs like quetiapine, aripiprazole, olanzapine, and risperidone are used with good clinical effect. They are used for severe IA which is either not controlled by the above two classes of drugs or where side effects have limited their use. It is important to keep the black box warning of sudden cardiac death in mind, which limits their use to particular patient population with IA where the benefits outweigh the risks. Other medications to be discussed will include beta blockers and psychostimulants.

When the IA is secondary to another disorder, in many cases the aggressive behavior subsides with appropriate pharmacotherapy of the primary disorder. This review and algorithm also includes evidence in support of pharmacotherapy of the IA itself, when treatment of the primary disorder fails to control aggression.

Critical to the success of the algorithm and effective pharmacotherapy of the IA in general, is careful monitoring of the IA episodes in the course of the treatment. There will be a discussion about having the patient maintain a diary and also monitoring for these episodes during follow up appointments, by using the Overt Aggression Scale by Yudofski or the Modified Overt Aggression Scale.

**Impulsive Aggression, Intermittent Explosive Disorder, Pharmacotherapy of Aggression**

## **I16 Clinical and Forensic Evaluation of Accountability in Homicide Cases: A Study on the Penal Court of Rome in Italy**

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After attending this presentation, attendees will improve the relationship between mental illness, crime, and the evaluation of accountability.

This presentation will impact the forensic science community by the evidence based model that is suggested in the forensic psychology and psychiatric area.

**Introduction:** Italian legislation on evaluation of accountability is introduced and the results of the explorative analysis are discussed. By exploring the interest in analyzing the way in which experts evaluate accountability for trial purposes in based on a growing need for evidence-based and scientific expert evaluation, this presentation will reflect about the relevance of evaluation of accountability for the scientific evidence in the court.

**Data Analyzed:** An in-depth content analysis was conducted on seven expert testimonies that evaluated accountability.

Seven reports were considered, regarding cases of voluntary murder (articles 575-577 of the Italian Criminal Law) tried in the Law Court of Rome (Italy) between 2002 and 2008. Each report is approximately thirty pages long and reflects the evaluation of a single expert witness. In two cases, the expert witness is assisted by a colleague exclusively for the purpose of test assessment.

**Methodology:** The content analysis of the expert testimonies follows the theoretical and applied principles of “Content Analysis” (Losito, 2001; Amaturo, 2005).

The unit of context is defined by the sentence; the unit of analysis is formed by a phrase. A series of categories were created for the purpose of content analysis, in order to classify useful areas of interest for clinical-forensic analysis.

The following results emerged from the explorative study:

1. The theoretical model that guides data acquisition and analysis is never explicit.
2. Evaluation criteria of accountability are never explicit; the only explicit literature reference is the DSM IV-TR.
3. Interviews and psychological tests are used as diagnostic instruments; however, they are not used to explore the meaning of crime in this study.
4. The areas of diagnosis most frequently explored are clinical symptoms (Axis I of DSM-IV-TR) personality (both according to the criteria of Axis II of DSM-IV-TR e independently of the manual).
5. In general, the areas diagnosed are not explored in reference to the fact or crime and/or to the relationship between author and victim; greater attention to these factors tends to arise in the final phases of the expert testimony.
6. The answers provided for the inquiries submitted are based on the evaluation of the symptoms and of the type and level of personality function, rather than on the assessment of the relation between possible personality disorders and the mental state of the defendant at the moment of committing the crime.

**Conclusion and Future Perspectives:** Although the sample is obviously quite limited, given the results that emerged from the study, more research is clearly needed in order to develop an integrated model of evaluation of accountability. Such a model would allow consideration and analysis of the action at the same time (Harre’ 1990; De Leo & coll. 1991, 1999, 2001, 2004) and the possible pathological functions of the subject that were active during the crime.

In this way, the expertise witnesses would be able to better respond to the juridical needs of evidence-based criteria between the crime and the mental state of the defendant during the crime.

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**Homicide, Accountability, Diagnosis**

## **I17 Case Study: Paraphilias Carried to the Limit — Child and Dog Victims of a Subject With Schizoid Personality Traits**

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After attending this presentation, attendees will understand how an individual’s paraphilic fantasy allowed a lonely 53-year-old man to use his own dog to sexually abuse an 11-year-old boy. In this case, the prosecutor along with the criminal profiling unit, through a behavioral analysis, explained the case to a judge, who finally condemned the man.

This presentation will impact the forensic science community by showing how the investigation of this case led physical evidence and behavioral evidence to the understanding of a man with schizoid personality traits with paraphilic fantasies, who conditioned his dog to respond to sexual erotic maneuvers and who later used his dog to sodomize

a child. This unique case is the only one of its kind ever documented, which seems more to be part of the plot of a psychological police thriller than a real criminal case.

The criminal profiling unit was called to assist in the investigation of an extremely complex case. Initial reports came from the preliminary account of an 11-year-old boy, who claimed he had fallen in the forest and had been accidentally injured in the anal area. However, the emergency services found injuries consistent with a possible sexual offense. After analyzing the samples, sperm was found that had a morphology that was not human and was possibly canine. Later, with the support of the profiler, the child was interviewed using the protocol RATAAC. In the interview, the child reported that while playing in the woods, a man had approached him and called a dog who penetrated the child’s anus with his penis. The offender then fled the area. The case was not “a dog who had raped a child” anymore. With this information, research activities were initiated and corroboration of information and it was during this profiling unit conducted a behavioral analysis of the facts, which yielded a report giving an account of the behavior one would expect the aggressor and as some investigative strategies. Finally, the man was identified, arrested, and the dog found.

Subsequently, the man’s psychological tests established the presence of schizoid traits. Meanwhile, the dog was tested and showed a conditioned sexual response to certain kinds of touching. The behavior and concern of the owner toward the dog during these tests suggested a link between pet and master that went beyond the normal link between dog and master. These aspects together suggested that the dog had been used as an object of sexual activity, which in turn become the means of satisfaction of his master, who then wanted to extend it to others.

The case, despite its difficulty, was brought to trial. The profiler attended court as an expert adviser to the prosecution. At the trial the main work was aimed at establishing the conditioning to which the dog had been subjected, that the defendant had used the dog as an implement to violate the child, and therefore the child had told the truth.

The case was decided in favor of the prosecution and the defendant convicted of the crime of “aggravated violent sexual intercourse with child under 14.”

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**Behavioral Analysis, Sex Crime, Schizoid Personality Traits**

## **I18 Writing Forensic Psychological Reports for Police Officer Applicants: First Responders in the War on Terrorism**

*Ronn Johnson, PhD\*, University of San Diego, 3525 Del Mar Heights Road, #302, San Diego, CA 91230*

The goals of this presentation are to introduce the IACP pre-employment guidelines as well as the Peace Officers Standards of Training (POST) as a practice foundation for conducting the forensic pre-employment psychological evaluation. This presentation will also explore the essential parts of a proper clinical interview where a significant portion of the report information is obtained. For example, an applicant’s job history and contacts with legal authorities are reviewed for the applied for position.

This presentation will impact the forensic science community by showing how long-term outcomes may be observed through a reduction in negligent hire cases highlighted through litigation for officer misconduct.

Police officers are vital front-line responders in the war on terrorism. Therefore, the recruitment, selection, and training of officers assume an essential role in national security. Two useful forensically-relevant references are available for examiners involved in the selection process (i.e., psychological pre-employment screening). The International Association of Chiefs of Police (IACP) and the Peace Officer Standards (POST) both offer guidance for qualified forensic examiners who must evaluate applicants for these safety sensitive positions. Psychological screening must now incorporate psychological issues related to national

security into the evaluation process. After attending this presentation, attendees will understand some of the practices used in conducting these evaluations as well as crafting the forensic psychological report that is usually submitted to the hiring authority with national security in mind.

The presentation has three primary learning objectives. First, attendees are introduced to the IACP pre-employment guidelines as well as of the Peace Officers Standards of Training (POST) as a practice foundation for conducting the forensic pre-employment psychological evaluation. Second, this presentation explores the essential parts of the clinical interview proper where a significant portion of the report information is obtained. For example, an applicant's job history and contacts with legal authorities are reviewed for the applied for position. The ability to manage or vulnerability to stress is examined within the context of an applicant's coping skills. Finally, the presentation covers writing of the forensic report with considerable attention devoted to the I-Section (i.e., integration section). For example, in some venues, the integration section is the only portion of the report that will be read either because of time or interest constraints on the part of the recipients or because of legal strictures. For example, Federal Rule 803 does not permit the jury to receive the entire report as a written exhibit. In addition, 803 restrict the use of learned treatises attached to the report allowing only the reading of report passages and Rule 702 requires that the psychologists' testimony be grounded in valid constructs and techniques applied to the data in the report rather than relying on biased or stitched together speculation.

These constraints make the integration of the data and reasoning of central importance to the report and the subsequent testimony. The informed use of data, reasoning, and recommendations are by-products of a culturally responsive model that would substantially improve the foundation needed to withstand intensive review during adversarial questioning and cross examination. A case study will be used to illustrate how this process takes place. When carefully crafted, the integration section represents the highest form of evidenced-based practice within this specific forensic context. It is a process that includes forensic research, psychological expertise, and relevant psycho-cultural aspects of the individual being evaluated.

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**Forensic Psychology, First Responders, Terrorism**

## **I19 Reviewing and Synthesizing Culturally Responsive Forensic Psychological Data in Pre-Employment Psychological Evaluations**

*Ronn Johnson, PhD\*, University of San Diego, 3525 Del Mar Heights Road, #302, San Diego, CA 91230*

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After attending this presentation, attendees will be able to articulate some of the data information sources frequently used in forensic psychological evaluations conducted on police applicants. The presentation will also cover how the process is explored for using other data to craft a culturally-responsive psychological interview format.

This presentation will impact the forensic science community by presenting how long-term results may be observed through work products noted by negligent hire or appellant hearing outcomes.

The forensic psychological examiner is often confronted with an extremely large data set (e.g., MMPI-2 RF Scales, personal history statement, background reports, etc.). The credibility and defensibility of any forensic evaluation is directly related to synthesis (i.e., sorting and prioritizing) of the most valid data available. And, it is at the integration section that salient cultural factors are at the greatest potential to either augment or detract from the decision-making of the examiner. Cultural factors are identified in several practice reference resources (e.g., APA, ABFP, and the DSM-5) used by forensic psychologists. The Culturally-Responsive Integrative Model (CRIM) may function as a practice guide for approaching the forensic evaluation process of police officers. It is hypothesized that forensic examiners exposed to the content of this session

will be able to apply this knowledge with the cultural context of the evaluation process.

There are three primary learning objectives expected from this presentation. First, following the completion of this presentation, attendees will be able to articulate some of the data information sources frequently used in forensic psychological evaluations conducted on police applicants. For example, background reports and consultation with background investigators can serve as a value sourced of informing used to frame the semi-structured interview of an applicant. Second, the process is explored for using other data to craft a culturally-responsive psychological interview format. For example, ethical standards set forth by the American Psychological Association require forensic psychologists to administer, adapt, score, interpret, or use assessment techniques, interviews, tests, or instruments in a manner and for purposes that are appropriate in light of the research on or evidence of the usefulness and proper application of these forensic evaluation approaches.

While forensic psychologist may elect use different psychological tests, they are still ethically-bound to be able to defend such evaluation tools under a legal standard (i.e., *Daubert vs. Frye*). In addition, a State's definition of mental health criteria for a police officer may directly impact a forensic psychologist's decision to use or not use certain data sources. In this case, a forensic examiner practicing in several jurisdictions or states may need to be ever mindful that there could be little mobility or transferability of standards from department to department. More importantly, these same factors may be rigorously examined during cross-examination during appeal hearings or subsequent litigation stemming from some action or inaction of an officer screen by the forensic psychologist.

Finally, a rubric is offered for weighing the relevance of information from divergent sources of information over time. For example, psychological tests validated for use with police populations include those that assess psychopathology such as the MMPI2-RF or PAI, those that assess normal psychological functioning such as the CPI, and those that measure cognitive abilities such as the Wonderlic. One forensically-relevant question is how does an examiner consider the problematic tests results in the absence of corresponding negative biopsychosocial information?

A case study will be used to demonstrate how this forensic decision making occurs. It is a process that includes research, forensic psychological expertise, and attention to cultural or racial factors associated with the individual being evaluated. The long-term results of this presentation may be observed through work products noted by negligent hire or appellant hearing outcomes.

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**Forensic Psychology, Pre-Employment Psychological Evaluations, Culturally Responsive**

## **I20 The Four D's of Decision Making of Police Officer Applicants in Forensic Psychological Evaluations**

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After attending this presentation, attendees will understand a review of the ethical forensic context that is used as a framework for exploring the four D's of decision making.

This presentation will impact the forensic science community by showing how the safety sensitive nature of police work requires that an applicant's behavioral patterns to be placed under a higher level of scrutiny.

Most accredited law enforcement agencies require a series of steps for all applicants that include a post-offer forensic pre-employment psychological evaluation. These evaluations frequently use guidelines from the International Association of Chiefs of Police (IACP) and at the same time relevant psychological dimensions stated in the Peace Officer Training Standards (POST). Both reference sources provide forensic examiners with

direction for using the “rule out” approach designed for identifying counterproductive behaviors related to an officer’s decision making. Why? Because police officers have to make appropriate judgments under Action Forcing Moments (Johnson, 2010) where split-second decision making is required during extremely stressful situations. Forensic psychologists conducting these evaluations at a minimum will rely upon several informational sources that include background investigation, personal history questionnaires (PHQ) and objective personality tests (e.g., IPI, MMPI2-RF, PAI, 16PF). Because the quality of an officer’s judgment is a critical selection factor, forensic psychological examiners must use samples of an applicant’s past behavior and decision-making as a foundation for rendering an opinion. The forensic psychological interview itself may last less than an hour but usually involves about five hours of the forensic examiner’s total time that is spent evaluating the applicant (e.g., reviewing files, crafting forensic interview framework, and writing the forensic report). There are many job-relevant areas where the forensic psychological interviewer can probe to secure a scientifically-informed basis for rendering an opinion about the quality of an applicant’s decision making and judgment. These job-relevant areas are hereafter referred to as the four D’s and include debt, driving, drugs, and drinking. This investigator hypothesizes that a rigorous review of the four D’s will significantly enhance the opinions and recommendations offered by forensic examiners working in these law enforcement settings.

There are four primary learning objectives for this presentation. First, attendees are presented a review of the ethical forensic context is used as a framework for exploring the four D’s. For example, guidelines from the American Board of Forensic Psychology stress that practitioners use assessment methods in the way that is appropriate based on research or evidence of their usefulness and proper application within a law enforcement personnel selection context.

Second, the safety sensitive nature of police work requires that an applicant’s behavioral patterns to be placed under a higher level of scrutiny. The standards for armed police officers are different than civilian and military personnel. Primarily because police officers have discretion in executing an arrest, using deadly force, engaging in pursuit chases, and having to respond to a wide range of diverse citizen contacts under stress. A review of an applicant’s history of judgment in the four D’s facilitates a forensic examiner’s understanding of a distinctive style expected as they would carry out the duties of an armed police officer.

Third, forensic practitioners document all data they considered and used while assessing the four Ds. This documentation includes background reports, letters and consultations, notes, assessment and test data, scoring reports, and interpretations in connection with the pre-employment evaluation. Finally, there are several practice benefits emerging from this presentation. These include sensitizing forensic examiners to ways of crafting probes in the domains of the four Ds based on an integrative discussion of the applicant findings. A forensic case study to demonstrate the practical application of conducting these types of evaluations will be presented. Evidence based practice prompts the recommendation of additional training for mental health professionals practicing in the area of forensic pre-employment psychological evaluations. At a minimum, this would include becoming familiar with POST, IACP, relevant state statutes, high profile cases, and the police psychology research literature.

**Forensic Psychology, Pre-Employment Psychological, Decision-Making**

## **121 Crafting Forensic Psychology Response Reports for Attorneys to Use in Appellant Hearings**

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After attending this presentation, attendees will gain information relating to the forensic practice standards pertaining to psychological reports conducted in law enforcement settings. The presentation also covers an assessment of the validity of the methods and reasoning used to evaluate the opinions, logic and linkages articulated in the Outside Evaluation (OE) report.

This presentation will impact the forensic science community by showing how a reduction in the information gap that results in the selection of personnel who are less likely to be vulnerable to the high stress associated with being a police officer.

Forensic pre-employment psychological evaluations must take place with considerable guidance from recognized professional sources (e.g., International Association of Chiefs of Police, APA, and the Police Officer Standards of Training better known as POST). The forensic examiner is responsible for rendering an opinion (e.g., meets, does not meet, suitable, or not suitable) that is submitted to the hiring authority or their representative. Most large departments, jurisdictions, cities, or states have some type of an articulated appeal process. Typically, the process includes the rejected applicant or appellant to secure an outside evaluation (i.e., OE report) that either may corroborate or offer a dissenting opinion from the original forensic psychological findings or recommendations. In the cases where there is a dissenting OE report, the examiner writing the initial disqualifying report may be called upon to write a forensic response (FR) report that addresses issues identified in the OE report.

There are three primary learning objectives associated with this presentation. First, attendees are briefed on the forensic practice standards related to psychological reports conducted in law enforcement settings. Second, the presentation covers an assessment of the validity of the methods and reasoning used to evaluate the opinions reached in the OE report. Third, the presentation examines the logic of the linkages articulated in the OE report. In this case, the clear and convincing evidence in the OE report that demonstrates a reasonable connection between job-relevant psychopathology, psychological test results, or any functional or judgment impairments that would preclude this appellant from carry out the full scope of the duties of an armed police officer.

The forensic response report is usually written for the attorney representing the hiring authority. The overall purpose of crafting this report is to assist with the cross-examination of the opposing expert as well as allow the initial examiner to have a better idea of the justification used in the original disqualification report. An approach to report writing for practitioners is presented, with practical strategies and examples provided to illustrate the use of the model in a forensically-based setting by outlining five concepts that, if utilized, hold promise to improve the quality and efficacy of response psychological reports.

It is hypothesized that attendees will leave the presentation with a better appreciation for the intricacies of the forensic process as it takes place in the circumscribed arena of police psychology. This process includes discussion of the session with the department’s attorney that may allow a refinement of the cross-examination or highlighting other issues associated with the findings or methods in the OE report. For example, some OE reports are plagued by misinterpretation of test reports, ignoring relevant laws, and glossing over or ignoring problematic behavior contained in the appellant’s problematic history. After attending this presentation, attendees will understand some of the principles of crafting FR reports within the context of pre-employment psychological evaluations. Although, the guidance offered here may have broader implications for forensic psychology in general.

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**Forensic Psychology Reports, Appellant Hearings, OE Reports**

## 122 Use of Law Enforcement Oath of Honor in Forensic Psychological Evaluations of Police Officers

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After attending this presentation, attendees will be able to articulate the relationship between key elements of the law enforcement oath and relevant job functions of a police officer.

This presentation will impact the forensic science community by improved precision in forensic evaluations. It is believed that public trust is enhanced by awareness that officers are being evaluated on their ability to fulfill standards consistent with what most would view as critical for these safety sensitive positions.

Forensic examiners responsible for psychologically evaluating police applicants or incumbent officers must draw upon several job-relevant sources. The International Association of Chiefs of Police (IACP) provides guidelines for conducting pre-employment psychological evaluations and fitness-for-duty evaluations. Another IACP source not often thought of within the context of the evaluation but is nonetheless instructive in the evaluation process is the law enforcement oath of honor. Experienced and qualified examiners using this oath as an assessment prism will be immediately struck by several ethical behavior elements. First, there is the self-imposed promise to “never betray my badge, my integrity, my character, or public trust.” Second, the oath fuels a commitment to “...always have the courage to hold myself and others accountable for our actions...” Third, there is the self-assurance that, “I will always uphold the constitution, my community, and the agency I serve.” These three areas collectively and separately offer a firm foundation for evaluating behavior, dispositions, knowledge, and skills that anyone functioning as a police officer should be able to demonstrate through the way they have functioned in the past. Why? A police officer’s duty carries critical responsibilities. All police officers must follow core values of the profession that are reflected in the aforementioned oath. A history of slippage in behaviors related to the oath would justifiably call into question a recruit or officer’s fitness to appropriately carry out the duties of an armed police officer.

There are three primary learning objectives expected from this presentation. First, attendees will be able to articulate some the relationship between key elements of the law enforcement oath and relevant job functions of a police officer. For example, background reports, personal history questionnaires, personnel records, oral panel statements, and psychological test results can serve as important sources of information used to frame the semi-structured interviews of an evaluatee. Second, the same oath may be used in connection with other data to craft a psychological interview format. Third, the guidelines set forth by the IACP and Police Officer Standards of Training (POST) require forensic psychologists to administer, adapt, score, interpret, or use assessment techniques, interviews, tests, or instruments in a manner and for purposes that are appropriate in light of the research on or evidence of the usefulness and proper application of these forensic evaluation approaches.

It is hypothesized that using the Oath of Honor as one assessment prism is a powerful mechanism for understanding an evaluatee’s capacity for consistently adhering to high ethical standards while functioning as a police officer. A case study will be used to demonstrate how the law enforcement oath can be integrated within the forensic evaluation process. This includes the opinions and recommendations contained in the final forensic report. The benefit to the field of practice may be observed through improve precision in forensic evaluations. In terms of community impact, the author believes that public trust is enhanced by awareness that officers are being evaluated on their ability to fulfill standards consistent with what most would view as critical for these safety sensitive positions.

**Forensic Psychology, Law Enforcement Oath of Honor, Ethical Issues**

## 123 Use of Police Culture and Ethics as a Decision Making Resource in Forensic Pre-Employment Psychological Evaluations of Police Officer Applicants

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The goals of this presentation are to introduce attendees to the police cultural factors that may compromise, increase vulnerability, or call into question the person being evaluated. Second, involves risk assessment for three types of misconduct that include malfesance, misfeasance, or nonfeasance. Third, a forensic case study as a practical example of using police ethics during an actual evaluation will be presented.

This presentation will impact the forensic science community by virtue of attenuation in the quality of recommendations coming from forensic examiners that ultimately result in the selection of police officers with lower risk assessment ratings for areas of concern.

Forensic psychologists conducting pre-employment psychological, second opinion, outside evaluations, and fitness-for-duty evaluations are expected to have substantial understanding of the law enforcement culture. Forensic skills used in these evaluations can use awareness of police ethics in formulating opinions while making recommendations to the hiring authority. Ethics pose a major leadership and training challenge within the police culture. For example, one of the elements of the police culture is a high level of allegiance and loyalty that functions as a framework that motivates an officers’ willingness to risk personal safety to protect and serve the public. A culture of allegiance and loyalty can also reinforce the “Blue Code” or an unwanted cognitive schema that includes the belief that questionable actions or misbehaviors must somehow be covered up. As a result, this cognitive framework could result in unprofessional and even illegal behavior to be accepted out of a misguided sense of loyalty. It must be emphasized that most psychologically screened police officers are ethical and honest. Police officers are members of a distinct work culture. It is a culture that reinforces certain personality traits that have been covertly endorses as necessary for this type of work. For example, police officers begin to develop a belief in an “us/them” framework that informs officers that while fellow officers are trustworthy (us), they should be cynical and ever cautious of non-police (them). Law enforcement training reinforces the inherent potential dangers stemming from police work (e.g., misconduct and poor quality of judgment). A forensic examiner is charged with the responsibility for evaluating for indicators that rule out those without or vulnerable to lapses in this particular police mindset. Unfortunately, high profile cases of police misconduct still occur. Such cases reduce public confidence and trust whenever questionable ethical misbehavior takes place.

In this presentation, several forensic psychological factors related to ethics as they apply to fulfilling the duties of an armed police officer are examined. There are three primary learning objectives of this presentation. The first learning objective includes introducing attendees the police cultural factors that may compromise, increase vulnerability, or call into question the person being evaluated. For example, these factors may include behaviors inconsistent with the police culture’s norms or values, corruption, favoritism, mistake prone, and misconduct.

The second learning objective involves risk assessment for three types of misconduct that include malfesance, misfeasance, or nonfeasance. The police culture is theoretically intended to shield individual officers. The culture is supposed to function as a base for all officers and protect them from erosion in their judgment. Lapses largely due to carrying out day-to-day duties while managing a boundary separation from distractions coming from the demands in their personal life. The third learning objective, the author presents a forensic case study as a practical example of using police ethics during an actual evaluation. Attendees are expected to be able to operate with an increased sensitivity to the role knowledge of police culture

and ethics can have on the selection process of which forensic examiners are a part.

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**Forensic Psychology, Police Culture, Blue Shield**

## **I24 A Decision-Making Approach for Offering Risk Assessment Ratings in Forensic Psychological Evaluations of Police Officers**

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After attending this presentation, attendees will be aware of the need to remain sensitive to negligent hire issues in selection of police officers.

This presentation will impact the forensic science community by using the Peace Officer Standards of Training's psychological dimensions as an evidence-based anchoring base for forensic examiners to use while making the risk assessment rating.

Risk assessment in the context of a forensic evaluation of police officer refers to the identification and psychological weighing of potential factors that are expected to compromise judgment or increase concern about stress vulnerability that can fuel misconduct. In the aftermath of a high profile police misconduct incident, once details are disclosed it becomes clear that there were matters that should have previously raised red flags for the department. The financial costs to departments and municipalities for police officer improper action reinforces the need to reduce the lawsuit risks where negligent hire can erode public trust. Insurance rates are also expected to rise in the wake of such cases. Following a high profile police-involved incident, attorneys representing the alleged victim are quick to request copies of all forensic psychological evaluations of the involved officers. What are they looking for or a better question might be, what is their forensic evaluator looking for? In this case, any part of an officer's behavior (i.e., on or off duty), in the distant past or recent can function as a basis for the claim that a particular officer should not have been hired or allowed to remain on the job. For example, an off-duty officer shot and killed his romantic partner with his service revolver. He was convicted of involuntary manslaughter. His blood alcohol count was below the legal limit. There was a long history of domestic violence in this relationship. The victim's family's suit claimed negligent hire on two primary bases. First, the officer had one occasion around the age of 12 when he imbibed a six-pack of beer as recorded on a form used by the department psychologist during the pre-employment psychological interview. The accused officer was said to have an alcohol problem that the department should have used to rule him out as an officer. Second, the department's psychologist failed to write a report on the accused officer.

There are three learning objectives associated with this presentation. First, attendees will be made aware of the need to remain sensitive to negligent hire issues in selection of police officers. For example, what is not as well-known is that police officers must pass a psychological screening in advance to their being sent into the department's academy. The screenings function to eliminate those applicants assessed as poorly suited or in the case of a fitness for duty evaluation, fit or unfit to return to work. The psychological evaluation provides a barometer of the evaluatee based on the original referral questions.

The second learning objective of the presentation uses the Peace Officer Standards of Training's (POST) psychological dimensions as an evidence-based anchor for forensic examiners to use while making the risk assessment rating. A practice-relevant forensic case study is used to demonstrate how the POST psychological dimensions can be used in this risk assessment process. The goal is that attendees from this presentation will have a greater understanding of the forensic elements associated with use of the psychological dimensions. The community is expected to gain from the enhanced forensic competencies of examiners who use this recommended approach to risk assessment ratings of police officers.

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**Forensic Psychology, Risk Assessment, POST**

## **I25 Forensic Applications of the Personality Assessment Inventory (PAI) With Pre-Employment Psychological Evaluations of Law Enforcement Applicants**

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After attending this presentation, attendees will understand the forensic-related standards associated with these types of evaluations, receive an overview of PAI scales and the development of the PAI report used for law enforcement.

This presentation will impact the forensic science community by improving upon the screening and selection process of peace officers who must interact with citizens in a wide range of stressful situations.

Research indicates that at least 80 percent of all law enforcement agencies use some form of personality testing during the psychological screening of applicants. The International Association of Chiefs of Police (IACP) recommends that at least two psychological tests be included in this forensic evaluation process. The Personality Assessment Inventory (PAI) is a standardized test that has been widely used in the evaluation process of law enforcement applicants. The PAI comes with a manual and software program to assist psychologists in the screening and selection of law enforcement applicants. This PAI resource contains a normative sample of PAI scores based upon more than 17,000 police officer applicants of various ethnicities from over 100 different agencies across the United States.

For example, Roberts et al. (2004) randomly selected a subsample of more than 3,000 police officer applicants as well as a cross-validation subsample of over 5,500 police officer applicants in an effort to test the PAI's usefulness in predicting poor performance. The psychological screening procedures resulted in the applicants in both subsamples to be administered a comprehensive psychological evaluation. This process included the California Psychological Inventory (CPI), the MMPI-2, the State-Trait Anger Expression Inventory (STAXI), the Johnson and Roberts Personal History Questionnaire (PHQ), the PAI, and a structured interview. The results obtained from the battery of tests administered, the applicants were assigned into one of five suitability categories – applicants who were assigned ratings of A, B, or C were later assessed as suitable recruits for hire. However, those assigned ratings of D or F were assessed as unsuitable. The PAI test results, Roberts et al. (2004) were used to craft a predictive equation to determine the likelihood that a police psychologist would rank an applicant as "poorly suited" for a career in law enforcement. Known as the Psychological Rating Risk Factor Statement (PRRFS), this probability index places an applicant into one of three categories of risk based upon her/his PAI responses: low risk ( $p \leq 24\%$ ), moderate risk ( $p = 25\% - 49\%$ ), and high risk ( $p \geq 50\%$ ). In general, the PAI appears to demonstrate potential usefulness in the police selection process.

A forensic case study of a police applicant will be used to demonstrate the utility of the PAI. The presenter recommends that forensic examiners become familiar with this assessment tool as well as the law enforcement report.

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**Forensic Psychology, PAI, Police Evaluations**

## **I26 Forensic Applications of the MMPI2-RF With Pre-Employment Psychological Evaluations of Police Officer Applicants**

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After attending this presentation, attendees will understand the forensic-related standards associated with these types of evaluations.

Attendees will also be introduced to the differences between the MMPI2 and MMPI2-RF tests and the use of these testing tools in law enforcement.

This presentation will impact the forensic science community by improving upon the screening and selection process of peace officers who must interact with citizens in a wide range of stressful situations. A forensic case study of a police applicant will be used to demonstrate the utility of the MMPI2-RF.

A large percentage of all law enforcement agencies rely upon personality testing during the psychological screening of applicants. This testing identifies the attitudinal, personality, and psychological aspects of functioning that may significantly interfere with an applicant's ability to carry out the duties of an armed police officer. Forensic tests of this type do not answer questions but generate hypotheses for follow-up review during the face-to-face forensic interview. The International Association of Chiefs of Police (IACP) recommends that at least two psychological tests be included in this forensic evaluation process. The MMPI2 and MMPI2-RF are standardized tests that are widely used in the evaluation process of peace officer applicants. Both the MMPI2 and MMPI2-RF come with manuals and software programs to assist forensic psychologists in the screening and selection of law enforcement applicants. This MMPI2-RF and MMPI-2 are important resources for work done in evaluating police officer applicants of various ethnic groups as well as diverse departments.

From a forensic standpoint, the MMPI has been subjected to extensive critical analysis. In 1965, Congress reviewed the MMPI through hearings because of issues raised about testing employees hired for government positions. The primary concern was whether a test like the MMPI invaded an individual's privacy during employee screening; however, Congress did not find any reason to outlaw the use of the MMPI. Although Congress failed to discover any reason to outlaw the use of the MMPI, the use of this tool to assess an applicant's suitability continues to receive scrutiny. For example, one of the questions is if the tool is designed to evaluate job performance, its normative data for police officers underrepresent women and minorities. The MMPI test elicits responses related to sexual orientation and religious attitudes and fails to measure the construct of conscientiousness (identified as one of the best predictors of job performance and work behavior). There is research indicating that the MMPI was found to be the most frequently used protocols for employment screening.

A side-by-side comparison of the MMPI-2 and MMPI2-RF is expected to highlight key features of both tests. In addition, the usefulness in providing data used in rendering forensic opinions and recommendations are expected to occur as a desirable by-product for examiners as well as the community.

A forensic case study of a police applicant will be used to demonstrate the utility of the MMPI2-RF. It is recommended that forensic examiners become familiar with this assessment tool as well as the law enforcement report.

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**Forensic Psychology, MMPI2-RF, Police Psychology**

## **127 Ethical and Professional Issues in Crafting Outside Evaluation Reports (OE Reports) for Police Officer Applicants**

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The goals of this presentation are to provide: (1) the forensic-related guidelines associated with conducting OE evaluations; (2) a recommended format for Outside Evaluation (OE) reports. For example, it may be appropriate to use the typical report format but the referral questions in these evaluations may require a format more consistent with the needs of the hiring authority; and, (3) suggestions for preparing for appellant hearings that may be held in connection with the OE report.

This presentation will impact the forensic science community by improving upon the screening and selection process of peace officers who must interact with citizens in a wide range of stressful situations by assuring professional responsible to conducting evaluations are properly informed about relevant ethical matters. A forensic case study of an OE report is used to demonstrate how ethical issues can arise.

Forensic psychologists must adhere to ethical and professional standards as they deliver services. According to the APA Committee on Professional Practice and Standards (2003), ethics "is defined as the rules or standards governing the conduct of members of a profession" (p. 595)." The main resources for standards of conduct for psychologists are the APA's (2002) Ethical Principles of Psychologists and Code of Conduct (referred to hereinafter as the Ethics Code). The American Board of Forensic Psychology has outlined recommended specific guidelines aimed at forensic psychologists. There are at least three areas of those guidelines that may be useful to forensic psychologists conducting evaluations in law enforcement settings. The areas include documentation and compilation of data considered, disclosing sources of information, bases of opinions, as well as comprehensive and accurate presentation of opinions in reports and testimony. Forensic psychologists are sometimes called upon to craft outside evaluation reports (OE reports) on appellants who have been disqualified or rejected on psychological grounds. It is also possible that an agency, county, or department may contract with a forensic psychologist to perform an independent evaluation of an appellant or incumbent officer. Some departments or hiring authorities have developed standards (e.g., minimum number of police evaluations, licensure, and experience in law enforcement) as pre-requisites for conducting Outside Evaluations; however, there is an absence of standards for conducting such evaluations as well as ethical guidance for qualified forensic examiners performing them.

A less experienced forensic examiner may be tempted to use methods or techniques they commonly rely upon in their customary work but be less aware of the ethical issues in this type of work. As a result, the examiner may unwittingly stumble into ethical dilemmas but still be held accountable for their actions or lack thereof. Second, a recommended format for OE reports is provided. For example, it may be appropriate to use the typical report format but the referral questions in these evaluations may require a format more consistent with the needs of the hiring authority. Third, suggestions are made for preparing for appellant hearings that may be held in connection with the OE report. For example, the entire evaluation has legal implications and the forensic examiner could be subpoenaed to provide testimony regarding the OE report. Examiners with limited experience in these areas may be caught unprepared for the often rigorous cross examination regarding a department's psychological screening standards, professional literature as well as other resources frequently used in police and public safety psychology.

The goal is that this presentation will impact the forensic examiners by sensitizing them to ethical, legal (e.g., negligent hire), and professional issues related to OE reports. The benefits to the community include improving upon the screening and selection process of peace officers who must interact with citizens in a wide range of stressful situations by assuring professional responsible to conducting evaluations are properly informed about relevant ethical matters. A forensic case study of an OE report is used to demonstrate how ethical issues can arise. The presenter recommends that examiners become familiar with problems identified by empirical studies of forensic reports.

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**Forensic Psychology, Ethical-Legal Issues, OE Reports**

## 128 Is This a Real Suicide Note? Authentication Using Statistical Classifiers and Computational Linguistics

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After attending this presentation, attendees will learn a method of suicide note authentication using both a quantitative, validated software tool Suicide Note Assessment Research (SNARE) and qualitative assessment based on database extraction. Attendees will learn how this method has been used in actual cases of death investigation and how it can be used in psychological assessment. Students will also learn how validation testing is performed for behavioral and linguistic datasets.

This presentation will impact the forensic science community by showing why a suicide note assessment is essential for investigating some deaths which could be classified as a homicide or suicide. The method presented here using SNARE illustrates validation testing for behavioral and linguistic datasets, and thus also advances the methodology of forensic science.

Identifying suicide notes is an extremely difficult task. Part of this difficulty is common to many identification tasks with low accuracy rates. First, if the object to be identified is rare or experience with the object to be identified is low, identification rates are typically low because the identifiers do not have a well-founded grasp of the identifying features. Second, if the object to be identified has low internal consistency, because the object has a wide range of class characteristics, then identification accuracy is predictably low, because the object can be mistaken for so many other types of similar objects.

Suicide notes are difficult to identify accurately because, first, they are rare in most investigators' experience, and second, they are not highly consistent or stereotypical. Suicide notes are found in only the minority of actual suicides (it is estimated that notes only occur in 10 to 15% of suicides).<sup>1</sup> Suicide notes contain elements of other text types, such as apologies and love letters, which can make them easily confusable with other text types. Suicide notes as a whole are internally inconsistent –no one feature set is present in all. It is not surprising then that psychiatrists and psychologists are claimed to have an accuracy rate for identifying texts as suicide notes or not at ranges from 50% to 70% as even this group of professionals may not have consistent experience with real suicide notes, and the experience is informed by a group of documents which are not necessarily similar to each other.<sup>2</sup> Bennell's work on police decision-making mentions the possibility of a quantitative analysis for verifying suicide notes.<sup>3</sup>

SNARE is a computational and quantitative tool for identifying and classifying suicide notes. SNARE currently has obtained an accuracy rate of 80% on a dataset of 400+ real suicide notes and 500 control documents. When the real suicide note data is limited to brief notes (45 words or less) the accuracy rate increases to 86%. The dataset has been vetted. The statistical classifier is a linear discriminant function analysis using leave-one-out cross-validation. The reported accuracy rates are the average of true positives for the real suicide note class and the control document class.

The quantitative method enables the analyst (death investigator, psychiatrist, or psychologist) to start from an objective point in the investigation, since the software analyzes the questioned text without input from the analyst. But given an error rate from 14 to 20%, the analyst should then use SNARE to collect a pool of comparative documents from the real suicide note dataset. By comparing the questioned document to real suicide notes with similar characteristics, the analyst can determine whether SNARE has made a classification error or not.

Methodologically, the most interesting aspect of this second step is the requirement that the analyst seek to disprove the SNARE classification. The two-step analysis based on a quantitative classification followed by

qualitative assessment allows for the application of analyst knowledge, preferably in line with Leenaars' work.<sup>4,7</sup>

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### Suicide Note, Computational Linguistics, Validation Testing

## 129 What Happens When a Dream Is Deferred and the Village Erodes: Application of a Diathesis-Stress Model to Risk and Protective Factors for Suicidality in Racial/Ethnic Minority Youth

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The goal of this presentation is to facilitate an understanding of how a stress diathesis model may be applied to risk and protective factors regarding suicidality for minority youth. After attending this presentation, attendees will better understand how this model applies to conceptualizing the literature's findings regarding societal, community and individual contributions to suicidality in minority youth age 10 to 24.

This presentation will impact the forensic science community by facilitating an understanding of the risk and protective factors of suicidality in minority youth. Specifically, this paper will explore potential individual, relational, community, and society-based factors relevant to suicidal behavior in this population. Recommendations for applying the diathesis-stress model to suicide prevention and intervention will also be provided.

According to the Centers for Disease Control and the National Institute of Mental Health, suicide is a major public health concern for youth; specifically, it is the third leading cause of death for individuals ages 15 to 24. Research indicates clearly that all demographic groups are susceptible to this public health problem. Over the last several decades, suicidality has become increasingly problematic for racial/ethnic minority youth, such that more are attempting and completing suicide; however, suicide within this vulnerable group is poorly understood. As a consequence of this, many warning signs and potential points of intervention are missed, resulting in preventable suffering and death for a notable segment of the population.

Currently, in majority and minority group studies, suicidality is approached as an issue arising from the individual alone or in relationship to others. Occasionally, certain cultural factors such as vulnerability to

academic problems, disproportionate incarceration rates, and health disparities are discussed; however, the effects of community and broader societal changes on mental health functioning for minority youth, particularly suicidality, are rarely considered. The diathesis-stress model may more comprehensively conceptualize the complex interactions between these various factors and suicide-related risk and protective factors for minority youth. According to this model, subsequent to exposure to negative life events, vulnerable persons are more likely to develop psychopathology. This may be due to actual or perceived disparity between contextual demands and resources. For example, an educational environment's demand for appropriate academic performance requires the individual have particular resources, such as sufficient cognitive ability and access to technologies and materials. Perceived disparity between demands and resources, meaning where one's resources seem deficient compared to the environment's demands, generates psychological distress. The greater the perceived disparity, the greater one's distress. Such distress varies widely in intensity and may range from mere irritation to complete hopelessness.

Factors such as age, gender, sexual orientation, and perceived social support are generally related to suicidality within youth populations. Risk and protective factors for suicide; however, may vary among groups of racial/ethnic minority youth. Under the diathesis-stress model, such group-specific factors may indicate changes in individual, relational, community, and societal demands and/or resources that have generated increasing and maintaining high levels of distress for minority youth. Added to increased exposure to suicide, these changes in contextual demands and/or resources, and the distress generated by the perceived discrepancy between the demands placed upon minority youth and their access to necessary resources may result in increased vulnerability to suicide.

This paper will review the public health and social science literature from 1990 to the present for trends in suicidality among minority youth. Empirically supported risk and protective factors among this population will also be reviewed. These trends and factors will be examined for their application to the diathesis-stress model of psychological functioning and distress.

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#### Minority Mental Health, Stress, Suicide

### 130 Adolescents and Crime

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The goal of this presentation is to present the solution for children in conflict with law, by analyzing socio-economic features, style of crime, characteristics of the family, and the reasons of the juvenile delinquency.

This presentation will impact the forensic science community by underlining the importance of rehabilitation of children in conflict and giving recommendations for the treatment that children in these situations need.

**Introduction:** Childhood and adolescence are rapid development cycles. Adolescents may not be aware of the meaning and the result of their behaviors because of their rapid biological and psychological fluctuations. Due to this agitated cycle, The United Nations Convention on the Rights of the Child states that every human being below the age of eighteen years is considered a child, unless under the law applicable to the child, majority is attained earlier. Therefore, any crime that they commit is assessed by this rule. The most important attribution that allocates child crime than adult crime is that childhood contains the adolescence which is considered a problematic or alternation cycle. The crime that is committed must be evaluated in the developmental period which the human being is in.

Some theories show that in adolescent criminal behavior, genetic, psychiatric, and psychological problems are important and at the current time it is believed that environmental factors play a more important role.

In recent years, the incidences of juvenile delinquency increases day-by-day. In Turkey, the incidence of juvenile delinquency is progressing rapidly. In order to understand when an action becomes crime known the component of crime, reasons of crime, and punishable situations, because all the actions must be considered within these parameters. The crime theory explains which behaviors are against the law and called crimes and which are not. Since juvenile delinquency is a social problem, there is an urgent need to find solutions to make a healthy society.

**Material and Methods:** The sample consisted of 121 children and adolescents between the ages of 12 and 17 who entered in the Turkish forensic system as children in conflict with the law between the years 2008 to 2011. All of the children were given psychological evaluations and all the data relating to these children and adolescents were taken from the reports of the court's psychologist.

**Results:** Of the 121 children included in this study, 89 (73.75%) are males and 32 (26.5%) female. Twenty-three (19%) of all children are between 12 to 14 years, 98 (80.1%) of them are between 15 to 17 years. In females the average age was 15.18 years. In males, this number was 15.93 years. Of all of the offenses, 92 (76%) were crimes against property and 29 (24%) were crimes against persons.

**Conclusion:** Previous studies considered children between the ages 16 to 17. In this paper 80.1% of the children were between the ages 15 to 17. These data are consistent with the literature. In this paper, 73.5% of the children in conflict with the law are male. It is possible that more boys become involved with the forensic system than girls because boys are more likely to spend time out of the home and out of family control.

A weakness of this study is the fact that all of the subjects came from one specific geographical region and court.

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#### Juvenile Delinquency, Children in Conflict With Law, Child

### 131 A Case Study Illustrating Sexual Grooming as Part of the Child Molester's Modus Operandi

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After attending this presentation, attendees will understand what constitutes sexual grooming, how this forms part of the pedophile's modus operandi in order to successfully offend against the victim as well as the application of grooming laws in South Africa and how this correlates with grooming as a legal concept in other countries.

This presentation will impact the forensic science community by providing law enforcement officers with a better understanding of grooming as part of the modus operandi of child molesters, enabling them as well as the community in general, to be able to identify these behaviors and prevent children from being abused, and to understand these behaviors from a cross national perspective.

Many pedophiles court children like an adult will court a partner with attention, affection, and gifts. In sex offenders, this behavior is referred to as grooming. The grooming process takes time until the child's inhibitions have been lowered and the offender progresses to sexual behavior. In South Africa, grooming is a crime under the Criminal Law (Sexual Offences and Related Matters) Amendment Act No. 32 of 2007.

Grooming behavior includes identifying with vulnerable victims, having access to children through, for example, baby sitting, seducing the child with attention, affection, and gifts. In general child molesters do not use force to engage children in sexual activities but rather count on different forms of psychological manipulation, grooming, and desensitization. Physical grooming entails the gradual sexualization of the relationship between offender and victim. The offender builds the child's trust, makes him or her feel good, and then starts to violate boundaries (e.g., progression from innocent touching to inappropriate touching or showing pornography to children). The victims' repeated acceptance of non-sexual touch early in

the grooming process could lead them to believe that they have given consent to more invasive sexual contact. In this way, the desensitization process seems to promote cooperation and reduces potential for disclosure. When confronted about engaging in such activities, pedophiles commonly justify and minimize their actions or make excuses while others use cognitive distortions or redefine their action as love and support.

The following case study will be presented to illustrate typical grooming behavior and cognitive distortions or excuses of a sentenced child molester in the South African context.

A 62-year-old male groomed his step granddaughter from the age of seven by gaining her and her parents' trust. He sexually molested her from the age of eight and sexually penetrated her from about the age of nine until eleven. He took photos of her vaginal area and was in possession of other child abuse images. He pleaded guilty on five charges of rape and manufacturing of pornographic material. He was sentenced to life for the rape charge, and five years for the charge of manufacturing of pornographic material.

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**Sexual Grooming, Child Molester, Sexual Abuse**

### **I32 From Classification to Clinical Practice: Current Psychiatric Understanding of Stalking Behavior**

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After attending this presentation, attendees will: (1) understand the legal and mental health definition of "stalking"; (2) review the epidemiology of stalking; (3) to review typologies and classification of stalking; (4) understand how stalking behavior is associated with DSM diagnosable disorders; and, (5) use our current understanding of stalking behavior to comment on risk management and treatment strategies.

This presentation will impact the forensic science community by reviewing stalking, an often overlooked behavior, that has a high potential for violent outcomes, from a psychiatric and treatment perspective.

Prior to the 1990's, relatively little research had been performed regarding the conceptualization, understanding, or treatment of stalking behavior. This is somewhat surprising given the high prevalence of stalking in the community. As many as 8% of women and 2% of men state that they have been stalked at some point in their lives and as many as one in four cases of stalking in the United States will culminate in significant violence. Public appreciation of stalking as a behavior was, and in some respects still is, largely limited to sensationalized cases involving the stalking of high-profile celebrities such as Madonna or Princess Diana. What constituted "stalking" has not always been clearly defined as a legal term. Although less than in earlier times, laws defining the crime of stalking can vary dramatically from state to state. It is no surprise, then, that the psychiatric understanding of stalking as a concept has lagged behind study of other potentially violent behaviors and is just recently emerging as an area of study.

As legal definitions of stalking are unique to each jurisdiction, there is no consensus definition of stalking in the mental health literature either. J. Reid Meloy's definition is one of the most widely cited and defines stalking as "the willful, malicious, and repeated following and harassing of another person that threatens his or her safety" (Meloy and Gothard, 1995). Other descriptive terms such as "obsessional following" have also come to be accepted as synonyms for this behavior. In general, the various definitions typically seek to describe a behavior that is: (1) a pattern of intrusive behavior or harassment; (2) a resulting implicit or explicit threat; and, (3) as a result, the threatened person experiences reasonable fear. Numerous typologies have attempted to classify staking behavior for identification, risk stratification, treatment, or other purposes. Most typologies have been based on the stalker's relationship with the victim, the context in which that

relationship is based, and the degree to which violence or the potential for violence is an issue. However, no single classification system has ever been agreed upon in the forensic community.

One of the most difficult obstacles in conceptualizing stalking is that it is a behavior that can be the manifestation of numerous currently understood mental health diagnoses. It does not exist as a stand-alone diagnosis in DSM IV-TR nor is there any current plan for inclusion in DSM-V. Those who exhibit stalking behaviors may be classified as having a multitude of different Axis I and Axis II diagnoses. While it is classically taught that obsessional followers have erotomatic delusions or de Clereambalt's syndrome (i.e., erotomania), only a small minority of perpetrators appear to fit this mold. Schizophrenia, schizoaffective disorder, bipolar disorder, delusional disorder, major depressive disorder, adjustment disorders, cluster B personality disorders, cluster A personality disorders, and others have all been seen to manifest in stalking behavior. Because of this complexity, treatment recommendations for stalking behaviors have often been generalized and inexact.

The goal of this presentation will be to review the most recent and accepted stalking typologies and attempt to place them in context of DSM diagnoses that are familiar to practitioners. Current recommendations regarding risk management in the stalking situation will be reviewed. Finally, the current recommendations and evidence base for management and treatment of stalking behavior in the context of typographical description and diagnosis will be described.

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**Stalking, Obsessional Following, Psychiatry**

### **I33 A Particular Case of Latticed Allegations in a Nursery School in Brescia, Italy**

*Cinzia Gimelli, PsyD, PhD\*, Viale Montegrappa, 29/C, Reggio Emilia, 42100, ITALY; and Melania Lugli, PhD\*, Viale Montegrappa 29/C, Reggio Emilia, 42121, ITALY*

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After attending this presentation, attendees will learn tools to conduct a proper interview in cases of alleged sexual abuse.

This presentation will impact the forensic science community by helping evaluators to better distinguish between correct and incorrect procedures in interviewing children.

Sexual abuse of children is difficult to identify. The phenomenon rarely emerges as objective evidence of the abuse and children are the only witnesses of the alleged facts.

Sexual abuse of children has become the subject of great community concern and the focus of many legislative and professional initiatives. This is evident by the expanding body of literature on sexual abuse, public declarations by adult survivors, and increased media coverage of sexual abuse issues.

There are various reasons why children can make false allegations and a false report may also occur without any responsibility on the part of the child, often due to incorrect interviewing procedures with the child who is the alleged victim of abuse. Moreover, interviewers do not always have enough expertise to avoid errors.

Research has had a tendency to concentrate on the suggestibility of children and neglected other topics that may be of great utility to those who investigate suspected cases of child sexual abuse. More attention should have been paid in the brief to the complexities of assessment. Too much emphasis has been placed on children and not nearly enough attention on remedial actions to minimize the problems associated with suggestion. Finally, research shows that it may be possible to develop reliable and valid techniques to assess child sexual abuse allegations.

The best way for interviewing children is the foundational structure of the interview itself. If the child is approached in the wrong manner with limited efforts to make them feel comfortable during the reconstruction of the incidents, the final product could be useless. It is important to encourage children to tell particulars as well as any detail of the alleged incidents in a spontaneous way.

Cross-contamination between children and/or their families could also, in part, explain the latticed allegation problem.

If most of the alleged victims know one another, then is it possible that children will talk among themselves about their allegation, resulting in contamination.

The purpose of this study was to examine a particular case of latticed allegations involving more than 20 victims between four and five-year-olds in a preschool in Brescia, a city in the northern part of Italy.

In this case, as in all latticed allegations, although some statements stem from real elements, the proliferation of reports is attributed to a range of mutual contaminations made, as this research has highlighted, mainly by bad interview techniques. This research compares the emerging Italian case studies on this phenomenon with the well established cases in the United States.

This presentation will also address how to consolidate the best practice in psychological forensic sciences and how it is important to integrate this practice together with the crime scene activities and lab analyses especially in cases of alleged child abuses.

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**Latticed Allegations, Brescia, Nursery School**

### **I34 Implementation of a Quality Improvement Program to Predict and Prevent Inpatient Violence**

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After attending this presentation, attendees will understand how to use the Broset Violence Checklist (BVC) to predict violence on an inpatient psychiatric unit and will consider methods of implementing quality improvement projects on inpatient psychiatric services

This presentation will impact the forensic science community by educating forensic scientists about acute violence prediction and strategies to implement systematic programs to reduce violence in hospital settings.

**Proposition:** This project will investigate the predictive value of admission BVC scores on imminent dangerousness in an acute psychiatric inpatient setting. In addition, methods behind the implementation of a violence reduction quality improvement program will be examined with subsequent proposals for improving and/or continuing staff satisfaction in quality improvement programs.

**Synopsis:** Bellevue Hospital Center, a public hospital in New York City with over 300 inpatient psychiatric beds, 20% of which are devoted to the treatment of incarcerated males from Rikers Island, implemented a quality improvement project, the Violence Reduction Pathway (VRP), on all of its adult psychiatry units in 2009. The goal was to identify patients who were at imminent risk of violence and then develop specialized treatment plans for those patients in order to reduce episodes of future violence. As part of the VRP, the Broset Violence Checklist (BVC) was used to assess imminent risk of violence.

The BVC measures the presence or absence of one of six behaviors/symptoms that have been shown to have significant relationships with violent episodes within 24 hours: confusion, irritability, boisterousness, physical threats, verbal threats, and attacks on objects. The creators of the BVC, which was designed based on medical records examined at a Norwegian maximum security forensic hospital,<sup>1</sup> recommended a cut-off score of two or greater as this yielded 63% accuracy in predicting violence and 92% accuracy in predicting non-violence within 24 hours of the score being received.<sup>2</sup>

The BVC has been validated in other inpatient psychiatric settings since its development,<sup>3,4</sup> although limits of reliability in reporting violent incidents and difficulties with prediction in certain populations,<sup>5</sup> such as female substance abusers have led to at least some concern about the scales'

generalizability.<sup>4</sup> Some have suggested changing the cut-off score in order to decrease the rate of false positives or false negatives.<sup>3,4</sup>

Although the VRP was implemented at Bellevue Hospital, there have not yet been any studies to explore its validity, impact on staff work flow, or effectiveness as a quality improvement project. Retrospective data collection on 100 consecutive admissions to the inpatient forensic psychiatric service will be collected. This will include demographic information, admission Broset scores, and information related to violence risk within the first 24 hours, specifically emergency medication administration, crisis management team interventions, and violent incident reports. The data will then be statistically analyzed to determine the predictive value of an admission Broset score of two or higher on the occurrence of each of these three events. In addition to the quantitative analysis, the qualitative arm of the study will describe the way in which the VRP was initially implemented and explore staff opinions about the VRP quality improvement project. A survey, distributed to all of the multidisciplinary staff involved in the VRP's implementation, will help characterize the logistical implementation of the project, the education provided to the staff about the project, the work load associated with its implementation, the perceived utility of the VRP, and any suggestions for improvement. The information provided from this study will help guide future policy decisions regarding inpatient violence risk assessment.

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2. Almvik *et al.* The Broset Violence Checklist: sensitivity, specificity, and inter-rater reliability. *Journal of Interpersonal Violence.* 2000;15(12):1284-1296.
3. Abderhalden *et al.* Predicting inpatient violence in acute psychiatric wards using the Broset-Violence-Checklist: a multicentre prospective cohort study. *J Psychiatr Ment Health Nurs.* 2004;11:422-427.
4. Bjorkdahl *et al.* Nurses' short-term prediction of violence in acute psychiatric intensive care. *Acta Psychiatr Scand.* 2006;113(3):224-229.
5. Woods *et al.* Piloting violence and incident reporting measures on one acute mental health inpatient unit. *Issues Ment Health Nurs.* 2008;29(5):455-469.

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#### **Violence Prediction, Broset, Quality Improvement**

### **I35 The Use of Brain Imaging in Legal Matters**

*Jonathan Manaoat, MD\*, PO Box 86125, Los Angeles, CA 90033*

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After attending this presentation, attendees will understand some of the principles of brain imaging. Moreover, recent brain imaging findings pertinent to the understanding of criminal behavior will be presented. In addition, applications of brain imaging in legal issues, especially court cases and legal precedent will be discussed. Lastly, the future applications of brain imaging in the forensic and legal setting will be discussed.

This presentation will impact the forensic science community by helping those in the field understand the basis of brain imaging studies and how they have been or may be applied to legal and forensic issues.

Following the decade of the brain in the 1990's, our understanding of the brain has grown immensely. In particular, advances in neuroimaging have worked to further our understanding of the structure, function, and correlations with typical development and disease processes. This paper will review the different brain imaging studies used towards these endeavors. This includes positron emission tomography (PET), single positron emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI), and anatomical magnetic resonance imaging (aMRI). Moreover, an assessment of the strengths and limitations of each technique will be presented, including the replicability and suggestions on how to improve the rigor of these studies.

Applications of these findings and the implications for criminal behavior will also be presented. A review of the legal precedents involving brain imaging will be conducted. An assessment of the present applicability of brain imaging to legal proceedings will be discussed. Moreover, the question of whether the legal standards of *Daubert* and *Frye* should be applied to these studies will be addressed.

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**Brain Imaging, Legal, Evidence**

### **136 Causing Parental Alienation is a Form of Child Abuse**

*William Bernet, MD\*, Vanderbilt Psychiatric Hospital, 1601 23rd Avenue South, Suite 3050, Nashville, TN 37212-3182*

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After attending this presentation, the attendees will understand the concept of parental alienation, how parental alienation comes about, and why parental alienation should sometimes be considered an example of emotional or psychological child abuse.

This presentation will impact the forensic science community by increasing the awareness of mental health and legal professionals regarding parental alienation; increasing the knowledge of mental health and legal professionals regarding emotional and psychological child abuse; and perhaps leading to the adoption of the proposal that causing parental alienation should be considered an example of child abuse.

Parental alienation is a serious mental condition that sometimes occurs in a child when his or her parents are engaged in a high-conflict divorce. In parental alienation, the child allies himself or herself strongly with one parent (the preferred parent) and rejects a relationship with the other parent (the alienated parent) without legitimate justification. Parental alienation should be considered a mental disorder because the child's rejection of the alienated parent is driven by the false or illogical belief that the parent is evil, dangerous, or not worthy of love. In contrast, parental estrangement is normal behavior, not a mental disorder. Estrangement is the rejection of a parent for good cause, e.g., abuse or neglect.

In some instances, parental alienation is brought about by the indoctrination of the child by the preferred parent against the rejected parent. If the preferred parent has indoctrinated the child in a knowing, purposeful, persistent manner to hate and avoid the alienated parent, that behavior should be considered emotional or psychological abuse of the child. In practice, these cases are complex: Parent A might accuse Parent B of physically abusing the child, so it is normal for the child to dislike Parent B. However, Parent B might accuse Parent A of indoctrinating or brainwashing the child, so the child's dislike for Parent B is based on a false belief.

Although emotional/psychological abuse of a child is not currently included in the *Diagnostic and Statistical Manual of Mental Disorders*, it is included in the *International Classification of Diseases*. Emotional/psychological abuse typically includes behaviors such as: rejecting the child; isolating the child from normal social experiences; terrorizing the child verbally and with threats of assault; berating the child; and overpressuring the child. Indoctrinating a child to dislike and reject a parent should be considered emotional/psychological abuse.

There is considerable international research to support this proposal. The work of psychologists, psychiatrists, and legal professionals from Canada, Germany, Italy, Spain, South Africa, the United Kingdom, and the United States will be summarized. In some instances, state and national governments have taken totally opposite positions regarding parental alienation. For example, the governments of Brazil and of the state of Querétaro, Mexico, have made it illegal to induce parental alienation in a child. In some cases, the European Court of Human Rights has recognized the serious nature of parental alienation. In contrast, the legislature of California has considered doing exactly the opposite, that is, making it illegal for a court to consider parental alienation in a child custody

proceeding. It is important that parental alienation be understood and recognized by mental health and legal professionals.

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**Parental Alienation, Child Maltreatment, Emotional/Psychological Child Abuse**

### **137 Matricide: Criminological Understanding Beyond Psychosis — An Italian Case Series Study**

*Felice F. Carabellese, MD\*, and Chiara Candelli, PhD, Section of Forensic Psychiatry, University of Bari, Piazza Giulio Cesare, 11, Bari, 70124, ITALY; Gabriele Rocca, MD, Section of Forensic Psychiatry, University of Genova, Via De Toni, 12, Genova, 16132, ITALY; and Roberto Catanesi, MD, Section of Forensic Psychiatry, University of Bari, Piazza Giulio Cesare, Bari, 70124, ITALY*

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After attending this presentation, attendees will be able to recognize some of the features of matricide and understand principles of forensic psychiatric assessment of matricidal criminals.

This presentation will impact the forensic science community by assessing the role of the mother-son bond in the etiology of matricide.

Matricide, or killing of one's own mother, is one of the rarest of reported murders, with a rate of about 2% of all homicides worldwide. Historically, this type of crime has been known since ancient times and also has a mythological reference (the Orestes complex). According to the literature, a son who kills his mother is usually a single, unambitious young man with an intense relationship with his mother, a lack of interest in other women, a feeling of social inferiority, and an absent or passive father. Matricide seems to be more common among or specific to individuals with severe psychiatric disorders, especially schizophrenia. Among schizophrenic offenders the paranoid subtype is the most common. Other diagnoses include mood disorders, substance abuse, and personality disorders. Many offenders suffered from psychosis at the time of the crime, and their acts were influenced by persecutory delusions and/or auditory hallucinations.

A history of a problematic relationship between the mother and son is typically present. Offenders often report feeling that their mothers were either ambivalent toward them, or excessively domineering; these relationships have been described as "mutually dependent but hostile." Matricides are classically committed in the victim's home, usually using a weapon, although asphyxia is also common. In some cases "overkill" is reported, involving extreme violence.

To better understand the characteristics of matricidal criminals and the psychopathological mechanisms related to this behavior, a study of ten matricides committed in Italy will be presented. The forensic psychiatric examination reports of the ten offenders (age range 21-53 years, mean age 37) were retrospectively analyzed to study several variables regarding the homicide features as well as offender characteristics and motives. The diagnoses were collected exactly as they appeared in the reports and evaluated on the DSM-IV-TR criteria. The assessment of the degree of criminal responsibility, according to the examining forensic psychiatrist, was extracted (full responsibility/diminished responsibility/no responsibility) and analyzed.

From a medico-legal point of view, there were two deaths by shooting, one deliberately provoked fall, three smotherings, two bashings, and two stabbings in which the injuries were due to more than one weapon or action.

Clinically, the most common diagnosis was schizophrenia (5/10); two offenders were found to have other psychoses and three perpetrators suffered from an Axis II disorder. Of the offenders, seven out of ten (7/10) were found not guilty due to insanity, one was judged to have a diminished responsibility, and two to be fully responsible.

It is interesting to note that although most of the cases were diagnosed with schizophrenia or other psychoses, not all the perpetrators who committed matricide had psychotic symptoms at the time of the offence

(i.e., delusional thinking or hallucinations). On the other hand, in all cases a “pathologic” mother-son bond was found. In particular, in accordance with the literature, the victims were ambivalent mothers, with an intruding, domineering but symbiotic relationship with their sons that was mutually dependent but intrinsically hostile. Against this background many different motives, both “psychotic” and non-psychotic, can trigger the crime. Each of these must be accurately described and analyzed to reconstruct the dynamics of the matricide and understand its genesis.

It is not always persecution delusions, therefore, that cause these crimes. Mental disease is not the only causal factor and is not enough, taken alone, to explain the crime. Indeed, the observation that different psychopathological pictures induced a “perverse,” dysfunctional bond over the years, leading to the same tragic epilogue, seems to confirm that something more needs to be searched for, presumably in the history of the mother and son, and especially the way their relationship developed and crystallized over the years, also due to the absence or passivity of the other parent.

A psychiatric-forensic study of the entire existential history of both people, as well as of the socio-economic context, is therefore needed to understand the roots of this tragic crime. The peculiar dynamics of the mother-son relationship, as well as their unique personalities and life experiences, are the key to cases of matricide.

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#### **Matricide, Psychosis, Mother-Son Bond**

### **I38 Parricide by Pre-Teens: Case Review and Study**

*Sandra Hah, MD\*, Los Angeles County, University Southern California, 2010 Zonal Avenue, Outpatient Department, 1st Floor, 1P-2, Los Angeles, CA 90033*

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After attending this presentation, attendees will be familiar with several cases of parricide by pre-teens and be better acquainted with the core issues that are present in the legal, ethical, and psychiatric dilemmas surrounding these cases.

This presentation will impact the forensic science community by providing a platform to discuss the challenges that child parricide cases present to the legal system, as well as highlighting the complex ethical issues that result when the courts must consider punishment versus rehabilitation. This presentation will also serve as a forum in which to discuss several salient psychological and forensic psychiatric issues that arise in child parricide cases.

Parricide is an extremely rare occurrence and parricide committed by pre-teens (children ages twelve and younger) is practically unheard of. On average, there are between two hundred to three hundred parricides a year which represents approximately two percent of all homicides. Only a few of these parricides are committed by minors and of these cases, there may only be a handful of parricides by a pre-teen. The vast majority of parricide cases involve sons murdering their fathers with a firearm. The smallest subgroup of parricide is daughters who commit matricide. There are several etiological theories on parricide by minors. Several studies show that minors who commit parricide are often victims of abuse (physical, sexual, and emotional) or violence, and that they murder as a way to end the abuse. Yet, another theory outlines a shame-rage cycle in the pre-teen that occurs as a result of a contentious relationship with the parent who is the victim of the parricide. There is a smaller subset of children who commit parricide as the result of a severe mental disturbance, and yet an even smaller subset of children who commit parricide for seemingly inconsequential reasons. This presentation will serve as an outline to highlight these theories, and illustrate that there may not necessarily be any pattern or predictability to parricide.

This presentation will also offer insight into the complex issues that must be addressed in the evaluation of the preteen from a forensic psychiatry perspective, as well as the trial proceedings. Several court proceedings and case law files will be reviewed as examples of the specific

legal dilemmas that will be discussed. These include; various defense strategies such as battered child syndrome (BCS), how to ensure a fair trial to a child, and how to determine whether the child knew the difference between right and wrong at the commission of the parricide. Ethical dilemmas reflect the strong emotionality of child parricide cases, and include the debate about the fairness of rehabilitating versus punishing children, and whether it is ethical to hold a developing child responsible for a crime for which they may not fully understand the consequences and outcomes. Forensic psychiatric issues focus on examining whether there are any patterns of behavior or psychiatric illness in children who commit parricide. Additionally this presentation will address whether there are any reliable risk factors that can be identified and used to predict this kind of dangerous behavior in a child.

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#### **Child Parricide, Forensic Psychiatry, Legal Defenses**

### **I39 Incest: Analysis of a Case**

*Gaye Ozmen\*, and Ahmet Yilmaz, Istanbul University Forensic Science Institute, Istanbul, TURKEY; and Coskun Yorulmaz, MD, Istanbul University, Istanbul University Forensic Science Institute, Department of Forensic Medicine, Cerrahpasa, Istanbul, 34099, TURKEY*

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After attending this presentation, attendees will be able to: (1) identify the definition and causes of incest; (2) identify the characteristics of offender and victim; and (3) understand the indicators of sexual abuse and the process of the event when it occurs identify the signals of abuse clearly with the presentation of points of sexual abuse and incest with a case. This case is different than the other cases in literature, with the behaviors of victim’s mother, who was active in speaking up, bringing the case to court, and preventing the victim from seeing her father.

This presentation will impact the forensic science community by presenting an incest case which is forbidden in every culture throughout history.

**Introduction:** Child abuse and neglect, a universal problem, causes physical and psychological damage that negatively affect well-being and life-long development of exposed children.

When an adult uses a child for sexual stimulation or satisfaction, this is considered sexual abuse. Likewise, child prostitution or child pornography, touching of the genital area, exhibitionism, pornography, and rape can all be forms of child sexual abuse.

The word “incest” comes from “incestare” which means contaminating and aspersing and comes from “incestus” which means dirty in Latin language.

Incest is a sexual relationship between people related by blood. Because it occurs within family and is also forbidden in every culture, it is hard to reveal incest. It is emphasized that offenders in incest cases are usually fathers, the mother of family is a passive family member and also usually aware of the incest case.

**Methods:** The case was a 13-year-old girl described as a very successful secondary pupil. She disclosed that she had been a victim of incest for one-and-a-half years by her father. It was said that the beginning of the incest case was with her father’s touching offer. She thought by threatening to tell, her father would stay away. Instead, the father began to abuse her and tried to destroy the relationship between the victim and her mother.

Her father began to beat her and tried to touch her sexually whenever he could. She kept quiet in order to keep her family together. After this situation, she disclosed that she began to have nightmares, eating and sleeping disorders. The offender father was born in 1960 and worked as a taxi driver. He had no sexual relationship with his wife for the last five years. He had nervous problems and been in treatment. Mother was born in 1963, house wife, she was economically dependent to her husband. She stated she had been raped by her husband at the beginning of their marriage but for the last five years they had no sexual relationship. She stated that she had found some pornographic scenes on her husband’s personal

computer after the case began.

The case arose when some relatives and neighbors told the mother that her husband had offered them sex. The mother talked to her daughter who had been very depressed recently. The daughter told her mother what had happened and the case was brought to law enforcement.

The literature about incest reveals that the mother of victim often chooses to keep the abuse secret, blame her child, and deny the facts because she is afraid of divorcing. In this case, although the mother was economically dependent on her husband, she was the pioneer person who brought the case to light by first bringing her daughter to the hospital and then contacting the police.

**Conclusions:** Incest is rarely revealed because it is forbidden and also it occurs within the family. This paper attempts to clarify the signals of sexual abuse and incest by describing a specific case. This case is consistent with the literature about the characteristics of offender and victim, but differs greatly in the attitude of the mother, which helped to reveal this case.

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**Incest, Sexual Abuse, Child**

## **I40 A South African Case Study Illustrating Multiple Intimate Partner Murder: Serial Murder or Not?**

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After attending this presentation, attendees will have an understanding of what serial murder is, what intimate partner murder is, and whether a murderer who committed multiple intimate partner murders can be classified as a serial murderer.

This presentation will impact the forensic science community by showing how law enforcement officers, psychologists, and profilers will have a better understanding of intimate partner murders and serial murders, and how intimate partner murderers can repeat these crimes and become serial murderers. It will enable attendees to identify danger signals and prevent more tragic incidents.

Intimate partner murders are often referred to as domestic murders or spousal homicide. This occurs when one person is murdered by their current, or ex-, intimate or romantic partner. Motives for such crimes typically include an element of jealousy, which may be due to real or feared abandonment by the victim. While some such perpetrators have a history of physical abuse towards their partner, a significant number of offenders have no history of violence towards their partners, and often there is no escalation of violence preceding the murder.

A serial murderer murders at least two people at different times and for a primarily intrinsic/psychological reason. Motives include anger, ideology, power, thrill, psychosis, and sexually based. Serial murderers tend to stick to one main method of obtaining and murdering their victims. They also tend to keep to a certain victimology in most instances.

The following case study will be presented to illustrate how one person committed three murders, two of which were intimate partner murders, and discuss whether he can be regarded as a serial murderer or not.

A 26-year-old man murdered his high school sweetheart by shooting her seven times in her office at the university where she worked. This occurred when she ended the relationship after eight years and he went to her place of work and murdered her with a pistol he stole from his brother. He was found guilty of the murder and sentenced to 15 years imprisonment.

After 12 years he was released from prison, and he soon became romantically involved with another woman. The relationship was tumultuous and violent, with alcohol abuse by both parties. After an

incident where he assaulted his girlfriend, she denied him any contact with their daughter. When he tried to reconcile with her, it resulted in a dispute after which he murdered his girlfriend by stabbing her twice and her mother three times, with a knife.

When the last two murder cases went to court, arguments were heard at sentencing about the possibility of this person being classified as a serial murderer. These were the second and third murders committed by him. There was a clear, inner psychological motive for the crimes which seemed to stem from his interaction with females he was romantically involved with. There were similar features between the first and second incidents: he followed the same *modus operandi* in the execution of the crimes, the victims were adult females he had close associations with, in the first incident he attempted to murder people who intervened, in the second incident he successfully murdered the mother. While serial murderers tend to prefer strangers as victims, there are numerous cases where people known to him fell victim to the murderer.

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**Intimate Partner Murder, Serial Murder, South Africa**

## **I41 A South African Case Study Illustrating an Intimate Partner Murder Staged as a “Muti” (Ritual) Murder**

*Elmarie A. Myburgh, BA\*, South African Police Services, 255 Schoeman Street, Pretoria, SOUTH AFRICA; Susanna M. Knoetze\*, South African Police Services, Investigative Psychology Unit, Criminal Records and Crime Scene Management, Head Office, Pretoria, SOUTH AFRICA; and Marina Genis, MA\*, Unit 149, 21 Sunset Avenue, Pineslopes, Lonehill, Ext 71, SOUTH AFRICA*

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After attending this presentation, attendees will have an understanding of intimate partner murder and muti murder and how it can be established whether an intimate partner murder scene was staged to look like a different crime.

This presentation will impact the forensic science community by showing how law enforcement officers, psychologists, and profilers will have a better understanding of intimate partner murders, muti murders, and staging of a crime scene.

Intimate partner murders are often referred to as domestic murders or spousal homicide. This occurs when one person is murdered by their current, or ex-, intimate or romantic partner. Motives for such crimes typically include an element of jealousy, which may be due to real or feared abandonment by the victim. While some such perpetrators have a history of physical abuse towards their partner, a significant number of offenders have no history of violence towards their partners, and often there is no escalation of violence preceding the murder.

A crime scene can be staged by an offender in an attempt to delay the identification of the body and to delay the investigation. Staging a crime scene is a high-risk behavior, because it implies that the offender is spending more time than is necessary with the body and thus risks discovery. While the offender makes these changes, he is revealing a lot about himself and is likely to provide investigators with more evidence than he is aware of, because he may be under great stress. Comparing injuries to the crime committed, or comparing the scene and forensic reports to witness statements, can help indicate staging.

The word “muti” is a Zulu word meaning medicine. Muti murder happens when human body parts are gathered for use in traditional African medicine and the person dies as a result of the wounds inflicted. The motivation for the use of muti is usually to improve an individual’s or community’s circumstances. The reason for using human body parts is that they are considered to be more powerful than the usual ingredients like roots, herbs, other plant material, animal parts, and seawater. Characteristically the traditional healer would consult the ancestors to determine the cause of the problem and then prescribe the treatment. The death of the individual is usually secondary to the injuries inflicted while

removing the body parts. The victim must be alive when the body parts are removed as this is believed to increase the power of the muti.

The following case study will be presented to illustrate how one person committed an intimate partner murder, and staged the crime scene to look like a muti murder.

Early one morning the naked body of an adult black female was discovered in an open area. The body was lying on its back and the head, breasts, and external parts of the vagina had been removed postmortem. There were bullet wounds in the torso and left forearm. The victim's clothes were not on the scene. It was clear that the body recovery site was a secondary crime scene. Since the hands of the victim were intact, her fingerprints were taken to establish her identity. The home of the deceased was inspected and the missing body parts were found in the bathroom. When her boyfriend was interviewed he confessed that they had a fight the previous night, during which she ended their relationship. He shot her and removed the body parts in an attempt to stage it as a muti murder.

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**Intimate Partner Murder, Staging, Muti Murder**

## **I42 The Influence of Personality Traits on Intrafamilial Homicide**

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After attending this presentation, attendees will learn whether the personality characteristics affect the homicide offenders or not, whether sociodemographic and criminological characteristics are related to the homicides, and which personality characteristics might be specific to the offenders of intrafamilial homicides.

This presentation will impact the forensic science community by figuring out the dominant behavioral traits in perpetrators committing intrafamilial homicides.

Several researchers have taken an interest in personal characteristics that may have influence on criminal activities. These studies are of great importance in terms of figuring out which behavioral traits are dominant in perpetrators committing the same crimes in different ways since there might be some differences in the personality traits of intrafamilial and other homicide offenders. Homicide in the family is the most severe type of murder. Personality disorders, cultural differences, and economic issues are some of the risk factors in partner homicides. The aim of this research is to compare the personal characteristics of the intrafamilial and other homicide offenders. The Minnesota Multiphasic Personality Inventory (MMPI) was performed in 93 voluntary male prisoners in Maltepe-Istanbul, accused of or sentenced for murder between 2000 and 2010. Personality traits of the perpetrators were grouped as dependent variants. Independent variants such as sociodemographic (educational background, economic conditions, etc) and criminological characteristics (offensive weapon types, crime scenes, and the causes of murder) were also analyzed via personal information forms filled by volunteers. Intrafamilial homicides were 32.3%, whereas other homicides were 67.7%. The results pointed out that 70% of the intrafamilial homicide offenders were over 36-years-old, whereas 73% of the other homicide offenders were younger than 35-years-old. When the birth places of the offenders were checked, a clear and expressive increase from Western to the Eastern part of Turkey was seen only in the intrafamilial homicide offenders, which might be directly related to sociodemographic and sociocultural characteristics of the region. When MMPI subtests were assessed, the highest score was obtained in

schizophrenia (Sc) and the lowest score was obtained in paranoia for both of the offenders of intrafamilial and other homicides. The difference between the arithmetic mean of intrafamilial homicide offenders and the arithmetic mean of other homicide offenders was statistically significant only in psychopathic deviate (Pd) and social introversion (Si). Although there are some research-related limitations such as the specificity of the behaviors, variety of crimes and the closed environment, this study is expected to facilitate further comprehensive research in the field.

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**Intrafamilial Homicide, Personality Traits, Turkey**



ATLANTA 2012

# QUESTIONED DOCUMENTS



ATLANTA 2012

## J1 A Study on Discrimination Methods of Black Ballpoint Pen Ink Lines on Paper

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The goal of this presentation is to compare ink discrimination methods UV light by VIS spectrum, thin layer chromatography (TLC), and high pressure liquid chromatography – electrospray tandem mass spectrometry (HPLC-ESI-MS).

This presentation will impact the forensic science community by making a comparison of different ink discrimination methods using UV light by VIS spectrum, TLC, and HPLC-ES as well as detailing how these methods can provide methodology, classification, and identification of ballpoint ink lines.

In forensic examinations of question documents, analyses of ink components and the dating of ink entries are often of considerable importance. Forensic ink examinations are principally concerned with the classification and comparison of complex chemical mixtures. The inks on a questioned document may be examined through analyses that include spectral examination, thin layer chromatography (TLC), high pressure liquid chromatography/mass spectrometry (HPLC/MS), and gas chromatograph/mass spectrometry (GC/MS). Fifty-six different types of black ballpoint pens manufactured from five country groups were collected. Experimental sampling was conducted by extracting samples from ink lines produced on white copy paper. The goal of this work is to investigate the degradation pathways of ballpoint ink dyes using UV light irradiation, TLC, and HPLC-ESI/MS. To study the effects of aging, the fifty-six ink samples on paper were created and stored with varying elapsed-time periods of one, two, and three years. As ink age determination is highly dependent on the composition of the ink and storage conditions of the document, two variables usually unknown in forensic document examination, the study samples were kept in darkness to simulate natural aging and to minimize variance in exposure to UV irradiation.

Individual characteristic data was identified among all of the fifty-six different types of black ballpoint pen ink samples on paper using the Infrared luminescence, ultraviolet light features, TLC, HPLC-ESI/MS, and GC/MS. Ink samples were first totally dissolved in methanol. Ink component reactions were observed and recorded using light radiation and filter pair sets in five groupings of (① 580nm, 420nm; ② 580nm, 550nm, 420nm; ③ 550nm, 580nm, 660nm, 380nm; ④ 580nm; and ⑤ 580nm, 550nm.) A mobile phase system for TLC analysis was conducted using n-butanol: ethanol: acetic acid at ratios of 6: 1: 2: 0.05, which were effective in separating nearly all dye mixture samples. In this system, the spot capacity more than 21 was achieved. Dyes of black ballpoint ink lines were analyzed using HPLC-ESI/MS and completely detected 15 samples of which the ten violet dyes identified were: ① crystal violet (CV, Hexamethyl pararosaniline); ② CV-CH<sub>2</sub> (Pentamethyl pararosaniline); ③ CV-2CH<sub>2</sub> (Tetramethyl pararosaniline); ④ CV-3CH<sub>2</sub> (Trimethyl pararosaniline); ⑤ CV-4CH<sub>2</sub> (Dimethyl pararosaniline); ⑥ CV+OH-2CH<sub>2</sub> (Tetrahydroxy methyl pararosaniline); ⑦ DLPM-3CH<sub>2</sub>(bis(4-(dimethylamino)phenyl)methanone); ⑧ DLPM-CH<sub>2</sub>; ⑨ DLPM+OH-2CH<sub>2</sub>; and ⑩ basic violet 14 and basic yellow 33, basic yellow 2 of yellow dye, basic blue 26, basic blue 9 of blue dye, and Megawhite PL of

fluorescence. The results of this study indicated that analysis of UV-VIS spectrum, TLC and HPLC-ESI/MS could make a discriminating tool of ballpoint inks dye for forensic purposes and can supply methodology for the classification and identification of ballpoints pen ink lines.

The absorption maximum of the spectral bands shifts hypsochromically from 580 to 560nm with UV irradiation. This hypsochromic shift (blue shift) of crystal violet absorption band is presumed to result from the formation of series of N-demethylated intermediates in a stepwise manner. Aging curves for the N-demethylated intermediates of crystal violet and equations from elapsed time and UV irradiation samples were identified. Crystal violet (hexamethyl pararosaniline) was transformed to its decomposition product pentamethyl pararosaniline by demethylation. Pentamethyl pararosaniline is transformed to tetramethyl pararosaniline and latter to trimethyl pararosaniline. UV accelerated aging mimics natural aging from a dye perspective and can be characterized by UV-Vis spectrum and HPLC-ESI/MS. Irradiation for 1.6h produces the same extent of degradation as what occurs naturally over a period of one year. The relative abundance of the intact dye molecule (m/z 372) decreases as the relative abundances of the degradation products (m/z 358, 344) increase with irradiation time.

**Question Document, Black Ballpoint Pen Ink, Discrimination**

## J2 Study on the Dye Components of Black Gel Pen Inks by HPLC-Tandem/MS

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After attending this presentation, attendees will learn about black gel pen discrimination methods.

This presentation will impact the forensic science community by identifying the dye components in black gel pen ink using high pressure liquid chromatography- tandem/mass spectrometry (HPLC-Tandem/MS).

In order to determine and identify the dye components in black gel pen ink, sixteen black gel pen inks produced from three different countries (Korea, Japan, and Germany) using an HPLC-Tandem/MS were analyzed. Nine different dyes at varying compositions within the sample inks were detected. The dye types included: methyl violet B base (MVB), crystal violet (CV), methyl violet (MV), acid orange 10 (AO10), sudan black B (SBB), victoria pure blue BO (VPBBO), patent blue VF (PBVF), acid red 52 (AR52), and aniline blue diammonium salt (ABDS). Among the dyes, CV, AO10, SBB, and VPBBO were found in all inks, while the other dyes were differentially observed. MV was detected in samples 6-8 (Korea), 11 and 14 (Japan); ABDS was detected in samples 1-3 (Korea) and 11 (Japan); and AR52 was detected in samples 1 (Korea) and 12 (Japan). PBVF was only detected in the Japanese ink sample 13.

Sample inks were divided into five groups by statistical analysis. Group 1: GP1, GP2, GP3 (Dong-A, Korea), and GP11 (Pental, Japan); Group 2: GP4 (Dong-A, Korea), GP5, GP9 (Monami, Korea), and GP10 (Japan); Group 3: GP6, GP7, GP8 (Monami, Korea), GP14 (Sakura, Japan), GP15, and GP16 (Germany); Group 4: GP13 (Zebra, Japan); and Group 5: GP12 (Pental, Japan).

An accelerated weathering test was performed with the black gel pens and the dye components of black gel pen ink were analyzed using HPLC-Tandem/MS (Table 23). The MVB and CV dyes in sample 1, 2, 3, 4, 5, 6,

and 7 were significantly decreased by UV light in a time-dependent manner. After one hour UV of light treatment, MLB dye detected 6.476 µg/kg at sample 1, 3.85 µg/kg at sample 2, 4.461 µg/kg at sample 3, 7.113 µg/kg at sample 4 and 10.547 µg/kg at sample 5. After seven hours UV light treatment, MLB dye detected 2.924 µg/kg at sample 1, 2.149 µg/kg at sample 2, 2.601 µg/kg at sample 3, 4.855 µg/kg at sample 4 and 3.652 µg/kg at sample 5. After one hour UV of treatment, CV dye detected 3.572 µg/kg at sample 1, 3.51 µg/kg at sample 2, 2.488 µg/kg at sample 3, 1.794 µg/kg at sample 4 and 2.479 µg/kg at sample 5. After seven hours of UV treatment, CV dye detected 2.803 µg/kg at sample 1, 1.15 µg/kg at sample 2, 1.224 µg/kg at sample 3, 1.09 µg/kg at sample 4, and 0.915 µg/kg at sample 5. The increased detection of several dyes following the weathering test might be caused by the degradation of some dyes into shared components or could be due to the sample pretreatment process for the HPLC-Tandem/MS trace-level analysis.

In this report, it was established an HPLC-Tandem/MS method for simultaneous determination of the dye components in black gel pen ink, and showed that black gel pen inks produced from three different countries contained common as well as unique dyes. It was also demonstrated that MVB and CV dye contents were significantly reduced by UV light. Based on the data described here, it may be possible to classify the manufacturing origin of black gel pens based on the ink dye components and provide a scientific foundation for dating of documents written with ink from black gel pens.

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**Question Document, Black Gel Pen Ink, HPLC-Tandem/MS**

### **J3 Studies in Ink Analysis and Line Crossing**

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After attending this presentation, attendees will be presented with an example of creating and validating a new analytical capability in their laboratory.

This presentation will impact the forensic science community by presenting an example on determining the suitability of different techniques for ink analysis and line crossing; and validating that analytical capability in their laboratory.

Ink identification and the order of ink layer deposition when lines cross are important determinations confronting questioned document examiners. Before performing case work, individual laboratories need to determine their own capability with the instrumentation on hand, validate analytical protocols, and write standard operating procedures. The results of this study will be used as a guideline for ink analysis in Utah Valley University lab.

A selection of conventional ballpoint and gel pens were purchased from office supply stores. In general, the analytical approach of these experiments is to characterize each line by determining the visible and infrared spectra of each line using a microspectrophotometer, a FTIR microscope, and a Raman microscope. Thin layer chromatography (TLC) was used to visualize the dyes and/or pigments, and examiner impressions of visible microscopic examination of ink deposition at the point of crossing are included. Because not every ink lent itself to unique identification by every analytical technique, a rubric is used to report the findings of useful application.

Inks of the same color are often made of different combinations of dyes and/or pigments. Although TLC destroys the sample it is useful to answer if two lines were made with the same ink. But it not useful to determine which ink is the top layer when lines cross.

Because gel inks are more fluid, they tend to penetrate paper where ballpoint inks tend to create layers on the paper's surface. Visually, gel inks produce more uniform coverage with little or no raw paper showing, whereas a significant area of uncolored raw paper remains after a line with a ballpoint pen has been written. When a ballpoint line crosses on top of a

gel ink line, only a small amount of randomly deposited ballpoint ink is deposited, resulting in a ballpoint line that lacks uniformity and edge definition. However, when the underlying line is made with a ballpoint pen, its line remains distinct and uniform when a gel ink is written over it. Although a gel ink will penetrate and color the raw paper left by the ballpoint ink, it will not disturb the underlying ballpoint ink. Spectral analysis in the visible region of ballpoint inks at the point of line crossing usually is the spectrum of the overlying ink. Inks at the point of crossing were evaluated with visual microscopy, FTIR, and Raman microscopy.

Because not every technique is meaningful with every sample type or situation, a rubric was created to guide appropriate analysis based on the evidence to be examined. Analytical accuracy was determined by comparing analytical results of known sample against the known expectation and the error rate is 100% – analytical accuracy. Robustness was measured by compiling the accuracy of the analytical results of several examiners who each analyze the same samples.

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**Ink Analysis, Microspectrophotometry, Raman Microspectrometry**

### **J4 Differentiation of Document Paper Based on Elemental Profiles Using Inductively Coupled Plasma-Mass Spectrometry, Inductively Coupled Plasma-Optical Emission Spectroscopy, and Multivariate Statistical Procedures**

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After attending this presentation, attendees will understand how trace elements can be used to differentiate different types of document paper, such as copy paper and recycled paper. Inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-optical emission spectroscopy (ICP-OES) are used to generate element profiles for different paper types from the same manufacturer. A combination of statistical procedures, including analysis of variance (ANOVA) and principal components analysis (PCA), are then used to differentiate the paper by type based on the trace elements present.

This presentation will impact the forensic science community by generating element profiles for different paper types. These profiles potentially offer greater discriminatory information than the physical and chemical methods currently used in paper analysis. Demonstration of the statistical procedures will provide an objective method that can be implemented in forensic laboratories for the analysis of questioned documents.

Paper analysis typically relies on comparing physical characteristics of the paper such as color, thickness, and brightness. However, due to improvements in the production process, these physical characteristics do not necessarily allow differentiation of paper. As a result, alternative methods for analyzing and comparing paper samples are necessary.

Trace elements present in paper originate from impurities in the raw materials, as well as from processes used during paper production. These elements may provide greater discrimination of paper by both type and production plant. However, due to the low levels of these elements, sensitive instrumental techniques are necessary for the analysis.

Preliminary research conducted demonstrated the potential of differentiating two different paper types based on element profiles generated using ICP-MS. However, the instrumentation is very expensive and analysis using this technique involves high running costs. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) may be a viable alternative to ICP-MS for this purpose. In this research, element profiles for a larger number of different paper types will be obtained using both ICP-MS and ICP-OES and multivariate statistical procedures will be used to

assess discrimination of paper according to type based on the elements present. Results will be used to assess the potential of ICP-OES compared to ICP-MS for this purpose.

In this research, two reams each of five different types of paper produced by the same manufacturer were purchased. Paper samples were microwave-digested using nitric acid and hydrogen peroxide prior to instrumental analysis. A subset of samples, representative of all paper types, was initially analyzed using ICP-MS in full scan mode to identify potentially characteristic elements for each paper type. Such elements were those that: (1) were present at levels above the instrument limit of detection; (2) were not present at significant levels in the procedural blank; and, (3) did not vary significantly in concentration within a ream of paper. The full sample set was then analyzed by ICP-MS using the selected ion monitoring mode and quantifying the elements of interest. Samples were then analyzed by ICP-OES, quantifying the same elements.

The resulting element concentrations were normalized according to the initial mass of paper digested, and separated into two data sets: the first contained element concentrations determined by ICP-MS and the second contained element concentrations determined by ICP-OES. Analysis of variance (ANOVA) was performed on each data set separately to assess variation of each element within a ream, between reams of the same paper type, and between reams of different paper types.

Each data set was then subjected to principal components analysis (PCA), which is a multivariate statistical procedure used to identify sources of variance within a data set. The PCA scores plot is a scatter plot in which chemically similar samples are grouped, with distinction from samples that are chemically different. The PCA loadings plots can be used to identify the variables contributing most to the variance in the data set. In this research, the scores plots were used to assess association of reams of the same paper type, with discrimination of different types, based on the element profiles. The loadings plots were used to identify those elements that were responsible for the association and discrimination observed. Both scores plots were also assessed to determine if one technique, ICP-MS or ICP-OES, offered improved discrimination of the paper types based on elemental profiles.

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#### **Questioned Documents, Trace Elements, Spectroscopic Techniques**

## **J5 The Examination of Suspected Artificially Aged Paper**

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After attending this presentation, attendees will be able to provide information regarding the application of commonly used methods to artificially age paper and apply physical, optical, and chemical techniques that may be used to distinguish the differences.

This presentation will impact the forensic science community by informing examiners of some of the characteristics that indicate that paper has been artificially aged. This kind of examination may be infrequent for some laboratories; therefore, this presentation will provide visual examples, methods to apply in an examination, and suggested conclusions.

The artificial aging of paper is a popular activity among hobbyists and specialty paper-makers who seek to reproduce the appearance of genuinely antiqued paper, and the plethora of craft books and websites that provide methods that can be easily applied at home attest to its popularity. These same, easy-to-use methods; however, are also employed by those who seek to create fraudulent documents that appear to have been produced from another time.

Determining the purported age of a questioned document is among the myriad examinations that can be conducted by a forensic document examiner. These examinations include establishing the introduction dates of various writing inks and machine printing processes, such as ball point

pen, typewriting and inkjet printing that may be present on the document in question, and whether the introduction dates comport with the alleged date of the document's production. Other dating determinations can be made by examining the actual paper for coded watermarks or whether the constituents of the paper's composition were available on its purported date. Numerous forensic texts and published articles have described many of these kinds of examinations for decades. Understanding the natural causes of paper aging and deterioration, and how to better preserve paper and books, has been an ongoing topic of study by conservationists for years. The focus of this study; however, is on a less-studied, specific aspect of dating determination - the appearance of the paper itself and the more common, widely-available methods that are used to artificially age paper for fraudulent purposes.

The natural paper aging process tends to produce observable, readily identifiable characteristics that significantly differ from the effects created through artificial paper aging. The current study employed some of the most common methods used to artificially age paper, such as soaking paper in coffee or tea, or applying lemon juice or milk prior to baking the paper in an oven. Other methods used may include intentional burn marks or man-made holes added to paper to simulate other artifacts of natural paper aging. Naturally-aged paper that is at least 60-years-old was compared to paper that has been artificially aged for purposes of this study, as well as paper that was suspected of artificial aging for fraudulent purposes from actual cases. Appropriate physical, optical, and chemical techniques that may be used to distinguish the differences between genuine and artificial aging features, such as paper texture, discoloration patterns, and ultraviolet properties will be described.

Following a comparison of the paper properties of genuine and artificial aging, recommendations will be provided regarding which physical properties a forensic document examiner can recognize or identify that would lead to definitive conclusions versus which features can be combined, or may be less reliable for aging purposes, but may nevertheless indicate that artificial aging has been attempted. The possibility for making errors in conclusions, and misinterpreting observations will also be discussed.

The findings of other research projects that address the long-term natural paper aging and accelerated paper aging studies, such as the American Standards and Testing Materials (ASTM) Paper Aging Research Program, as well as studies conducted by the Library of Congress will also be included.

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#### **Artificial, Paper, Aging**

## **J6 The Challenges of Examining Liquid Soaked Documents**

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After attending this presentation, attendees will be aware of the challenges when handling, examining, and preserving documents that have been liquid soaked or previously liquid soaked.

This presentation will impact the forensic science community by providing useful information on the challenges in handling liquid soaked documents or rehydrating liquid soaked documents that have dried in an unusable state.

There are numerous conditions in which a disputed document may be found and the impact that these may have on an examination requires that they each must be dealt with individually. This presentation will offer a glimpse into the limitations, but ultimate success, of one case in which a previously soaked document was reconstructed and examined. Liquid soaked documents for the purposes of this presentation are not only those that are still in a wet or in a damp condition, but are also those that have been previously wetted and then dried. In either case, the document examiner must be knowledgeable of how to properly preserve and record the evidence for an optimal examination.

In this particular case, the document in question was originally an 8.5''

X 11" ruled paper bearing hand printing and drawings. It was folded numerous times and then left out in a harsh Northern environment for approximately a year. Upon initial examination it was thought that unfolding and reconstruction of the evidence might not be successful due to the number of folds and its extremely dry condition. At best, it was thought that only portions might be reconstructed. The current ASTM standard, Standard Guide for the Preservation of Liquid Soaked Documents, ASTM E2711-11, advocates the use of submersion, using an appropriate liquid. Among other equipment, atomizers, trays, and pliable screening are also suggested. As is also the case with charred documents, other publications in the field have advised the application of a glycerin and water mixture to the evidence to facilitate the preservation process. In this instance, a 10% glycerin and water mixture was prepared and an atomizing process was begun. After placing the item in a glass dish, the mixture was slowly and lightly applied to both sides of the folded mass. The examiner then allowed the mixture to fully soak into the document before attempting any sheet separation. A plastic wrap was placed over the dish to prevent drying and approximately an hour later the unfolding process began. Using tweezers, each layer was carefully unfolded, taking care to visualize paper edges and avoid tearing at folds. With a multifold document, this process was challenging, but with a repeat of the atomizing process over several days, the document was successfully recreated, save for two portions in the middle section.

The permanent conservation of the final product is crucial in these types of cases and the proper assessment of the item at hand and the appropriate use of an atomized mixture and drying substrate is important to this process. To finalize the examination, glass plates provide the necessary viewing and yet firm substrate for the rest of the forensic and legal processes to follow. In order to prevent the plates from slipping and causing possible damaging movement of the document, the edges are taped. Attendees will learn how to successfully approach and perform these stages of preservation and also to apply them in similar types of cases to include charred evidence.

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**Liquid Soaked Documents, Questioned Documents, Preservation**

## **J7 Current Bank Check Scanning Practices**

*Jane A. Lewis, MFS\*, 544 East Ogden Avenue, Suite 700-289, Milwaukee, WI 53202*

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After attending this presentation, attendees will learn about the history of the law regarding check clearing for the Twenty-First Century, also known as Check 21. The examination of images of checks with current bank check scanning practices will be described. Implications of scanned low resolution versus higher resolution images of checks as they relate to forensic document examination will be presented.

This presentation will impact the forensic science community by presenting information about the existing bank check scanning practices. Attendees will be made aware of the resolution and image types used by banks to scan and save images of checks.

According to ASTM Standard Guide for Examination of Handwritten Items – E2290-07a, 7.5.1, *if the original is not submitted, evaluate the quality of the best available reproduction to determine whether the significant details of the writing have been reproduced with sufficient clarity for comparison purposes and proceed to the extent possible. If the writing has not been reproduced with sufficient clarity for comparison purposes, discontinue these procedures and report accordingly.*

Low resolution (200dpi or less) images do not reproduce significant details necessary for a proper examination by a forensic document examiner. ASTM E2290-07a describes the potential significant handwriting features considered by forensic document examiners. *Note 6 - Among the features to be considered are elements of the writing such as abbreviation; alignment; arrangement; formatting; and positioning; capitalization; connectedness; and disconnectedness. Cross strokes and dots, diacritics and punctuation; direction of strokes; disguise;*

*embellishments; formation; freedom of execution; handedness; legibility; line quality; method of production; pen hold and pen position; overall pressure and patterns of pressure emphasis; proportion; simplification; size; skill; slant or slope; spacing; speed; initial, connecting, and terminal strokes; system; tremor; type of writing; and range of variation are other forms of handwriting features considered by forensic document examiners.*

Forensic document examiners in the United States, Canada, Australia, and Europe were asked to contact their banks to ask five questions:

1. What resolution are checks scanned at your bank?
2. Is the image type black and white or grayscale?
3. Are the images saved as jpegs or tiffs?
4. What is the make and model of the scanner?
5. What is the name of your bank?

Results were analyzed to determine the current bank practices for scanning checks since implementation of Check 21 in 2004.

The most common scanning resolution in the banking industry is 200 dpi. Banks most often save images in black and white rather than grayscale. Files of check images were saved as either jpeg or tiff formats.

A case study example of a sample check scanned at 100 dpi, 200 dpi, 300 dpi, and 800 dpi will be presented. Forensic document examiners benefit from the best quality images of questioned checks. The better the resolution, the more handwriting details are available for examination. Images with resolution of 300dpi are superior to the current bank scanning resolution of 200 dpi.

It is recommended that the banking industry consider adopting a standard of scanning checks at 300 dpi or higher resolution. Forensic document will appreciate the current state of bank check scanning practices in the United States and internationally.

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**Document Examination, Scanning, Checks**

## **J8 Determining Reliability and Frequency of Trough Pattern in Gel Ink Pens**

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After attending this presentation, attendees will understand the frequency of trough patterns in blue and black gel ink pens, their reliability, and value as a physical characteristic when influenced by various substrates.

This presentation will impact the forensic science community by providing further knowledge to the Forensic Document Examiner (FDE), by enhancing their non-destructive examination techniques in casework where the determination and differentiation of a writing instrument is vital.

To become a FDE, a two year minimum training program must be completed under the tutelage of a trained FDE. The training program includes a multitude of tasks that must be successfully mastered. One of those tasks is the ability to classify the type of writing instrument used in the preparation of a document; for example, a ball point pen from a roller ball pen or a roller ball pen from a gel ink pen.

When examining the earliest gel pen lines, an acceptable assumption (at initial inspection) was that a visible trough in the ink line was indicative of a gel pen being used. The term "trough" is described as a "long narrow or shallow channel" according to the dictionary. Over the course of numerous examinations, it was discovered that this visual identification is not always reliable. Some gel ink pens do not demonstrate a trough pattern. Consequently, research had to be conducted to determine the reliability and frequency of trough patterns produced by gel ink pens.

In forensic document examination, both destructive and non-destructive techniques can be used to determine and differentiate writing instruments and inks. For the purpose of this research, only non-destructive techniques were utilized. Eighteen gel ink samples were handwritten on three types of substrates using multiple brands of blue and black gel pens. After careful examination of the samples, the trough observations were documented and divided into four categories based on trough presence.

These categories were: no trough observed, trough observed some of the time, trough observed most of the time, and trough observed all of the time. The observations from all three substrates were used to calculate the trough frequencies. In addition to observing how the substrate may have influenced the trough pattern, the gel pen brand and ink color were considered and the frequency was calculated as well.

An analysis of the results determined that the substrate influenced the presence of a trough pattern, and that a trough pattern was unreliable as the only physical feature to differentiate gel ink pens from other types of writing instruments. Therefore, it is recommended that trough patterns, only in conjunction with other physical characteristics, be used to successfully classify gel ink pens.

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#### **Frequency, Trough, Gel**

## **J9 Hidden Data: A Barcode Primer With Casework Examples**

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After attending this presentation, attendees will be able to understand the history, use, and examination of 1D and 2D barcodes, and will have a foundation upon which to develop their competency in assessing and deciphering barcodes.

This presentation will impact the forensic science community by providing a broader, updated understanding of the current usage of barcodes in which examiners may encounter.

Upon initial consideration, the barcode (in its various forms) is a printed element used for automatic identification and data capture (AIDC). It would be perfectly natural for a Questioned Document Examiner (QDE) to shy away from all but a perfunctory comparison of size and format. Unfortunately, there is no technical profession that specializes in deciphering barcodes. That the task will fall to the QDE is evident in the incorporation of barcode reading software in the newest versions of equipment marketed to forensic labs. A certain level of competence regarding barcodes will be necessary for an examiner to properly assess, document, and present the physical features and data of a barcode. That competence must include an understanding of the history of barcodes, the types of barcodes and their various applications, technical limitations in their assessment, and an understanding of the types of data that may be revealed.

The history of the barcode is a long one. The first documented consideration was in the late 1940s. Their first use was in tracking train cars (clearly a limited application). However, it was not until the mid-1970s that the grocery industry began to see the possible cost savings of scanning products and put out a request for proposals to a variety of technology companies. As late as 1980, the implementation of “UPC” barcodes for food stores was seen as a failure. Albeit at a crawl, the technology moved into more and more stores, and then eventually spread to other industries. The military began to require that all purchases contain a barcode (“Code 39”). The U.S. passport application system generates a barcode that summarizes the form data, as do some medical records. The creation of the tech-savvy Japanese marketplace, the “QR code” is now seen on coupons and products throughout the U.S.

Today, there is a great variety of barcodes. The simple “1D” barcodes hold a small amount of data in a simple to read format; for experienced users, some can be deciphered at a glance. These barcodes may be used to impart only a small amount of information, or they can be used to access information in a centralized system. The more advanced “2D” barcodes can hold far, far more information. The most common of these, the PDF-417, is based on an international standard and a correctly encoded PDF-417 can be read by any standard reader (or by a scan/software solution). Efforts

to encrypt the PDF-417 for identification documents have had limited success because this solution requires that all verifiers obtain equipment for decryption.

The QDE may encounter barcodes on a variety of documents: state driver licenses, state insurance documents, applications (the information is summarized in a barcode), passports, commercial and industrial products, and countless others. Both the simple and more advanced barcodes typically hold data. While some barcodes serve as pointers to information in a central system, the majority hold the actual data. In the case of travel and identity documents, and certainly medical records and other forms, comparing the data in the barcode to the text on the document immediately reveals if there is a difference.

The first limitation a QDE may encounter with a barcode is access to equipment to decipher the entire barcode. The “readers” used for point of sale (POS) acceptance of documents and by some law enforcement entities extracts the “important data” and presents it in a summary form. More data is often present and may be revealed (and made useful) given the use of a decoding system. The assessment of barcodes is also limited by the lack of information regarding methods for incorporating non-encryption based enhancements and/or security elements. This limitation must be addressed on a case-by-case basis through contact with the issuing authorities for the genuine documents. Unfortunately, there is often a very small group of individuals who have this information and they are difficult to find.

At the FDL, it was determined that counterfeiters are making readable barcodes and including them on counterfeit documents. This is unfortunate because at POS locations, clerks often rely on the scan to verify the document, and never actually look for any of the security features. The barcodes produced by the counterfeiters include the name/address/date of birth in readable form. Fortunately, they also contain data that can help lead investigators to their source. To date, there have been a limited number of cases at the FDL in which barcodes played a significant role. A summary of those cases will be presented.

Although barcodes are not new, their role in the examination of documents is. Given the potential for identifying altered or wholly fabricated information on a document, passing them off as a “data element” and hoping that another technically-trained individual will handle them is no longer appropriate.

Currently, most questioned document examiners are not assessing barcodes when they are present on suspect documents. Both the physical features and the deciphered data of a barcode must be considered in order to provide a full assessment of a document. By developing competence regarding barcodes, QDEs will be better able to identify evidence of alterations, counterfeiting and unauthorized production.

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#### **Questioned Documents, Barcodes, Data**

## **J10 Frequency of Occurrence in Handwriting and Hand Printing Characteristics — Research Methodology**

*Thomas W. Vastrick, BS\*, 380 South State Road 434, Suite 1004-132, Altamonte Springs, FL 32714-3866*

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After attending this presentation, attendees will have a better understanding of how to apply various scientific principles to research so as to achieve maximum validation of any research efforts.

This presentation will impact the forensic science community by instructing and enforcing the need for the use of proper research methods.

Research is the backbone of any science. People often hear the word “exact science” thrown around in courts and elsewhere, but the cold, hard, fact is there is no such thing as an exact science, as that would necessitate a body of knowledge that never changes or advances. For any science to maintain their integrity and legitimacy, the profession must proactively seek to advance – whether through use of new technologies, new methods, or the

reconsideration of established practices under the beneficial eyes of further experience.

Leading the way are those of the profession that take the time to conduct research. One can look at any journal, scientific magazine, or various organizational websites to see that there are countless meetings in every corner of the world providing presentations of work from professionals.

But how much of this work is truly research that will impact the advancement of the science? Yes, there is room for “I had a case,” and “Let me show you a really neat new gadget,” but advancement requires committed time and study into areas and thoughts that may be new and unexplored. It can require imagination that is out of the ordinary. One thing is for sure: effective research requires proper planning as to the methodology used. Is the method a valid method? Is the method the most valid method? What are the weaknesses and strengths of the method? Can the limitations be quantified in any way? Are there experts in other areas, such as statisticians, that can help in the validation process?

In January 2011, a large-scale, two-year research project was initiated. One of the goals of this study was to utilize the most valid methods. In so doing, subject matter experts were utilized in order to insure that proper methods were indeed being used. It is the purpose of this presentation to walk attendees through the development of the methods used in this study. One of the first issues was determining how many specimens would be needed to provide an adequate sampling of the population in the United States. Numerous questions arose in dealing with this issue. For example, what criteria were used to determine adequate numbers? Should a random selection process be used or some other method? Is there more than one method that would be considered statistically valid? Is there a good-better-best of the valid methods? What are the criteria that needs to be addressed in order to obtain the best sampling, if not random? What authorities and standards apply to this problem?

Another issue addressed was what features within handwriting would be utilized in the study. Again there were numerous concerns that affected the product of this portion of the study. One of the concerns was whether the cataloguing of each characteristic would be reproducible. To deal with this problem, features were initially listed that were thought to be objective in nature. For example, whether a certain stroke curved clockwise, curved counterclockwise, or was relatively straight would be used. Whether a stroke was long or short was not used, as it was thought that this kind of feature was too subjective. Next the selected features were put through a pilot test by having attendees at the 2011 ASQDE meeting categorize one set of specimens in order to determine whether any of the characteristics displayed an unacceptable level of non-reproducibility. These were but a few of the issues that had to be addressed in order to maximize the value and integrity of this study. The presentation will detail these issues and how they were addressed.

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#### Research, Statistics, Principles

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## J11 Portable Document Format (PDF) Technology in 2011

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*Joseph L. Parker, MSA\*, 518 Pinegate Road, Peachtree City, GA 30269*

After attending this presentation, attendees will have a greater understanding of “Portable Document Format” (PDF) digital file technology in 2011, to include PDF history, basic PDF file structure, PDF technical applications, PDF file security, worldwide PDF use, and PDF technical features that forensic document examiner should consider when examining many contemporary questioned documents, particularly when related PDF files exist.

This presentation will impact the forensic science community by providing additional knowledge of contemporary PDF technology, specifically the potential application of PDF technology software features in determining the origin(s) and production method(s) used to create PDF-

related questioned documents. The increased knowledge should enhance the thoroughness, accuracy, and overall efficacy of forensic document examiner during examinations of documents and materiel involving PDF technology.

Forensic document examinations most often involve disputes concerning the origin and genuineness of questioned documents. The proliferation of “Portable Document Format” (PDF) digital file technology documents today, particularly the expansion of PDF use in digital document archiving, suggests forensic document examiners will increasingly examine questioned documents that involve PDF technology. A review of PDF technology literature was performed and a summary of this information is provided. The summary includes an overview of PDF technology and related historical highpoints; a description of fundamental PDF file structure and the development of specialized PDF file subsets; developments up to the present concerning PDF document file security and related considerations; and

Adobe® Systems Inc., introduced the initial PDF technology in 1993. A chronology is given of the PDF technology significant points of evolution that followed through to today; of great significance was the adoption of PDF as an ISO standard by the International Organization for Standardization in July 2008. Also discussed are the specialized subsets of PDF files that were developed to meet the distinctive needs of respective customer groups: (1) PDF/X (2001) supports Graphic Designer/Print Professionals and Creative professionals; (2) PDF/A (since 2005) supports records managers, compliance managers, and archivists; (3) PDF/E (since 2008) supports architects, engineers, and the construction and manufacturing fields; (4) PDF/VT (since 2010) supports variable data/transactional printing (i.e., bank statements); (5) PDF/UA (begun in 2011) supports government and industry to meet disabled user needs; (6) PDF/H (since 2008) not a standard, but a best practices guide for Healthcare; (7) PAdES—standard for PDF digital signature security to meet European standards; and, (8) U3D—PDF technology supporting embedded 3D files for interactive and other 3D data. Finally, a summarization of PDF technology security considerations is related, including the recent warnings projected on an international level. Experimentation was conducted to identify PDF technology features having particular value to forensic document examiners for the examination of PDF-related questioned documents and their associated PDF files.

The initial experimentation tested whether PDF text and imaging materials could be accessed and acquired on the Internet. This initial experimentation confirmed the ease and simplicity involved in acquiring PDF materials from the Internet and further demonstrated the ease with which these items could be used to create fictitious documents. The fictitious PDF documents created, and other PDF materiel, were then tested to identify what types of information could be derived from PDF files by forensic document examiners using PDF software features readily available. The testing of these controlled/known PDF materiel identified features that forensic document examiners may employ during examinations of known/suspected PDF document files to determine the time(s), date(s) of PDF creation, and editing; method(s) employed in creating PDF documents, i.e., scanning printed documents versus software conversion of text or text and image combination files directly into PDF files; identification of various software programs used in the creation of PDF documents and sub-components; and the possibility of identifying individuals involved in creating and editing/altering PDF files and related documents. Experimentation confirmed that existing PDF software tools can be used productively for questioned document examinations by forensic document examiners operating at a “software user” level, and that it is not essential for forensic document examiners to transcend this somewhat “elementary” level of expertise in understanding PDF technology, to a deeper level of technical understanding, such as comprehending computer programming code.

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#### PDF Files, PDF Archives, PDF Documents

## **J12 A Case Study of a Specialized Gang Alphabet and the Transfer of Characteristics From That Alphabet Into the Normal Daily Writing Habits of a Gang Member**

*Pamela Zilly, BS\*, and Sarah Zarnick, MSFS\*, Nebraska State Patrol Crime Laboratory, 1233 Arapahoe Street, Lincoln, NE 68502*

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After attending this presentation, attendees will become familiar with a specialized alphabet used in the writings of a gang claiming affiliation with the Almighty Latin Kings. Attendees will gain some background information about the Almighty Latin Kings and see examples of a transfer of habits between the gang alphabet writing and a member's normal daily writing.

This presentation will impact the forensic science community by raising awareness of forensic document examiners of a specialized alphabet and how remnants of the specialized alphabet can be displayed in the normal habitual writing of a person that often uses that alphabet.

In September 2002, three gunmen entered a Nebraska bank and, in a botched robbery attempt, shot and killed four bank employees and one customer. Four individuals were later apprehended, three suspected of being the gunmen and one suspected of being the lookout and planned getaway driver. What followed was a costly and extensive investigation and series of trials, resulting in three of the defendants being sentenced to death and the lookout receiving five life sentences.

During the investigation, it was discovered that the four individuals arrested all claimed affiliation to the Almighty Latin Kings gang. Over the course of a number of months, investigators seized numerous handwritten documents that were submitted to the Nebraska State Patrol Crime Laboratory. Among them were several that were written in an alphabet previously unseen by the document examiner working on the case. The complex letters of the alphabet were written fluidly and naturally and the writings were extensive. The alphabet was deciphered and the texts decoded; the documents were found to contain the bi-laws and various rules of the gang. Of additional interest, the document examiner observed within the extensive known writing of the lead gang member a number of natural, fluid embellishments resembling those seen in the specialized alphabet. Over the course of the investigation and trial preparation, a number of handwritten letters were sent to various witnesses, the media, and family and friends of the defendants, sent from various correctional facilities. Examination and comparison to known writing from the defendants identified many of the letters as having been written by three of the four defendants.

Although the bulk of the letters were written by three of the defendants, one letter that alluded to an additional murder taking place before the botched robbery was only partially written by one of the gunmen. That letter contained an insertion written by a different person. The insertion was the critical text about the previous murder.

This case contained a number of interesting elements including a specialized alphabet, transfer of characteristics between the specialized alphabet and the natural writing of one gang member, information about the culture and rules that controlled the gang, and a critical insertion in an incriminating letter. Some of these elements will be shared during the course of this presentation.

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**Specialized Alphabet, Gang Writing, Writing Characteristics**

## **J13 Dynamics of Stroke Direction in Genuine and Simulated Signatures**

*Linton Mohammed, MFS\*, Rile, Hicks, & Mohammed, 100 Oceangate, Suite 670, Long Beach, CA 90802; Bryan Found, PhD, La Trobe University, School of Human Biosciences, Bundoora, Melbourne, Victoria 3086, AUSTRALIA; Michael Caligiuri, PhD, University of California, San Diego, Department of Psychiatry, 9500 Gilman Drive (0603), San Diego, CA 92093; and Doug Rogers, PhD, La Trobe University, Bundoora Campus, Melbourne, 3086, AUSTRALIA*

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After attending this presentation, attendees will learn empirical data about the dynamics of stroke direction.

This presentation will impact the forensic science community by providing Forensic Document Examiners (FDEs) with information that can be used in real casework.

A handwriting pattern is a sequence of ballistic strokes comprised of a series of upstrokes and down strokes, which may or may not be concatenated. Forensic Document Examiners (FDEs) consider the movement, shape, and formation of upstrokes and down strokes when determining the authenticity of handwriting. Osborn (1929) noted that upward connecting strokes were significant for the comparison of movement impulses and the relative smoothness of downward strokes was indicative of genuine or fraudulent writing.

In this study, each of 60 writers provided ten genuine signatures and 15 simulations of three model signatures. The model signatures were in three styles – text-based (where all the allographs are legible); mixed-style (where two or more allographs are legible); and stylized (where no more than one allograph is legible). The signatures were collected on specimen checks, which lay on a digitizing tablet that was connected to a laptop computer running MovAlyzeR® software. The resulting database comprised 600 genuine signatures and 1350 simulations in hard copy form and in electronic form with dynamic data.

The data were processed to extract five parameters from each pen stroke: stroke duration; stroke length; stroke velocity; normalized jerk; and, average pen pressure. Kinematic data for the upstrokes and down strokes were coded on the basis of the direction of the velocity trace for a given stroke. For each writer, the average value of each of the five kinematic scores was calculated for upstrokes and down strokes for the genuine and simulated signatures yielding 240 scores for each kinematic parameter.

A mixed model analysis of variance (ANOVA) was used to test main effects of writer style (text-based, mixed, stylized) and condition (genuine and simulated). Upstroke-down stroke difference scores were calculated and t-tests were used to evaluate differences between genuine and simulated conditions for each writer group.

Results supported previous findings showing differences between upstrokes and down strokes for genuine signatures along several kinematic parameters including stroke length (19% longer for upstrokes), stroke velocity (15% higher for upstrokes), and pen pressure (14% lower for upstrokes) across writer styles. The study revealed new findings on differentiating simulated from genuine signatures based on analysis of upstroke/down stroke ratios. Specifically, it was found that the ratio for stroke length was significantly greater in simulated than genuine signatures for stylized writers, but lower in simulated signatures for text-based or mixed writers. For stroke velocity an increase was observed in the ratio (from 18% to 31% greater velocity for upstrokes) from genuine to simulated signatures for stylized writers. Lastly, it was found that stylized writers exhibited lower pen pressures for upstrokes than down strokes (11%) for simulated signatures, which was not observed for genuine signatures. For all other writer groups, consistently lower pen pressures for upstrokes than down strokes were observed for both genuine and simulated signatures. Using existing tools, FDEs can evaluate stroke length and pen pressure from known and questioned historical documents for judgments of authenticity. These findings suggest that accurate measures of stroke length and calculating the upstroke/downstroke ratio or difference can increase the scientific rigor of judgments of authenticity.

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**Handwriting, Dynamics, Strokes**

## **J14 An Elemental Approach to Forensic Document Examination**

*Fary L. Lee, MS\*, Foster & Freeman, USA Incorporated, Summit Forensic Document Laboratory, PO Box 207, Eden, UT 84310; and David Tobin, Owen Lang, MFS, and Michael Zontini, BA, Foster & Freeman, USA Incorporated, 46030 Manekin Plaza, Sterling, VA 20166*

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After attending this presentation, attendees will learn about the use of the Elemental Composition Comparator (ECCO) that may be applied to forensic document examination. Introduced is the theory and application of the ECCO that employs Laser Induced Breakdown Spectroscopy (LIBS) for the elemental examination of paper, banknotes, inks, metallic foils, and coins. The results of this research will be presented.

This presentation will impact the forensic science community by enhancing the understanding of the discriminating power of this methodology, as it relates to the examination of questioned documents.

The substitution of a page within a multiple page document such as a will or contract, counterfeiting of paper banknotes and coins, and associating ransom or extortion notes, have all been the focus of forensic document examination. The comparison of the elemental composition of questioned documents, determined by LIBS, can further assist the forensic document examiner in the examination of questioned documents. LIBS, also known as Laser Induced Plasma Spectroscopy (LIPS) is basically an emission spectroscopy technique where atoms and ions are primarily formed in their excited states as a result of interaction between a tightly focused laser beam and the material sample that can be used to quickly determine the elemental composition profiles of a gas, liquid, and solid samples with minimal sample preparation. This technique is based on the analysis of spectra emitted by atomic species and the excitation of those elements by creating a plasma using a low energy laser source. This technique is based on Atomic Emission Spectroscopy (AES) which can be used to determine the elemental composition of a substance. Described simply, AES is achieved by measuring the light emitted from an electronically excited atom as it drops from a high energy state to a lower energy state. LIBS has evolved quickly over the past twenty years and is experiencing exponential growth in interest and finds today a growing number of applications. The advantages of LIBS are that the technique is relatively non-destructive, requires very little sample preparation, and the spectra can be obtained instantaneously. The disadvantages of LIBS are that the limit of detection is presently only 4-10 parts per million (ppm) and the percent composition of trace elements cannot be determined to the level of accuracy required for forensic analysis. However, LIBS can be used as a quick test when specific elements can be used to identify a sample.

ECCO uses this analytical technique called LIBS. LIBS, as previously stated is an atomic emission technique that determines which elements are present in the target sample. This means that the record of a spectrum is based on the breakdown of a sample that was created by a laser. This will then allow a comparison of the elemental composition of sample materials based on the spectra that they give. ECCO uses a high power pulsed infrared laser that is focused onto a sample raising the temperature by up to 15,000°C which produces a micro-plasma of the sample. The micro-plasma contains excited atoms and ions of the elements within the sample. The plasma then emits ultra-violet and visible colored light, which is collected by the spectrometer. The peaks seen on the spectra are called atomic emission lines. Each element has its own signature or pattern of atomic emission lines of both wavelength and intensity. Therefore, it is possible for elements to be identified from the peaks that are present. The atomic spectrum of the constituent elements of the target sample provides a material "fingerprint".

ECCO using LIBS offers significant advantages in speed, sensitivity and cost effectiveness over other processes such as x-ray fluorescence, scanning electron microscope, and mass spectroscopy. Examinations conducted with the ECCO are not only fast and simple to perform, but require minimal sample preparation, gives an immediate spectra of the

elements down to low parts per million, and the examination process is only minimally destructive. This presentation will show the varied applications of ECCO in the elemental analysis of questioned documents such as security paper, office paper, envelopes, differentiating between genuine and counterfeit banknotes and coins, pencil leads and toners.

**Elemental Composition Comparator, Laser Induced Breakdown Spectroscopy, Forensic Document Examination**

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## **J15 Kinematic Evidence of Parkinsonism in the Handwriting of Patients With Alzheimer's Disease**

*Michael P. Caligiuri, PhD\*, University of California, San Diego, Department of Psychiatry, 9500 Gilman Drive (0603), San Diego, CA 92093; and Linton A. Mohammed, MFS, Rile, Hicks, & Mohammed, 100 Oceangate, Suite 670, Long Beach, CA 90802*

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After attending this presentation, attendees will understand why some individuals with Alzheimer's Disease (AD) exhibit handwriting characteristics that resemble Parkinson's disease.

This presentation will impact the forensic science community by providing: (1) a neurobiological understanding of why signatures change in Alzheimer's disease; and, (2) an empirical support for a difference in handwriting and signatures between two forms of dementia.

The principle handwriting impairment in early Alzheimer's disease (AD) has a cognitive-linguistic basis comprised of lexical or semantic errors, word selection, and phonological substitutions. The literature on handwriting among patients with mild or early AD generally suggests little or no graphic motor impairment. Later in the course of the disease writing samples can show more graphic motor disturbances. While there have been numerous published works characterizing the linguistic aspects of handwriting impairment in AD, debate remains as to whether the decline in handwriting in AD reflects the pathological change in frontal cortical integrity, giving rise to cognitive and psychomotor deficits or pathological change in sub-cortical basal ganglia integrity, giving rise to Parkinsonian features. This distinction has relevance to forensic document examiners charged with the task of authenticating signatures and handwriting from older individuals with suspected dementia.

A study was conducted of handwriting kinematics in AD. AD patients were sub-grouped according to scores on a standard clinical assessment that suggested either the presence or absence of Parkinsonian neuropathology. Patients with clinical signs of Parkinsonism comprise a subtype of AD known as Dementia with Lewy Bodies or DLB. The study goals were to determine whether kinematic analyses of handwriting movements support previous literature that handwriting is preserved in AD and to identify kinematic parameters in signatures and handwriting that might distinguish AD from DLB. A standard laboratory assessment of handwriting was employed. Briefly, subjects were instructed to draw concentric circles, write series of the letter 'l' and alternating "lleelle", write a standard sentence, and sign their signature five times using an inkless pen on a digitizer tablet. MovAlyzeR® software was used to acquire and process multiple kinematic variables from each pen stroke.

Results indicated that as a group, AD writers exhibited longer stroke durations, lower stroke velocities, and greater number of acceleration peaks (inversions) per stroke. Overall, these features were not significantly different from those of healthy writers. However, AD patients were more variable as a group than healthy writers suggesting that some AD patients may have significantly impaired handwriting. One likely source of this variation could be the presence of motor impairment consistent with the provisional diagnosis of DLB. Further analyses of signature and handwriting movements in DLB patients showed slower movement velocities, longer stroke durations, decreased stroke length, and an increased number of acceleration inversions versus non-DLB AD patients. The present findings indicate that motor aspects of handwriting may be

impaired in AD patients, particularly those who met clinical criteria for DLB, and that the nature of this impairment may not have a solely cognitive or linguistic basis.

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**Alzheimer's Disease, Handwriting Kinematics, Parkinsonism**

## **J16 Basics of Typography for the Forensic Document Examiner**

*Peter V. Tytell, BA\*, Forensic Research, 116 Fulton Street, Suite 2W, New York, NY 10038-2712*

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After attending this presentation even the experienced forensic document examiner should have an increased level of familiarity with systems used internationally for type measurement, typestyle classification, as well as with the nomenclature used to describe the classifying features of type designs, and how these fundamentals of typography can ultimately be used in practical applications for forensic examinations requiring the differentiation of similar designs. This introductory presentation should provide awareness of some areas for the kind of further education, training, and experience needed to achieve the knowledge, skills, and abilities of the domain-specific expertise required in actual case work.

This presentation will impact the forensic science community by providing an historical perspective on typographic development over the half-millennium plus since the Gutenberg Bible (ca. 1454) as well their calligraphic and inscriptional underpinnings. Socio-economic and aesthetic trends as well as technological advances will be considered.

This presentation will seek to provide forensic document examiners and other attendees with an overview of forensically relevant technical and design aspects of typography. In an age when the overwhelming majority of documents submitted for examination are produced on computer printers using adapted or evolved versions of traditional printers' type styles, the forensic document examiner needs a basic knowledge of typography.

The domain-specific terminology developed by typographers and printers over the centuries will be emphasized because of the role of language in comparison type examinations in facilitation of the perception and labeling of significant features and patterns, both the similarities and the differences. The use of correct and specific terminology should also increase effectiveness in note taking, report writing, and demonstrative presentations.

Aspects of various typographic classification systems will be considered. Traditional classification systems have been historically based, such as the Association Typographique Internationale (ATypI) system based on the work of Maximilien Vox, and the similar, but more restrictive, British Standards Classification of Typefaces (BS 2961). Such systems are subject to criticism as inadequate in an age of computer-aided revivals, hybrids, and combinations of type designs and other material from diverse centuries and sources. The various versions of the PANOSE system, originating from the work of Benjamin Bauermeister, are largely measurement based with minimal subjective decisions or typographic background required. The system devised by Catherine Dixon from England considers the various design sources along with a series of formal attributes, factors that can combine in the patterns of commonly seen groups of type styles.

A variety of criteria have been used for these various classification systems, and their relevance to forensic document examination is that they provide the examiner with strategies of comparative measurements and an extensive starting list of selected characteristics for focused attention in the analysis and comparison of type. Covering both the major elements as well as fine and subtle features of the designs, these classifying characteristics include: overall style, posture, treatment of terminals, including presence and style of serifs, weight, proportion, degree of contrast and axis of contrast or stress, stroke variation, midline placement and x-height, as well as features of certain key characters.

Some information on typewriter type styles will also be provided because of the prolific use of these machines in creating documents in the

last century that still need to be examined in this century, and also the ongoing use of this technology in certain areas. Accordingly, the presentation will also address classification systems developed by Ordway Hilton, David A. Crown, Joseph Haas, and Interpol for monotone typewriter type styles, as well as the overall style classifications developed by Gerry de la Durantaye and Philip D. Bouffard.

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**Typography, Classification, Differentiation**



ATLANTA 2012

# TOXICOLOGY



ATLANTA 2012

## K1 Hair Analysis of Amphetamine Using SPE and LC/MS/MS

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After attending this presentation, attendees will learn about the extraction of amphetamine (and related compounds) in hair using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to provide data on these compounds in hair samples.

This presentation will impact the forensic science community by offering analysts in forensic facilities a method that permits small samples of hair to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both LC/MS/MS and solid phase extraction.

**Method:** Extraction (SPE) was performed on mixed mode column (C8/SCX) conditioned with methanol, deionized water, and pH 6 buffer (3mL, 3mL and 1mL, respectively) prior to sample loading. Samples of decontaminated hair (10mg) were digested in 1M NaOH (containing deuterated analogues) for one hour at 70°C. The samples were cooled and glacial acetic acid (100µL) was added. Each solution was adjusted to pH 6 with 0.1M phosphate buffer (4mL) and applied to a conditioned SPE column. After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1M), and methanol (3mL of each, respectively). Each SPE column was dried and eluted with 3mL of a solvent consisting of methylene chloride/isopropanol/ ammonium hydroxide (78:20:2). After elution, 200µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC/MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine, methamphetamine, MDA, and MDMA (and deuterated analogues), respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm biphenyl analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 30% acetonitrile in four minutes and then back to 5% for re-injection. The total run time for each analysis was less than five minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of amphetamine related compounds.

**Results:** The limits of detection/quantification for this method were determined to be 0.05ng/ mg and 0.1ng/mg, respectively for amphetamine (and other analogues). The method was found to be linear from 0.1ng/mg to 10 ng/mg ( $r^2 > 0.999$ ). Data is presented to show that recoveries of amphetamine were found to be greater than 95% for all the amphetamine analogues. Interday and Intraday analysis of the amphetamine analogues were found to < 8% and < 12%, respectively. Matrix effects were determined to be < 6% for the amphetamine analogues. Concentrations of amphetamine in real samples of hair were found to range from 1.2ng/mg to 1.3ng/mg. Other amphetamine analogues (methamphetamine, MDA, and MDMA) were not found to be present in the hair samples.

**Conclusion:** The use of this new procedure for the analysis of amphetamine and related compounds will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC/MS/MS to provide data from small amounts of hair sample.

**Hair, LC/MS/MS, SPE**

## K2 Evaluation of the Immunalysis Tapentadol Enzyme Immunoassay Kit

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After attending the presentation, attendees will understand the performance of a new tapentadol enzyme immunoassay (EIA) screening kit, using a chemistry immune analyzer with a UPLC-TQD for confirmation of all urine samples.

This presentation will impact the forensic science community by demonstrating the applicability of the Immunalysis Tapentadol EIA screening kit for the consistent detection of tapentadol in urine samples.

Tapentadol (Nucynta®) is a schedule II synthetic opiate that is often used as an analgesic for moderate to severe pain. It is excreted in urine as unchanged drug (3%) and as metabolites: N-desmethyltapentadol (13%), tapentadol glucuronide (55%) and tapentadol sulfate (15%).<sup>1</sup> Urine drug testing of pain patients plays a significant role in monitoring their prescribed medication. Tapentadol's availability as a prescription drug in the United States and its potential for abuse similar to other opioid agonists has led to the development of a homogenous enzyme immunoassay to screen for this drug in urine.<sup>2</sup> Currently, Immunalysis has developed an immunoassay kit for tapentadol. This study aims to evaluate and validate the Immunalysis Tapentadol EIA screening kit, at a cutoff concentration of 200 ng/mL.

The Tapentadol EIA was validated by determining the linearity, precision, accuracy and carryover of tapentadol standards and controls using a chemistry immune analyzer. Linearity was assessed in the range of 200-20,000 ng/mL (n=5). The precision and accuracy of the screening kit were evaluated at the proposed cutoff concentration (200 ng/mL) and at 25% above and below cut-off concentrations (250 ng/mL and 150 ng/mL, respectively) for tapentadol. Controls (n=10) were tested on three separate runs on three consecutive days. Carryover was assessed by screening certified negative urine (n=5) following the injection of urine fortified with tapentadol up to a concentration of 40,000 ng/mL. For the parallel study, de-identified urine specimens (n=300) from pain management patients that were previously confirmed positive or negative for tapentadol were obtained. All 300 patient samples were tested with the Immunalysis Tapentadol EIA kit at the cut-off concentration for tapentadol. All urine samples were then analyzed on a Waters Acquity UPLC-TQD for tapentadol and its metabolites (N-Desmethyltapentadol, Tapentadol Glucuronide and Tapentadol Sulfate) at a confirmation cut-off concentration of 100 ng/mL.

The immunoassay kit was found to be linear up to 1,000 ng/mL. The inter-assay and intra-assay precision of the Immunalysis Tapentadol EIA screening did not exceed a coefficient of variation of 10%. Accuracy was determined to be within ± 25% of each target concentration tested. No carryover was observed in the negative urine samples preceded by 40000 ng/mL of tapentadol. A total of 125 true positives and 167 true negatives were confirmed, by UPLC-MS/MS, based on free tapentadol concentrations only. No false negatives were demonstrated in the parallel study and only eight samples produced a false positive result. The immunoassay exhibits a cross reactivity with N-Desmethyltapentadol (2%) and tapentadol glucuronide (25%).<sup>2</sup> Similarly, it was found that the Tapentadol EIA was also cross reactive with tapentadol sulfate at 25%. The accuracy of the kit improved when examining total tapentadol

concentrations versus free tapentadol (128 true positives, 167 true negatives, 0 false negatives, 5 false positives). The sensitivity and specificity results obtained for the parallel study (sensitivity=100% and specificity = 95.4%) compare to the expectations per the package insert.<sup>2</sup>

The results of this study demonstrated that the specificity of the tapentadol immunoassay kit is based off of not only tapentadol, but its metabolites as well. The assay demonstrated good agreement with the UPLC-TQD confirmation results (97.3%). The Immunalysis tapentadol EIA appears to be a reliable homogenous enzyme immunoassay for the detection of tapentadol in urine.

#### References:

- <sup>1</sup> Bourland J, Collins A, Chester S, Ramachandran S, Backer C. Determination of Tapentadol (Nucynta®) and N-Desmethyltapentadol in Authentic Urine Specimens by Ultra-Performance Liquid Chromatography- Tandem Mass Spectrometry. *J Anal Toxicology*. 2010; 34: 450-457.
- <sup>2</sup> Immunalysis Corporation. Tapentadol Enzyme Immunoassay Package Insert. Revision A: April 2011.

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#### Tapentadol, Homogeneous Enzyme Immunoassay, Validation

### K3 Validation of Liver Oxymorphone Analysis Using LC/MS/MS: Comparison With Associated Blood Concentrations in Fatal Intoxications

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The goal of this presentation is to present a validated method for the detection and quantification of oxymorphone in liver tissue.

This presentation will impact the forensic science community by demonstrating an assay which provides reproducible quantification of oxymorphone in liver tissue. The assay has application in forensic and postmortem toxicology laboratories.

Oxymorphone, a semi-synthetic opioid analgesic derived from thebaine, is a  $\mu$ -opioid receptor agonist and indicated for the relief of moderate to severe pain. It is also an active metabolite of oxycodone and has about six to eight times the analgesic potency of morphine. With the introduction of oral formulations in 2006, oxymorphone has become widely abused. The West Virginia Office of the Chief Medical Examiner has seen a dramatic increase in incidence of accidental deaths related to oxymorphone. Most quantification methods focus on the analysis of oxymorphone in blood or urine. The objective of this study was to develop and validate a reliable method for the quantification in a common supplemental specimen, liver tissue.

Oxymorphone standards ranging from 0.5 – 500  $\mu\text{g}/\text{kg}$  and four control samples ranging from 6 – 300  $\mu\text{g}/\text{kg}$  were prepared in drug and ethanol negative liver homogenate. Internal standard, d3-oxymorphone, was added prior to extraction. Solid phase extraction utilizing Trace-B columns was employed to process the calibrators and controls. Dried eluents were reconstituted in 100  $\mu\text{L}$  of a mixture of water/acetonitrile/formic acid (99:1:0.1) and transferred to high recovery autosampler vials for LC-MS/MS analysis. All chromatography was performed using an ultra-performance liquid chromatography (UPLC) system with separation achieved on an UPLC HSS T3 2.1 x 100 mm (1.8  $\mu\text{m}$ ) column. For each analysis, column temperature was maintained at 40°C. All chromatographic runs were performed using linear gradients where mobile phase A was water with 0.1% formic acid and mobile phase B consisted of acetonitrile with 0.1% formic acid. Initial conditions of mobile phase A/B (99:1) were maintained for 0.5 minutes. Mobile phase B was then increased to 30% over 1.5 minutes. It was then increased to 100% over 1.0 minute and maintained for 1.0 minute. The system was then

returned to initial starting conditions and held for 0.9 minutes until the subsequent injection. Total run time for each injection was 5.0 minutes. Tandem MS analysis was carried out using a TQ Detector with ionization in electrospray positive mode. Oxymorphone and d3-oxymorphone were analyzed using multiple reaction monitoring (MRM) with argon as the collision gas. The source temperature was set at 150°C and desolvation temperature was 400°C. Desolvation gas was maintained at 800 L/h and cone gas was set to 11 L/h. One quantification and two target transitions were monitored for both oxymorphone (302->284, 302->227, 302->242) and the deuterated internal standard (305->230, 305->245, 305->287).

As part of the validation, studies to determine potential interference, ion suppression/enhancement and carryover were conducted. The calibration model, limit of detection, lower limit of quantitation, linear range, precision and accuracy were established from calibrators and controls prepared and analyzed on five different days with four replicates each day. The linear range was shown to be 5 to 500  $\mu\text{g}/\text{kg}$  for oxymorphone in liver homogenate. The limit of detection (LOD) and lower limit of quantitation (LLOQ) were determined to be 5  $\mu\text{g}/\text{kg}$ .

Liver specimens from thirty-three cases were analyzed using this validated method. For each, 3.0 g of water was added to 1.0 g of liver tissue. Samples were homogenized, extracted and analyzed. The oxymorphone concentrations ranged from 39 to 1740  $\mu\text{g}/\text{kg}$  for liver and 5 to 546  $\mu\text{g}/\text{L}$  for blood. A comparison of blood and liver oxymorphone data in fatal intoxications will also be presented.

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#### Oxymorphone, Validation, Liver

### K4 Driving Under Impairment With Hydrocodone, Carisoprodol, Topiramate, and Phenytoin

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After attending this presentation, attendees will be familiar with the driving under impairment with prescription drugs, hydrocodone, carisoprodol, topiramate, and phenytoin in one case.

This presentation will impact the forensic science community by providing understanding of the effects and interactions of hydrocodone, carisoprodol, topiramate, and phenytoin. These drugs are common prescription drugs in United States and the combination of them will definitely impair the driver's performance.

A 38-year-old female was charged with DWI following a mid-morning traffic accident. Breath alcohol levels were negligible; however, she did not perform well in the Standardized Field Sobriety Tests (SFST) such as the one-leg stand (OLS) and the horizontal gaze nystagmus (HGN). In fact, the driver failed all tests administered at the scene: she could not walk in a straight line, balance on one foot, and underestimated elapsed times. In the HGN test nystagmus appeared at 40 degrees and her eyes lacked both smooth pursuit and convergence. Additionally body and eyelid tremors were noted. Substantial miosis of the driver's pupils (2.0mm) was recorded. Because of the driver's refusal to submit to having a blood sample taken, no further testing was performed; however, the driver volunteered that she had taken hydrocodone, carisoprodol, topiramate, and phenytoin pills at 6:30 a.m. the morning of the incident. Her stated medical problems included a herniated disk, hypertension, and epilepsy.

Based upon the drugs the driver claimed to ingest, her poor performance in the SFSTs is readily rationalized. Carisoprodol and hydrocodone play a very important role for the noted impairment. Carisoprodol use may result in side effects that include drowsiness, analgesia, euphoria, sedation, dizziness, muscle relaxation, anxiolysis, and somnolence. Meprobamate, a metabolite of Carisoprodol, produces impaired perception, sluggish reaction time, confusion, inattentiveness,

slurred or thick speech, lack of balance and coordination, unsteadiness, and difficulty standing, walking, or exiting vehicles have also been noted in persons taking carisiprodol.

Hydrocodone is a narcotic analgesic and its effects on motor skills are well documented. These effects include: dizziness; lightheadedness; stupor; nausea; sweating; drowsiness; constipation; vomiting; and, euphoria. Topiramate and phenytoin are anticonvulsants used to treat patients suffering from epilepsy. The effects of topiramate include sedation, dizziness, ataxia, speech difficulty, and nystagmus. Overdose of topiramate can cause confusion and sluggishness. Additionally, phenytoin can cause horizontal gaze nystagmus (HGN), indicating that the result for the HGN test may produce false positives for patients taking phenytoin. Some common side effects such as dizziness, lethargy, and drowsiness might impair driving; however, most patients can tolerate them quite well.

Based upon on a Drug Recognition Expert's (DRE) opinion, she was under the influence of a central nervous system (CNS) depressant and a narcotic analgesic therefore impairing her ability to operate a motor vehicle. A jury convicted the defendant of driving while impaired with prescription drugs: hydrocodone, carisoprodol, topiramate, and phenytoin.

**Driving While Impaired (DWI), Prescription Medicine, DRE**

## **K5 Validation and Comparison of the Microgenics and Immunalysis Buprenorphine EIA Kits**

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After attending this presentation, attendees will gain knowledge of the validation and performance comparison of two buprenorphine EIA screening kits, at a cut-off of 5ng/ml using a chemistry immune analyzer and a UPLC-TQD for confirmation.

This presentation will impact the forensic science community by demonstrating the applicability and performance of the Microgenics and Immunalysis buprenorphine screening kits using a cut-off of 5ng/mL in authentic urine samples.

Buprenorphine is a semi-synthetic opioid that is closely related to morphine. It has recently been prescribed for treating opioid-dependence.<sup>1</sup> Around 10-30% is excreted in the urine primarily as conjugated metabolites. There is a need for specific assays with low detection limits.<sup>1</sup> A sensitive and rapid immunoassay is critical for drug screening situations. This study aims to validate and compare two buprenorphine EIA kits at a cut-off of 5ng/mL.

Both kits were validated based on linearity, precision, accuracy and carryover. The linearity study was completed by running five replicates at nine concentrations ranging from 5-500 ng/mL. Precision and accuracy were tested on controls at concentrations of 3.75, 5 (cut-off) and 6.25 ng/mL. Ten replicates of each were tested on three separate runs on three separate days. The carryover study was performed by injecting certified negative urine (n=3) following the injection of urine fortified with buprenorphine up to 1000 ng/mL and observing the response of the samples. For the parallel study, de-identified patient urine specimens (n=400) that were previously confirmed positive or negative were obtained. Each sample was screened by both kits. All 400 samples were confirmed on a UPLC-TQD for buprenorphine, buprenorphine-glucuronide, norbuprenorphine and norbuprenorphine-glucuronide.

The Microgenics kit displayed linearity up to 100ng/mL, while the Immunalysis kit was linear up to 15ng/mL. Inter-assay and intra-assay precision for the Immunalysis kit demonstrated a lower coefficient of variation (<8%) for all concentrations compared to the Microgenics kit (<13%). The inter-assay and intra-assay accuracy was determined to be

within  $\pm 25\%$  for Microgenics and  $\pm 20\%$  for Immunalysis from the target concentrations. No carryover was observed for either kit. The 200 previously confirmed positive samples all screened positive with the exception of one negative by the Immunalysis kit. From the 200 previously confirmed negative samples, five screened positive by Immunalysis and 16 by Microgenics. The confirmation results demonstrated the accuracy of both kits. A total of 203 true positives, 193 true negatives, one false positive, and three false negatives were observed for Immunalysis. Microgenics displayed the following results: 205 true positives, 183 true negatives, 11 false positives and one false negative. The sensitivity and specificity results obtained for the parallel study were 98.5% and 99.5%, respectively for Immunalysis and 99.5% and 94.3%, respectively for Microgenics. Both sets of sensitivity and specificity results compared well with the predictions of the respective package inserts.

The results of this study imply that both kits are reliable yet vary in screening ability due to cross reactivity with certain metabolites. Microgenics and Immunalysis were both in good agreement with the confirmation results at 97% and 99% respectively. Norbuprenorphine was present by UPLC-TQD confirmation in 201 of the confirmed positive samples out of 206 total positives by Immunalysis and Microgenics. The Immunalysis kit, that has significant cross reactivity with this metabolite, is likely to display a more ideal performance when norbuprenorphine is present. The Immunalysis kit proved to be more specific yielding less false positives while the Microgenics kit proved slightly more sensitive with less false negatives.

### **References:**

- <sup>1</sup> Kacinko S, Jones H, Johnson R, Choo R, Concheiro-Guisan M, Huestis M. Urinary Excretion of Buprenorphine, Norbuprenorphine, Buprenorphine-Glucuronide, and Norbuprenorphine-Glucuronide in Pregnant Women Receiving Buprenorphine Maintenance Treatment. *Clinical Chemistry* 2009; 55(6):1177-1187.

### **Buprenorphine, Enzyme Immunoassay, Validation**

## **K6 Conversion of Codeine to Dihydrocodeine During Toxicological Analysis of Urine**

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After attending this presentation, attendees will be aware of the risk for conversion of codeine to dihydrocodeine in toxicological samples as an artifact of the sample preparation.

This presentation will impact the forensic science community by presenting suggestions to minimize this conversion in laboratory settings and to prevent misinterpretation of results obtained after analyzing for total opiates.

Codeine is the most frequently prescribed oral opiate and is also commonly found in combination with multiple other drugs such as acetaminophen and aspirin. It is available over the counter in Canada and Asia. Codeine, like other opiates, is conjugated with glucuronic acid in the liver as one pathway of metabolism allowing codeine to be excreted by the kidney. This is important when urine samples are tested in forensic laboratories because the glucuronide must be hydrolyzed before the opiates can be analyzed using gas chromatography/ mass spectrometry (GC/MS).

The procedure implemented to hydrolyze, extract, derivatize, and analyze codeine found in urine samples has been demonstrated to cause a small percentage of the codeine to convert into dihydrocodeine, which is visualized by analysis on the GC/MS. The procedure involved enzyme hydrolysis using  $\beta$ -glucuronidase followed by derivatization of keto-opiates (such as hydrocodone, hydromorphone, oxycodone, etc.) with

hydroxylamine, to allow their separation from codeine during analysis on GC/MS. Codeine should not react with the hydroxylamine. Both the enzymatic hydrolysis and the derivatization with hydroxylamine require incubation at high temperatures. An acetate buffer (pH 6) is used to prepare the sample for hydrolysis and a phosphate buffer (pH 5.5) is used to ionize the drug for solid phase extraction. A mixed bed column is used to clean the sample and a mixed elution solvent of methylene chloride, isopropanol, and ammonium hydroxide is used for elution. The sample is then dried down and derivatized with BSTFA before being analyzed on the GC/MS.

Continued investigation of the conversion determined that approximately 0.2% of the codeine was converted to dihydrocodeine using this procedure. It is forensically important to determine how this conversion is occurring and determine if it is possible to prevent. The steps in the procedure preceding SPE were assessed to determine the likely cause for the conversion. In the standard procedure 0.5mL of  $\beta$ -glucuronidase and 0.5mL of pH 5.5 acetate buffer is used to hydrolyze the glucuronide and 0.1mL of hydroxylamine is used to derivatize keto-opiates. Both of these steps also involve an incubation period, two hours for  $\beta$ -glucuronidase and 20 minutes for hydroxylamine. Reagent volumes and incubation times were varied to determine the effects on the extent of conversion. Incubation times of one hour and two hours were tested for both compounds while the  $\beta$ -glucuronidase incubation was also lengthened to three hours. For each of these times the incubation temperature was tested at the original 50°C and at 24°C. The pH of the acetate and phosphate buffers were varied between four and six, and volumes of 0mL, 0.5mL, 1mL and 2mL were added to the sample.

Both the glucuronidase hydrolysis and the hydroxylamine conversion were evaluated to determine which step was responsible for the formation of dihydrocodeine. Initially, the volume, length of incubation and temperature of the glucuronidase incubation were investigated and appeared to have no effect on the extent of formation of dihydrocodeine. The conditions for the conversion of the formation of the keto-opiates were investigated. These conditions include the volume, length of incubation and temperature of the incubation. Less codeine converted to dihydrocodeine when a lesser concentration of hydroxylamine was added and when the samples were incubated for a longer period.

Hydroxylamine is a reducing agent, which may be the reason for the conversion of codeine to dihydrocodeine. Further research will investigate the use of other derivatizing agents to determine an alternate method for the separation of keto-opiates for analysis on GC/MS.

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**Forensic Science, Codeine, Dihydrocodeine**

## **K7 Differential Mobility Spectrometry as a Tool to Improve Mass Spectral Library Searching Scores by Removal of Isobaric Interferences**

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After attending this presentation, attendees will understand how differential mobility spectrometry (DMS) can be used advantageously to pre-separate isobaric compounds and therefore allow the removal of interfering peaks in generated MS/MS spectra that are used in library searching; improving the purity scores obtained for the library hit.

This presentation will impact the forensic science community by enabling a more confident confirmation of the identification of drug compounds in urine.

Rapid and reliable screening methods for drugs of abuse are required for the detection of xenobiotics in forensic intoxication cases. Multi-targeted screening by LC/MS/MS uses MRM triggered Information Dependent Acquisition (IDA) MS/MS spectra, which are used to confirm the identity of detected drugs based on mass spectral library searching.

Matching MS/MS spectra generated from real samples to library spectra can be impeded by the presence of interfering isobaric compounds

in the sample matrix. These isobaric interferences having similar m/z to the analyte will fragment producing extra peaks in the sample spectrum not present in the library spectrum. This results in a reduced score and reduced confidence rating.

DMS separates ions on the basis of the difference in their migration rates under high versus low electric fields and requires the application of an intense asymmetric electric field known as the DMS separation field, typically in the megahertz frequency range. Ion filters based on planar DMS can be integrated with the inlet configuration of most mass spectrometers and are able to enhance the quality of mass analysis by reducing chemical noise and pre-separating ions of similar mass. Using this technology, we have shown the separation of isobaric interferences that, in the absence of the device, would have been transmitted into the mass spectrometer and fragmented at the same time as the analyte.

Data was obtained using a linear ion trap system coupled with an LC system. The ion source region of the mass spectrometer was modified for incorporation of various DMS analyzers. The standard ceramic orifice plate was replaced with a modified ceramic plate that included provisions for sealing a DMS cell. The mass spectrometer analysis consisted of an MRM detection using scheduled MRM algorithm and product ion dependent scans using the linear ion trap, automatically triggered to collect full scan MS/MS fragmentation spectra. The MS/MS data were collected using low, medium, and high energy fragment ions. A 1250 compound Forensic Drug Library was searched to provide identification and confirmation.

Comparisons of IDA triggered product ion spectra generated from urine samples both with and without the use of the DMS were made. An improvement in the MS/MS spectrum generated with the use of DMS was seen for amphetamine detected in urine, by removal of the interfering ions at m/z 77, 107, and 109. This improved the quality of the spectrum for searching against the forensic drug library, producing 100% purity score for an amphetamine match when compared to only 40% when performing the same experiment, on the same sample but without the use of the DMS device. Other examples where improvements in MS/MS spectra were gained by the use of the DMS device, compared to without using the device, included product ion spectra triggered for m/z ions that corresponded to lidocaine, indomethacin, and fentanyl. Library match purity scores for these compounds improved from 79 to 90%, 14 to 78% and 32 to 89% respectively, allowing for a higher confidence in the identification of these compounds. Comparisons of product ion spectra generated from urine samples both with and without the use of the DMS show that fragment ions are detected and falsely represented in the resulting product ion spectrum in those experiments performed without the use of the DMS. The DMS, therefore, removes these isobaric interferences, improving the mass spectral library searching scores.

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**Differential Mobility Spectrometry, Drug Screening, LC/MS/MS**

## **K8 Incidence of Fetal Drug Exposure in Alabama: 2004-2011**

*Jack R. Kalin, PhD\*, Alabama Department of Forensic Sciences, 2026 Valleydale Road, Hoover, AL 35244-2095*

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After attending this presentation, attendees will learn the incidence of fetal drug exposure in Alabama from 2004 through June of 2011 and by extension, the extent of maternal drug use proximate to the delivery of stillborn fetuses and/or newborn infants.

This presentation will impact the forensic science community by pointing out how maternal drug use during pregnancy manifests in fetal drug exposure and represents a significant public health problem. This presentation describes toxicological findings in specimens collected from stillborn fetuses and newborn infants as a measure of the extent of drug exposure in utero and, by extension, of maternal drug use proximate to delivery. In some instances, maternal blood was also available for analysis and findings were compared to the stillborn fetuses. This differs from the

more common practice of conducting toxicological examinations with meconium.

The Alabama Department of Forensic Sciences (ADFS) provides forensic laboratory services to the law enforcement community in Alabama (pop. 4.66 million), which includes approximately 600 state, county and local police, sheriff departments, district attorneys, coroners, and medical examiners. Laboratory records from cases submitted to ADFS were reviewed for instances of fetal demise, regardless of cause, for the period 2004 through June of 2011. Cases were selected where blood and/or tissue specimens were available for toxicological examination, the purpose being to identify instances of in utero drug exposure. Thirty-two cases were identified statewide, excluding Jefferson County (pop. 700,000).

Toxicological examinations included headspace analyses for ethanol and related volatiles, immunoassays for common drugs of abuse and both liquid-liquid and solid-phase extractions followed by analysis with GC/MS and/or LC/MS/MS. Where significant, quantitative analyses were conducted.

Twenty-one cases were positive for one or more drugs and/or metabolites; autopsies were conducted in 18 of 21. Five full-term live births were followed by death due to drowning, asphyxiation (2x), acute drug intoxication (within minutes), and/or delayed drug intoxication while in the hospital (after 24 hours). One case involved fetal death due to the mother sustaining blunt-force injuries in a traffic incident.

Six drugs and/or metabolites were identified in one case; four were identified in two cases and one – three were identified in the remainder. Cocaine and/or benzoylecgonine (BE) were the most prevalent substances identified followed by methamphetamine and amphetamine. Levamisole, a common adulterant present in street cocaine, was identified in some cases where cocaine was present. In one case, cocaine and/or metabolites, methamphetamine, and amphetamine were all identified. A summary of drug incidence is provided herein. This presentation will include toxicological findings, causes, and manners of death, and body weights for each individual case.

Analyte	Incidence	Analyte	Incidence
Cocaine/metabolites	10	Chlordiazepoxide	1
Methamphetamine/amphetamine	6	Chlorpheniramine	1
Levamisole	3	Clorazepam/7-aminoclonazepam	1
Citalopram/Escitalopram/Desmethyl-	2	Etoridate	1
Dextromethorphan	2	Meperidine	1
Diazepam/nordiazepam	2	Meprobamate	1
Diphenhydramine	2	Methadone/EDDP	1
Hydrocodone	2	Oxycodone	1
Lidocaine	2	Promethazine	1
Acetaminophen	1	Pseudoephedrine	1
Bupivacaine	1		

In two cases, maternal blood was also available for examination. Results are provided herein.

CaseNo(s)	Fetal Blood	Maternal Blood
*****01/102	Methamphetamine, 650 ng/mL Amphetamine, 250 ng/mL	Methamphetamine, 220 ng/mL Amphetamine, 100 ng/mL
*****680/048	Acetaminophen Cocaine, 210 ng/mL Benzoylecgonine, 480 ng/mL Levamisole	Cocaine, 65 ng/mL, <10 ng/mL (+24h) Benzoylecgonine, 1700 ng/mL, 59 ng/mL (+24h) Methylecgonine Meperidine Nomeperidine

### Fetal Drugs, Drugs in Utero, Drugs During Pregnancy

## K9 A Comparison of Alprazolam Levels in Blood and Urine

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The goal of this presentation is to understand that there is an apparent correlation between the concentration levels of alprazolam in blood and urine specimen.

This presentation will impact the forensic science community by showing that there is an apparent correlation between urine and blood levels of Alprazolam that may assist the toxicologist in determining the possible intoxication.

It is generally accepted that blood levels of drugs are better indicators of impairment and urine concentrations cannot be correlated to levels of impairment; however, in many cases urine may be the only specimen received by the laboratory. Any correlation between urine and blood levels would assist the toxicologist in determining the possible level of intoxication.

Alprazolam is a common benzodiazepine that is used for the treatment of depression, anxiety disorders and panic attacks. Common side effects of the drug include drowsiness, confusion, hypotension, and tachycardia. Typical blood concentrations in persons using the drug therapeutically range from 0.005 - 0.05 mg/L; however, in cases of abuse or over dosage the levels may range between 0.1-0.4 mg/L. Alprazolam is commonly seen in cases related to intoxicated driving where urine may be the only specimen available. Labs typically use blood to determine the concentration of drugs that are present; however, in the case of alprazolam, urine specimens may work just as well. The goal of this study is to show that there is an apparent correlation between the levels of alprazolam in the blood and urine that is not typically seen with other drugs.

A total of 55 cases, from 2011, from the Southwest Institute of Forensic Science in Dallas, Texas were used for the study, all of which had previously had alprazolam quantitated in the blood specimen. The corresponding urine specimens were extracted for comparison using a liquid-liquid extraction method with two control standards, alphaprodine for an internal standard and cholestane for an external standard. The drug was first extracted into n-butyl chloride, followed by a back extraction into 1 N hydrochloric acid. Finally, the samples were concentrated into a chloroform layer and 2.5 µL of the chloroform layer, containing the extracted drug, was injected onto a gas chromatogram-flame ionization detector. The instrument uses a split injection and contains two different columns, a 100% Dimethylpolysiloxane (DB-1) and a (5%-Phenyl)-methylpolysiloxane (DB-5) column; quantitation of the drug occurs on the DB-1 column but the concentration on the DB-5 column should be consistent. Alprazolam was measured at its respective retention time, approximately 20 minutes, and a concentration was generated based on a previously defined curve. The results indicate that approximately 76% of the urine specimen contained alprazolam levels that were within ± 0.05 mg/L of their corresponding blood level concentration. When therapeutic levels and abuse levels were categorized and evaluated, the results remained consistent. The urine and blood level concentrations for alprazolam do have an apparent correlation unlike most other drugs. In the absence of a blood specimen, a urine specimen could be quantitated for alprazolam and potentially be used to determine the approximate value of the blood concentration. Further studies will be done to broaden the scope of these initial findings.

### Alprazolam, Urine, Concentration

## K10 Distribution of Methadone and Its Metabolites in Plasma and Blood Cell

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After attending this presentation, attendees will learn that data derived from the analysis of plasma do not represent the concentration of methadone in whole blood.

This presentation will impact the forensic science community by illustrating that methadone, perhaps most other drugs and their metabolites, is distributed between plasma and blood cell. Therefore, whether plasma or whole blood should be selected as the test specimen depends on the test objective.

Methadone has long been adopted as the primary substitution drug for "treating" heroin addicts elsewhere and recently in Taiwan. Accurate determination of this compound and its metabolites in patients' blood provides valuable information helpful to safe and effective implementation of the substitution therapy policy.

Simulated samples (drug-free whole blood spiked with the analytes of interest) were first separated into plasma and blood cell portions. Resulting whole blood, plasma, and blood cell samples were used for developing effective sample preparation approaches to determine the distribution characteristics of the analytes of interest in plasma and blood cell portions. Clinical samples collected from patients under treatment were then analyzed to: (a) validate the findings derived from simulated samples; and, (b) study factors — e.g., treatment dosage, time lapse between drug intake and sample collection, and genetic variations, such as ABCB1 (C1236T, C3435T, and G2677T/A) and CYP2C19 (G681A and C990T) — that may affect the distributions. Typical sample preparation steps included: (a) addition of internal standards (MTD-d9 and EDDP-d3); (b) deproteinization twice by acetonitrile; and, (c) extraction with isopropanol/hexane (1:8, v/v) at pH 10.2 (carbonate buffer). Extracts were dried, then reconstituted with ethyl acetate for analysis. GC-MS was used as the primary analytical method, while a significant number of samples were also analyzed by LC/MS/MS (triple quadrupole configuration) to confirm the accuracy of data derived from GC-MS analysis. Since the concentrations of the commonly monitored metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP), in most clinical specimens were below the GC-MS quantification limits, MTD data were more fully evaluated.

The validity of a set of analytical data was examined by comparing the compatibilities of: (a) finding of the whole blood sample and the sum of the findings of the plasma and the blood cell samples; and, (b) the analytical findings derived from the GC-MS and LC-MSMS for those clinical specimens that have been analyzed by both methods. Data derived from simulated samples indicated: (a) 80% (standard deviation = 0.52%; n = 5) of MTD and 77% (standard deviation = 0.95%; n = 5) of EDDP in whole blood were found in plasma; and, (b) portions of MTD and EDDP (especially MTD) on the blood cells can be removed by washing the blood cell with phosphate buffer (pH 7.4). Data derived from GC-MS and LC-MSMS methods for the analysis of clinical specimens were found compatible. Among the 26 clinical specimens studied, the amount of MTD found in the plasma portion ranged from 70 to 86%. Specimens were re-collected from a small group of patients (n = 5). Data derived from the follow-up analysis of these specimens appeared to indicate the observed inter-patient variations (in MTD distribution) was unlikely caused by differences in treatment dosage or the time lapse between drug intake and specimen collection. It is currently being examined.

### **Methadone, Distribution, Blood**

## K11 Alcohol Intoxication

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After attending this presentation, attendees will be informed about HS-GC/MS analysis of alcohols with specific toxicity and case reports.

This presentation will impact the forensic science community by providing details on the analysis and validation alcohol intoxications demonstrating a multi-disciplinary approach to the toxicity evaluation process.

Methanol, also known as methyl alcohol, wood alcohol, wood naphtha, or wood spirits, is a chemical with the formula CH<sub>3</sub>OH (often abbreviated MeOH). It is the simplest alcohol, and is a light, volatile, colorless, flammable liquid with a distinctive odor very similar to, but slightly sweeter than, ethanol (drinking alcohol). Methanol, is a commonly used organic solvent, the ingestion of which has severe potential ramifications. It is a constituent in many commercially available industrial solvents and in poorly adulterated alcoholic beverages. Toxicity usually occurs from intentional overdose or accidental ingestion and results in metabolic acidosis, neurological sequelae, and even death. Methanol toxicity remains a common problem in many parts of the developing world, especially among members of lower socioeconomic classes.

The pathological effects of methanol are attributed to the accumulation of the toxic metabolites formaldehyde and formic acid. Thus, symptoms of methanol poisoning may be delayed by 12 hours or more from the time of ingestion and may be accompanied by a severe metabolic acidosis. The early non-specific symptoms of nausea, headache, abdominal discomfort, generalized weakness, and deteriorating conscious level can be accompanied by visual impairment. This picture can then proceed to blindness, coma, and death in association with a profound metabolic acidosis. Recommended management aims to delay methanol metabolism by using intravenous ethanol infusion; to reduce methanol levels with hemodialysis; to control metabolic acidosis; and to support cardiorespiratory function.

A case is presented involving a transient loss of consciousness resulting from self-administered denatured alcohol (methylated alcohol). A 60-year-old woman was found unconscious at home, with a needle mark on her leg. A bottle of purple liquid was found next to the body. The compound was identified and quantified by headspace gas chromatography coupled to mass spectrometry. Headspace Gas Chromatography Coupled to Mass Spectrometry (HS-GC-MS) is one of the most commonly used techniques for the analysis of volatile compounds. At the intensive care unit, specimens of whole blood and centrifuged blood serum were collected at arrival to the emergency clinic. Post-dialysis serum sample from the second day and whole blood samples from the third day were collected for toxicological analysis, and stored at 4°C until analysis. A sample of the purple liquid from the bottle found near the patient was also collected. A sample of whole blood was collected at the clinic a week after the incident and was sent for toxicological analysis for the control and confirmation of the treatment.

The purple liquid found in the bottle was diluted 100 times and tested for ethanol and methanol using the headspace-GC/MS method. The whole blood sample collected at the arrival was found to be un-fit for the analysis due to clotting and low volume, the centrifuged blood serum was preferred instead. Remaining specimens were also tested for methanol and ethanol, respectively.

Methanol, a volatile compound that was identified by the following ions, m/z 29 and 31 after chromatography on a HP5-MS capillary. A headspace gas chromatographic-mass spectrometric (HS-GC-MS) method was developed for detection of ethanol and methanol in blood and quality control and validation was performed. The linearity ranges of the method were 0-2g/L for ethanol and methanol. The limit of detection was 0.03g/L

for ethanol and methanol. The limit of quantitation was 0.05g/L for ethanol and methanol. The range of recoveries was between 94%-120% for both ethanol and methanol.

A similar case that was studied involved alcohol intoxication which included a child who was abused and intoxicated continuously with anti-freeze. The case was brought due to nausea, dizziness, and fainting of the child and was suspected of a case of intoxication. After, using the same method mentioned above, it was clear by the qualitative analysis carried out that the child was intoxicated with methanol and ethylene glycol.

Another interesting case of alcohol intoxication was also studied using this method. The analysis was carried out on blood samples sent by the military. The person was found unconscious near an anti-freeze storage unit. The method was used for qualitative analysis and iso-propyl alcohol was detected.

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#### **Alcohols, Headpace, Intoxication**

### **K12 The Optimization of a Sol Solution for Its Use With Molecular Imprinting for the Extraction of Illicit Drugs**

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After attending this presentation, attendees will learn the solidification process of a sol gel along with factors affecting gel solidification and network strength. The process surrounding the molecular imprinting of sol gels will also be shown, with preliminary data on the imprinted sol gel's uptake of the target drug molecule.

This presentation will impact the forensic science community by demonstrating how the use of a novel sol gel can be molecularly imprinted with illicit drugs to enhance the selectivity and extraction efficiency in comparison to current techniques due to its high stability, retention capacity, and affinity for the imprinted target molecule.

According to the 2009 National Survey on Drug Use and Health (NSDUH), over 20 million Americans, aged 12 and older, were said to be current users of illicit substances, an increase from 2008. With the rise in drug abuse, the frequency of forensic drug testing is becoming more prevalent. Drug testing can be performed on variety of biological specimens, such as urine, to determine the presence of any residual illicit substances and to conclude if the presence of such substances were the cause of criminal behavior. When testing for the presence of drugs, samples must undergo an extensive sample preparation process in order to pre-concentrate the analyte, as well as to minimize the detrimental effects of sample matrix interferences. Such procedures can prove to be a challenging task for many forensic toxicologists due to the complexity of biological specimens.

Solid phase extraction (SPE) is the most common technique for drug extraction and preparation, yielding high recoveries and clean extracts of the target drugs; however, SPE offers only generic selectivity, often extracting other matrix interferences, complicating instrumental identification and quantification. In order to improve extraction selectivity, molecularly imprinted polymers (MIPs) have been developed. Recently, studies have shown that the use of molecular imprinting for the extraction of illicit drugs yields better recoveries than SPE, as they display higher molecular recognition for the template molecule. It was also found that MIP provides an enhanced sensitivity with a more superior limit of detection than that of SPE. Furthermore, MIP has a higher ability for matrix reduction, removing a larger amount of background and interferences existing from biological matrices.

The polymer chosen to be molecularly imprinted for this research is a sol gel. Through the sol gel process, a silica based network is created which can and has been utilized for the production of ceramic or glass particles, surface bound coatings, and SPE cartridges. Sol gel chemistry has received an increased amount of attention in recent years due to its high thermal stability and increased intermolecular interactions between target analytes and itself. Another advantage is its high and adjustable porosity, which allows for high retention capacity of the extracted drug. Furthermore, sol gels are also very easy to prepare and can solidify at ambient temperatures. Other polymers that have been experimented with for the purpose of molecular imprinting produced swelling which causes a distortion of the cavities that are left behind after imprinting the target drug molecule. On the other hand, using a sol gel with molecular imprinting, allows for a higher selectivity than imprinting on other polymers because of the negligible swelling that sol gels produce.

An optimal sol gel was created by varying the various key ingredients. The ideal molar ratio of the participating ingredients was determined and the sol gel's ability to maintain its shape during supercritical fluid extraction (SFE) demonstrated strength and stability of the network. A scanning electron microscope (SEM) was used to view the gel's homogeneity and its porosity. This new optimal sol gel has a high porosity allowing for the enhancement of the gel's surface area, and thus, its retention capacity. Preliminary data will be provided demonstrating the molecular imprinting capability of the novel sol gel, as well as factors affecting gel solidification and network strength. This newly developed sol gel is highly stable and highly porous, making this an ideal candidate for molecular imprinting, and thus, a providing a promising future for the capability of drug extractions.

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#### **Sol Gel, Molecular Imprinting, Toxicology**

### **K13 Evaluation of Lin-Zhi International EDDP Enzyme Immunoassay for the Determination of Methadone Metabolite in Urine**

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The goal of this presentation is to inform attendees of the performance of the Lin-Zhi International EDDP [2-Ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine] Enzyme Immunoassay for the detection of this methadone metabolite in urine.

This presentation will impact the forensic science community by providing an evaluation of the performance of the Lin-Zhi International EDDP Enzyme Immunoassay offering the field of toxicology alternative choices for the rapid detection of methadone metabolite in urine.

In this presentation, an evaluation of a new EDDP [2-Ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine] Enzyme Immunoassay [EDDPI] for the detection of this methadone metabolite in urine is presented. The Lin-Zhi assay is based on competitive antibody binding between EDDP in urine and glucose-6-phosphate dehydrogenase labeled EDDP. When EDDP is present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH resulting in an increase of measured absorbance at 340 nm.

The EDDPI was evaluated by testing 362 urine specimens collected from pain management clients and substance abuse treatment patients. All specimens were tested with the assay in a chemistry system auto-analyzer using two different calibrator sets. One calibration set contained 0 and 150ng/ml (cut-off calibrator) of EDDP and the other set contained 0 and 300ng/ml of EDDP. Controls containing 0ng/ml of EDDP, and -25% (negative control) and +25% (positive control) of the 150ng/ml and

300ng/ml cut-off calibrators (Lin-Zhi) were analyzed with each batch of specimens. All urine specimens were then analyzed by HPLC/MS/MS for EDDP with a 25ng/ml LOQ.

Approximately 42% (151) of the 362 specimens yielded positive results by the EDDPI at 150ng/ml and/or 300ng/ml cut-off values. Of these specimens, HPLC/MS/MS confirmed the presence of EDDP above 25ng/ml in 151 specimens; however, at the 300ng/mL EDDPI cut-off, nine specimens yielded positive results when EDDP was present at <300ng/mL as determined by HPLC/MS/MS. Similarly, at the 150ng/mL EDDPI cut-off, three of the nine specimens also yielded positive results when EDDP was present at <150ng/mL by HPLC/MS/MS. These three specimens contained 121, 68, and 29ng/mL EDDP. No specimen yielding a negative EDDPI result contained EDDP above 25ng/ml by HPLC/MS/MS. Therefore, when applying a 150ng/ml cut-off, the EDDPI demonstrated a sensitivity of 1.00, a specificity of 0.986, and an overall agreement with HPLC/MS/MS results  $\geq 150$ ng/ml of >99%. When applying a 300ng/ml cut-off, the EDDPI demonstrated a sensitivity of 1.00, a specificity of 0.959, and an overall agreement with HPLC/MS/MS results  $\geq 300$ ng/ml of 97.5%. The intra-run precision of EDDPI as determined from the absorbance rates of the negative and positive controls yielded CVs of  $\leq 2\%$  (n=8); while inter-run precision of the controls yielded CVs of  $\leq 6\%$  (n=21). EDDPI demonstrated no cross reactivity with drugs of abuse or popular prescription drugs added to urine at 100mg/ml. The Lin-Zhi EDDPI provides a precise, reliable method for the routine detection of methadone metabolite in urine specimens.

**Enzyme Immunoassay, EDDP, HPLC/MS/MS**

## **K14 Deaths Involving Methylenedioxypropylamphetamine (MDPV) in Upper East Tennessee**

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After attending this presentation, attendees will learn about the abuse of MDPV and the significance of blood and urine MDPV concentrations in actual postmortem cases.

This presentation will impact the forensic science community by providing actual case studies of deaths involving recreational MDPV abuse and the respective MDPV concentrations in postmortem blood and urine.

Two deaths involving the drug 3,4-methylenedioxypropylamphetamine (MDPV) are reported from the Upper East Tennessee region. MDPV is a synthetic stimulant that affects the central nervous and cardiovascular systems. MDPV is one of the constituents commonly found in "bath salts," the other being mephedrone (4-methylmethcathinone). Bath salts are legal, cheap, and readily available in the Upper East Tennessee region and the effects have been compared to those of methamphetamine. To date no postmortem MDPV biological concentrations have been reported in the literature. A qualitative and quantitative analysis for MDPV was performed by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry on two postmortem cases.

A 46-year-old white male was found dead on the floor by his partner after injecting and smoking bath salts in the days prior to his death. He was reportedly nauseous, weak, and vomiting for several days but refused medical attention. The decedent had a history of diabetes mellitus and his blood sugar was elevated days prior to his death. He had a history of known drug abuse including opiates, heroin, and methamphetamine; however, the weekend before his death was the first time he tried bath salts. Ten vials of bath salts labeled Drone IV were collected from the scene, analyzed, and

found to contain the bath salt constituent, MDPV. Analysis of the femoral venous blood and urine revealed MDPV concentrations of 39ng/mL and 760ng/mL. Metoclopramide was also present in the femoral blood at a concentration of 490ng/mL. No mephedrone was detected in the Drone IV vials, blood, or urine. At this time no cause of death has been established.

The second fatality was a 40-year-old white male found dead who had a history of drug and bath salt abuse. The deceased was alleged to have been smoking and/or snorting bath salts prior to death. He was HIV positive and undergoing hormone therapy treatment for gender reassignment. Analysis of the femoral venous blood revealed a MDPV concentration of 130ng/mL, dextromethorphan of 250ng/mL, and a butalbital concentration of 5.1 $\mu$ g/mL. The urine MDPV concentration was 3800ng/mL. Dextromethorphan, guaifenesin, bupropion, diphenhydramine, phenothiazine metabolites, barbiturates, and caffeine were also present in the urine. No mephedrone was detected in the blood or urine. At this time no cause of death has been established.

Toxic or lethal ranges for MDPV have not been established in the literature. A study by Ojanoerä monitoring opioid-dependent patients undergoing opioid substitution treatment screened each subject's urine for MDPV.<sup>1</sup> The median MDPV concentration from nine positive urines was 160ng/mL with a range of 40 to 3900ng/mL. Urine MDPV concentrations from our postmortem cases are within their documented recreational abuse range. The other drugs and/or combination of the drugs present do not solely explain the causes of death in these two cases. Preliminary pathological findings indicate other contributing factors for their causes of death.

Postmortem blood and urine MDPV concentrations in cases with bath salt abuse is presented. Literature and preliminary autopsy findings suggest that the deceased were recreational abusers and that other factors contributed to their deaths.

**Reference:**

1. Ojanoerä et al. Urine analysis of 3,4-methylenedioxypropylamphetamine in opioid-dependent patients by gas chromatography-mass spectrometry. *Ther Drug Monit.* 2011; 33(2):257-63.

**Methylenedioxypropylamphetamine (MDPV), Bath Salts, Postmortem Blood and Urine Concentrations**

## **K15 Fatal Intoxication With Amiodarone: Report of Two Cases**

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The goal of this presentation is to describe and discuss a case of fatal intoxication with amiodarone.

This presentation will impact the forensic science community by increasing awareness of toxicity of this widely used drug and its potential hazardous effect.

Amiodarone has become a widely used class III antiarrhythmic drug and is effective in treating both supraventricular and ventricular arrhythmias. It has been found to be more effective than other antiarrhythmic drugs in maintaining sinus rhythm, but because of its serious and potentially life threatening side effects, it is not the drug of first choice. Common side effects of amiodarone treatment are bradycardia, pneumonitis, liver function disturbances, hyperthyroidism, hypothyroidism, photosensitivity, cornea deposits, etc. Torsades de pointes and AV conduction disturbances are seldomly seen.

When amiodarone has been absorbed, it is metabolized in the liver to produce the active metabolite desethylamiodarone. Due to its lipophilic nature, it has strong tissue affinity and a large volume of distribution. In chronic users, high concentrations are found in fatty tissue, liver and lung and lower concentrations in kidneys, heart, skeletal muscle, thyroid gland and brain. The distribution to these tissues is relatively slow, and therefore

a steady state of tissue concentration is reached only after two months. The relation between serum concentration and clinical effect is not very clear. The therapeutic concentrations of amiodarone in serum are 1-2.5 mg/l and serum concentrations > 2.5 mg/l have been associated with an increased risk of toxicity. The most commonly used oral dose is 200 mg per day. In Denmark due to potential risk of toxicity, only cardiologists are allowed to prescribe amiodarone.

**Case 1:** A 69-year-old woman was found dead at home in her bed. She had been prescribed amiodarone for atrial fibrillation. Three days earlier she was discharged from the hospital, where she was subjected to a catheter ablation in the left atrium. Previously, she complained of chest pain and a shortness of breath, and twice she was seen by the doctor from the emergency unit, who thought she was all right. At autopsy there were two small holes in the foramen ovale and discoloration in the septum and back wall as follows from the operation. Histology examination showed that the SA-node, the AV-node, and the bundle of His was normal. Routine drug testing was performed on the peripheral blood, and it revealed the concentration of amiodarone was 2.8 mg/kg. The medical examiner concluded that the cause of death was heart rate disturbances likely related to amiodarone intoxication.

**Case 2:** A 48-year-old woman was found dead at home. She was taking amiodarone for atrial fibrillation, and had gone through a catheter ablation in the left atrium two weeks earlier. Two days before her death, she was administered an electrical cardioversion at the hospital, and was discharged the next day. In the evening she called the hospital, because she had a feeling, her heart was not beating correctly. She was told to contact her general practitioner in the morning. The autopsy revealed a slight enlargement of the left atrium with discoloration of the wall, and arteriosclerosis of the left coronary artery and stenosis of the LAD. Histology examination revealed a normal SA-node, AV-node, and the bundle of His. Drug testing was performed on the peripheral blood, and the concentration of amiodarone of 4.0 mg/kg was found. The cause of death was established to be heart rate disturbances probably in relation to amiodarone intoxication.

**Discussion:** These two cases illustrated deaths due to unintended intoxication with amiodarone. The cases show how doctors need to keep the possibility of intoxication with amiodarone in mind when prescribing amiodarone to patients with persistent atrial fibrillation. Despite amiodarone's long history and current widespread use, there are no broadly accepted evidence-based monitoring recommendations. Since the serum concentration has little relation to the clinical effect, measuring it usually is of limited use. However, in such cases the measurement of concentration of amiodarone in serum can be considered in order to prevent the intoxication and death.

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#### **Amiodarone, Intoxication, Death**

### **K16 Extraction of Methamphetamine From Postmortem Blood Samples by Molecularly Imprinted Polymers for Selective Solid Phase Extraction**

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After attending this presentation, attendees will have the opportunity to learn a new way to extract methamphetamine from a complex matrix involving putrefactive amines.

This presentation will impact the forensic science community by presenting information on a new extraction method called MIP-SPE. The

imprinting technique creates high affinity binding sites in MIP. The selectivity of MIP-SPE towards target molecules will be reported in this presentation.

Methamphetamine, a sympathomimetic amine, is a commonly encountered controlled substance in the forensic science community. When postmortem samples are analyzed, particularly blood and tissue, false positives may occur on the enzyme-linked immunosorbent assay (ELISA) screen, or the enzyme-multiplied immunoassay technique (EMIT), which are well known screening tests used by many forensic science laboratories. The false positive from the screen is confirmed with gas chromatography/mass spectrometry (GC/MS). One of the explanations for the occurrence of false positives is the appearance of putrefactive amines in postmortem samples, which may produce cross reactions with the ELISA test. Putrefactive amines, including 1-phenethylamine, 2-phenethylamine, putrescine, tryptamine, and tyramine, have similar chemical and physical properties as methamphetamine and are produced during putrefaction, a step in decomposition in which microorganisms breakdown proteins. Therefore, putrefactive amines have the potential to interfere with the interpretation of ELISA test results of methamphetamine. In this research, methamphetamine was extracted from known ELISA positive blood samples, some that have been treated with known putrefactive amines, putrefactive amines and methamphetamine, and methamphetamine only. The two extraction methods include a liquid-liquid extraction and a molecular imprinted polymer cartridge for solid phase extraction (MIP-SPE), which is designed to be specific towards amphetamines. Liquid-liquid extraction is a well known method for drug extractions but can be less selective and has the potential to use a large quantity of solvents. The liquid-liquid extraction of choice involves multiple washes and a back extraction, which further cleans the extract. The extracts were analyzed by GC-MS in order to determine which extraction method has a more significant specificity towards methamphetamine, which was determined through the absence or the decreased number of putrefactive amines. After attending this presentation, the attendee will know if the MIP-SPE is a more specific extraction method for postmortem blood sample. After method validation, actual postmortem samples will be collected at various postmortem intervals and evaluated with the new validated MIP-SPE method to further confirm the conclusions found in the earlier research. The removal of putrefactive amines from an analysis could reduce the potential for misinterpretation or interference when evaluating methamphetamine results from postmortem samples.

Putrefactive amines are potential interferences when methamphetamine is analyzed from postmortem samples in forensic toxicology. This research will evaluate the application of MIP-SPE for selective extraction of methamphetamine from postmortem samples. The objective is to see if MIP-SPE can successfully eliminate putrefactive amines from postmortem blood samples.

MIP-SPE is a new extraction method. The imprinting technique creates high affinity binding sites in MIP. The selectivity of MIP-SPE towards target molecules will be reported in this presentation.

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#### **Methamphetamine, MIP-SPE, Putrefactive Amines**

### **K17 Capillary Electrophoresis and Capillary Electrochromatography Mass Spectrometry for Chiral Drug Detection**

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After attending this presentation, attendees will understand some principles of how chiral drugs can be separated and detected using capillary electrochromatography – time-of-flight mass spectrometry (CEC-TOF-MS).

This presentation will impact the forensic science community by discussing one of the novel chiral separation techniques called capillary electrochromatography mass spectrometry (CEC-MS).

Worldwide issues with poisoning and death from clandestine drug manufacturing make it important to develop methods that can not only detect low levels of drugs but also determine their chirality. In this way, law enforcement can better track users and victims of these products. In addition, the field of chiral toxicology has become increasingly important as researchers and practitioners recognize the importance of defining the precise role structure – reactivity relationships play in drug activity. As a result, a large amount of research has been conducted in the analysis of chiral drugs. The goal of this project is to develop methods to detect these drugs using Capillary Electrophoresis (CE) and Capillary Electrophoresis Mass Spectrometry (CE-MS) techniques.

Trace detection of pharmaceutical compounds typically employs several analytical techniques, including gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). For chiral separations, these techniques may utilize specific stationary phases or may require derivation to diastereoisomers. Capillary electrophoresis can be a powerful alternative for the separation of neutral and chiral drugs through the use of guest-host reactions and micellar-solute interactions. It is proposed that CE-MS with an electrospray ionization (ESI) source would be a useful technique for separating and analyzing this class of drugs. Capillary electrophoresis-mass spectrometry has a number of important advantages for toxicological analysis including low sample consumption and the potential for highly efficient chiral analysis. Generally electrophoretic techniques are restricted to the analysis of charged molecules. However, in the past 20 years, a variety of electrophoresis techniques have been developed which are suitable for the detection of neutral and chiral drugs including micellar electrokinetic capillary electrophoresis (MEKC) and capillary electrochromatography (CEC).

Proper identification of unknown drugs is a critical issue in forensic drug analysis. While capillary electrophoresis with UV, electrochemical or fluorescence detection can be used to presumptively determine the presence of a particular compound, for absolute identity of trace levels of these compounds, mass spectrometry coupled to chromatography is necessary. While a number of useful procedures have been developed for the detection and screening of compounds by capillary electrophoresis/mass spectrometry, to date applications involving neutral or chiral drug detection by MEKC procedures have been problematic. These samples require separation via a detergent or cyclodextrin based pseudo-stationary phase that can be incompatible with electrospray ionization methods that require volatility. One potential solution for this issue is to operate the CE-MS system in a partial filling mode to avoid spraying the reagent into the spectrometer. However, the issues of timing of the capillary filling and the potential instability of a bimodal buffer make this a difficult technique.

Alternatively, capillary electrochromatography (CEC) can be used. This procedure is a novel technique which permits the detection of neutral compounds by combining the high efficiency of CE with outstanding selectivity of HPLC. In CEC, the capillary column is packed with an HPLC type stationary phase. Separation occurs via sample partitioning between the packed stationary phase and an electrodriven mobile phase. An efficient way to produce this stationary phase is through in-situ polymerization into a so called polymer monolith<sup>2</sup>. When coupled to mass spectrometry, the procedure has been shown to provide efficient and sensitive detection of drugs and their metabolites in biological fluids.

In this study, monolithic CEC-MS will be developed and compared with partial filling MEKC for the application of chiral drug detection in complex matrices. The CEC stationary phase will be developed by bonding chiral selectors onto the silica inner wall of a capillary or through attachment of these selectors to carbon chains on acrylate monomers. In this way CEC-MS can be developed as a powerful technique for chiral toxicology as well as other applications in pharmacological analysis.

#### **Capillary Electrochromatography, Mass Spectrometry, Chiral Drug Detection**

## **K18 A Spectroscopic Investigation of the Binding of Benzodiazepines to Human Serum Albumin**

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After attending this presentation, attendees will understand how fluorescence spectrophotometry can be used to study the interactions of different benzodiazepine derivatives with human serum albumin. Attendees will become aware of the varying binding affinities of different benzodiazepines for human serum albumin and some important parameters used to characterize the binding.

This presentation will impact the forensic science community by providing further pharmacological and toxicological information on benzodiazepines, a class of drug that is commonly used therapeutically and is increasingly being abused in social settings.

Benzodiazepines are commonly prescribed central nervous system depressants which are found in a wide variety of different medications from sedatives, hypnotics, to amnesiatics, and anticonvulsants. Benzodiazepines are increasingly being used as recreational drugs often in combination with other drugs such as opiates and alcohol. Human serum albumin is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and was the method utilized in the current research to study the interaction of different benzodiazepines with human serum albumin. The quenching of albumin fluorescence by nine benzodiazepines were analyzed at three different temperatures, 24°C, 30°C, and 37°C. The nine benzodiazepines used in this study were alprazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam. Varying concentrations of each benzodiazepine were incubated with a 2.42µM solution of human serum albumin. The albumin solution was prepared in a 0.05M Tris buffer at a pH of 7.4. Benzodiazepines were tested at each temperature at concentrations ranging from 19.82 µM to 198.2 µM. Each concentration was analyzed five times. A fluorescence spectrophotometer with thermostated cell holder was used to measure fluorescence. The excitation wavelength was set at 290nm and the emission wavelength range was set at 300-500nm.

Quenching mechanisms associated with the binding between a quencher and a macromolecule can be static or dynamic. Stern-Volmer analysis of the data was used to characterize the quenching mechanism. Comparison of the nine benzodiazepine derivatives established that all derivatives exhibited a quenching rate constant of  $\gg 2 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ , indicating that static quenching was present. Static quenching as opposed to dynamic quenching signifies that a relatively stable complex is being formed between the benzodiazepines and the human serum albumin. Analysis of the fluorescence quenching using double log plots allowed the determination of the number of binding sites (n) and binding constants (K<sub>b</sub>). The benzodiazepines that showed significant variation in binding affinity were diazepam and flurazepam. Diazepam, for example had a binding constant of 114.1 at 37°C whereas flurazepam had a binding constant of  $1.808 \times 10^6$  at the same temperature. Diazepam had an “n” value close to one whereas flurazepam had an n value closer to two, possibly indicating the existence of more than one binding site. Van’t Hoff analysis of the data was also used to calculate the thermodynamic parameters of binding and provided evidence of spontaneous binding and information on the involvement of hydrogen bonding and hydrophobic interactions in the binding of these drugs to albumin. The eventual goal is to use this information to determine if the affinity of these drugs for albumin can

influence the analysis of these drugs in blood/plasma using current methods of sample preparation and analysis.

**Forensic Science, Benzodiazepines, Fluorescence Spectrophotometry**

### **K19 Multi-Analytical Measurement of Drugs of Abuse in Vitreous Humor With Evidence Biochip Arrays**

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After attending this presentation, attendees will be able to see the application of biochip technology to the screening of common drugs of abuse compounds in small amounts of vitreous humor.

This presentation will impact the forensic science community by introducing cutting-edge technology for screening very small quantities of vitreous humor for drugs of abuse. This is pertinent in cases where no blood or urine is available and for screening for drugs in small children.

**Introduction:** To accommodate the demand for tests and the appearance of new drug classes, new analytical methods are required for clinical, regulatory, toxicological, and forensic applications. The use of screening methods enabling the rapid and simultaneous detection of multiple drugs of abuse facilitates the application to regulatory control as only positive results require confirmation. Evidence biochip array technology provides a flexible platform for the simultaneous determination of multiple analytes from a single sample across a number of matrices including vitreous humor.

**Methods:** The Drugs of Abuse I blood array was used to analyze the vitreous humor from 81 subjects that had gone through postmortem examination in the NHS Grampian region and the results compared against confirmatory GC/MS and LCMS/MS methods. The Drugs of Abuse I blood array facilitates the simultaneous detection of the following drug classes using 20µL of vitreous humor – methamphetamine, amphetamine, barbiturates, generic benzodiazepines, lorazepam, methadone, PCP, opiates, cocaine metabolite, and THC. Cut-off's were chosen by evaluation of a set of standards manufactured by spiking across a calibration range (5-200ng/mL) of the drug/metabolite of interest into bovine vitreous humor.

**Results:** The biochip screening method showed a high degree of agreement with the confirmatory methods with an overall average of 92% of results being in concordance with those generated from confirmation. Of the various drug classes: six agreed with confirmation in over 96% of cases (amphetamine, barbiturates, benzodiazepines, cocaine metabolite, methadone, and PCP); two others agreed with confirmation in over 90% of cases (lorazepam and methamphetamine). The opiates test was correct in 82% of the cases with THC correct in 67% of the cases. The lower specificity of the THC detection in vitreous may be on account of the proportion of THC and THCCOOH distributed in the vitreous humor compared to blood. Discrepant results were both falsely negative and falsely positive in relation to the chosen cut-offs for these two drug classes.

**Conclusion:** The data from this small pilot study indicate that the biochip drugs of abuse blood arrays can potentially be modified to allow for the drugs screening of vitreous humor. This is potentially very useful in cases where postmortem blood and/or urine may not be available, for example in victims of traumatic injury. Further investigations may improve the agreement for opiates and THC either by a different choice of cut-off concentration or possibly by performing a quick sample extraction procedure. This technology enables the generation of quantitative abused drug profiles and represents a useful tool for application in forensic and postmortem toxicological settings.

**Biochip Array, Vitreous Humor, Drugs of Abuse**

### **K20 Pharmacokinetic Postmortem Evaluation Reveals Death of a Toddler From Carbamazepine to be Accidental Rather Than Criminal**

*Fran Gengo, PharmD\*, Meghan Rowcliffe, PharmD, and Michelle Rainka, PharmD, DENT Neurologic Institute, 3980 Sheridan Drive, Amherst, NY 14226*

After attending this presentation, attendees will understand principles of clinical pharmacokinetics regarding the concentrations of carbamazepine that would be expected in its treatment of epilepsy. These include the effect of dosage changes, interactions with drugs that induce carbamazepine metabolism, and drugs that inhibit carbamazepine metabolism. Knowledge of dosage changes and drug interactions provide critical insight for interpretation of toxic concentrations measured at time of autopsy.

The presentation will impact the forensic science community by providing an understanding of how pharmacokinetic variables can affect relationships between dose and concentrations can provide insights for interpretation of toxic concentrations

Carbamazepine and phenytoin are two antiepileptic drugs that are prescribed for both partial seizures and generalized tonic clonic seizures.<sup>1</sup> These two drugs require therapeutic drug monitoring during treatment to ensure efficacy and prevent undesired adverse effects. When administered concomitantly, there is potential for a pharmacokinetic drug-drug interaction to occur. Rare cases of toxicity due to concomitant administration have been reported in the literature. Described herein are the circumstances and autopsy findings of a 23-month old child with a history of epilepsy whose death was caused by acute carbamazepine intoxication. This child suffered from seizure disorder since the age of one. Because of an intolerance to phenobarbital and mephobarbital (two other antiepileptics), he was placed on carbamazepine and phenytoin. The child died five months later and the cause of death was determined to be carbamazepine toxicity. The postmortem serum concentration of carbamazepine was 23.7mcg/ml, which is significantly higher than targeted therapeutic levels of 5-12mcg/ml.<sup>1</sup> The toddler's caregiver was then alleged to have intentionally overdosed the child causing acute toxicity, but events leading up to the death of this child included a myriad of dosing changes including discontinuation of phenytoin two weeks prior to the time of death.

Carbamazepine is converted to an active metabolite, carbamazepine 10,11-epoxide, through oxidation which is then metabolized to carbamazepine diol, the inactive metabolite.<sup>2</sup> Phenytoin is a potent inducer of the liver enzyme CYP3A4, which predominantly metabolizes carbamazepine, causing higher carbamazepine epoxide/carbamazepine and carbamazepine diol/carbamazepine ratios in children receiving both drugs.<sup>2</sup> <sup>3</sup> A decrease in plasma concentrations of carbamazepine is correlated with the dosage of phenytoin being administered.<sup>4,5</sup> Also, because carbamazepine has a unique property of auto-induction, the dose is increased gradually based on serum levels drawn every two to four weeks after initiation of therapy.<sup>1</sup> Average steady state concentrations of carbamazepine decrease by 50% after three weeks of administration.<sup>6</sup> A study conducted by Duncan et al. observed the effects of phenytoin discontinuation on concomitant carbamazepine therapy. It was found that upon discontinuation, total carbamazepine concentrations increased by a mean of 48% after four weeks of phenytoin removal and the ratio of carbamazepine epoxide/carbamazepine decreased.<sup>7</sup>

During the five months prior to death, the child's carbamazepine dose was increased from 150mg/day to 500mg/day. Over this same period of time the child was also treated with medications that can alter the pharmacokinetic disposition of carbamazepine. Pharmacokinetic modeling was used to estimate expected carbamazepine serum levels based on carbamazepine dosing orders from the time carbamazepine was started, the known effects of concomitant drugs on the pharmacokinetic disposition of carbamazepine and the timing of the addition and/or removal of these other

drugs. Estimates of carbamazepine concentrations from the pharmacokinetic model were compared to actual measured concentration. Estimated concentrations correlated well with measured concentrations. This demonstrated that carbamazepine concentrations measured at autopsy were the concentrations expected based on the dosing history and timing of the addition and removal of other drugs known to effect the pharmacokinetics of carbamazepine. The concentrations of carbamazepine measured at autopsy were concentrations were expected concentrations based on the prescribed doses as predicted by the pharmacokinetic model. Deliberate overdosing of carbamazepine was ruled out and with this insight the child's caregiver was exonerated.

This case underscores the importance of understanding the pharmacokinetic history of drugs with narrow therapeutic indices in the interpretation of toxic drug concentrations measured at autopsy.

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#### Carbamazepine, Toxicity, Postmortem Drug Levels

## K21 Analysis of Synthetic Cannabinoids by Mass Spectrometric Methods Coupled With Accurate Mass Determination

*Dima Sbenaty\**, 1507 Buckingham Drive, Murfreesboro, TN 37129; and *Vanessa Hobbs, MS, and Ngee-Sing Chong, PhD, Middle Tennessee State University, PO Box 68, Chemistry Department, Murfreesboro, TN 37132*

After attending this presentation, attendees will learn about the role of accurate mass determination in gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS) methods for analyzing synthetic cannabinoid products. The synthetic cannabinoid compounds of herbal products along with the full range of organic compounds present in the gas and particulate phases of smoke samples of the herbal products will be discussed. The findings and methodologies will contribute to future development of a protocol for analyzing banned synthetic cannabinoid substances and assessing their toxicological effects.

This presentation will impact the forensic science community by emphasizing the advantages of accurate mass determination in mass spectrometric analysis of illegal drugs and how it will be beneficial to the drug enforcement and forensic communities by providing rapid and reliable analysis of synthetic cannabinoid substances. Sound analytical methods based on mass spectrometry for characterizing products seized in police

raids and assessing the levels of the related drug compounds in the urine and smoke samples will contribute to effectiveness in drug enforcement and toxicological studies of the synthetic cannabinoid products, respectively.

The use of illegal synthetic cannabinoids has become increasingly popular over the past several years. These drugs have been successful in providing a "high" just like marijuana and yet these drugs and their metabolites can circumvent detection by most standard drug testing methods for natural cannabinoids. As a result, herbal products containing the synthetic cannabinoids such as JWH-018 and JWH-073 have become more appealing to drug users. These synthetic cannabinoids have shown harmful health effects in the human body and may even cause death from high concentrations of toxic compounds accumulated via extended drug abuse or accidental overdoses through haphazard manufacturing practices involved in these illegal products. It is therefore critical for these drugs and their metabolites to be identified and quantitatively determined by law enforcement and clinical laboratories. The study aims to develop an efficient and reliable method that will identify the synthetic cannabinoids in a forensic testing laboratory.

Forensic analysis of illegal compounds usually involves methods that could provide unequivocal evidence in a court of law. Most current methods involve GC/MS and LC/MS for analyzing commonly abused drugs like cocaine, heroine, and methamphetamine. Although both GC/MS and LC/MS have been reported for determining synthetic cannabinoids in the herbal products and urine samples,<sup>1-3</sup> there has not been any study of cannabinoid distribution in the gas and particulate phases of the smoke inhaled by the users. In this study, the smoke of the herbal products containing synthetic cannabinoids is studied in order to estimate the range of doses of the active ingredients in commonly available products. The GC/MS method developed in this project also relies on accurate mass determination to provide greater confidence in MS analysis based on quadrupole mass analyzers. The goal of developing such a method is that the analytical chemist can either bypass the GC analysis or use a short GC column to achieve rapid analysis to cope with the sample throughput issue in most forensic laboratories. Furthermore, the possibility of a portable mass spectrometer with accurate mass analysis can be conducive toward the development of mobile laboratory testing of illegal drugs for the law enforcement community. GC/MS instrumentation is fairly bulky and is too cumbersome for crime scene analysis. Therefore, it is beneficial to develop "GC-less" MS methods for onsite analysis in order to exclude possible contamination when crime scene samples are collected and brought back to the laboratory for GC/MS analysis. Also, accurate mass analysis is more efficient in unequivocal identification of target compounds relative to GC that is prone to chromatographic co-elution. Preliminary analysis of this study has shown that software-based accurate mass determination using quadrupole mass data allow highly specific and accurate mass-to-charge analysis to the third decimal places for the parent and fragment ions of commonly abused drugs, thus allowing reliable determination these banned substances.

There is great variability in the drug concentrations found in different herbal blends of synthetic cannabinoids. The amount of JWH-018 ranged from 4.09±0.04 mg/g herbal blend in K2 Blond to the highest concentration in Ultra Cloud 10 of 41.80±0.62 mg/g of herbal blend. The amount of JWH-073 ranged from 6.11±0.27 mg/g of herbal blend in K2 Blond to the highest concentration in Ultra Cloud 10 of 13.20±0.24 mg/g of herbal blend. Low concentrations of JWH-018 in K2 Blonde are likely compensated by the presence of JWH-250, a compound not found in the other 4 herbal blends studied. When the smoke samples were analyzed, it was found that the synthetic cannabinoids were not detectable in the gas phase but were detected in the particulate phase collected using a cascade impactor. This indicates that JWH-018, JWH-073, and other synthetic cannabinoids are inhaled in the particulate phase with a particle size fraction of less than 0.25 µm. The analysis also revealed that most of the flavor or scent additives for the herbal products were detected in the particulate phase. Further analysis using LC/MS with a time-of-flight mass spectrometer and ion mobility instrumentation allows the complete characterization of the synthetic cannabinoids and their related metabolites

and by-products in the mainstream smoke samples.

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### **Illegal Synthetic Cannabinoids, Accurate Mass Determination, Mass Spectrometric Analysis**

## **K22 Analysis of Volatile Organic Compounds Emitted During Aerobic Decomposition of Various Swine Tissues**

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After attending this presentation, attendees will learn about the analytical methods for the analysis of analytes released from decomposing animal tissues and organs. Cryogenic and sorbent preconcentration of ultratrace analytes are used in conjunction with gas chromatography-mass spectrometry (GC/MS) and pre-evacuated bottle or canisters for sample collection. The identity of the volatile organic compounds (VOCs) discovered and their relationship to the decomposition process will be discussed. The findings and methodologies will have important implications in understanding the chemistry of decomposition and the postmortem interval (PMI) in addition to improving the efficiency in search and recovery of dead human or animal remains.

This presentation will impact the forensic science community by providing important information on the profile of chemical compounds emitted from various tissues of animal carcasses, correlating the specific parts of the carcass with unique identities of compounds released, and their relationship to the different stages of the decomposition process. Knowledge related to the decomposition process could also improve the search and recovery of victims as well as provide crucial evidence for convicting or acquitting suspects in murder trials.

In a recent trial case, emitted volatile organic compounds (VOCs) found in the trunk of the car used by the accused for transporting human remains was introduced as evidence for the first time. However, just like with any new evidence, lawyers were able to discredit this evidence due to lack of knowledge and the need for further research. The goal of this research is to elucidate the decomposition mechanisms of animal carcasses that might yield forensic clues in the investigation of human remains.

Variable environmental conditions, the presence of microorganisms and the disposal mode of the animal or human remains, as well as the amount of time elapsed once the decomposition process has taken place greatly influence the emission profile of the VOCs. Statheropoulos et al.<sup>1</sup> and Vass et al.<sup>2</sup> independently measured more than 100 VOCs that were released during the decomposition of human remains. However, these studies involve the presence of body bags or other burial conditions including the soil matrix, insects, and microorganisms that are typically associated with decomposition. In this study, the influence of environmental media (e.g., soil, sand, water) in the decomposition process was minimized by conducting the study of decomposing tissues in glass vessels that are configured with a leak-proof design for efficient collection of compounds emitted from the degradation of tissues. Samples were collected approximately three times a week over a period of ten weeks

using pre-evacuated bottles or canister that were analyzed by gas chromatography-mass spectrometry (GC/MS) with analyte enrichment on a 3-trap preconcentrator.

The predominant classes of compounds found in decomposing tissues include sulfur-containing compounds, esters, and aldehydes. The data shows that aldehydes are formed more readily in organs and in general, aldehydes with five or six carbons, i.e., pentanal, hexanal, and 3-methyl butanal were found at the highest concentrations. Several ketones and esters were found in the muscle and skin samples whereas 1,1-difluoroethane was found to be characteristic in bone decomposition. The presence of sulfur-containing compounds such as carbon disulfide, methanethiol, dimethylsulfide, dimethyldisulfide, and dimethyltrisulfide could be linked to the biochemical degradation of sulfur-containing amino acids like methionine, cystine, and cysteine that constitute the swine tissues or organs. The presence of dimethyldisulfide and dimethyltrisulfide may be linked to cystine, which is due to the dimerization of two cysteine units and is commonly found in collagen (e.g., connective tissue) and keratin (hooves and hair).

The cryofocusing GC/MS technique based on a 60-meter column with dimethylsiloxane stationary phase was able to detect alkanes with carbon numbers ranging from 3 to 14 as well as aliphatic alcohols with 2-8 carbons. The detection limits of the compounds released from the decomposition of porcine tissues are generally in the 0.1 to 20 parts per billion levels. With the current study of tissue-specific or organ-specific decomposition studies, valuable information will be gleaned for strengthening the credibility of evidence involving odor analysis pertaining to human decomposition in court.

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### **VOC Analysis, Decomposition Chemistry, Animal Carcass**

## **K23 Is Your Urine Really Dilute? An Analysis of Normal Urine Creatinine Measured by HPLC-UV Below the Cutoff Limit of 20 Mg/Dl**

*Brad T. Holden, BS\*, Heather L. Workman, BA, and Erica A. Guice, MS, Western Slope Laboratory, 1197 Rochester Road, Suite K, Troy, MI 48083*

After attending this presentation, attendees will understand a simple method for the detection of creatinine in urine samples, how creatinine concentrations is used in clinical medicine to determine adulteration of samples, factors effecting creatinine concentration in urine, and hear a proposal to change the cutoff limit of adulterated urine samples.

This presentation will impact the forensic science community by addressing concerns of the Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines for urine adulteration by reviewing the creatinine concentration of a population of unknown advanced toxicology urine samples.

In clinical and forensic toxicology laboratories there is a need to establish criteria for identifying a random urine sample submitted for drug testing as being adulterated by using creatinine analysis. According to SAMHSA, the cutoff value mandated for workplace drug testing is 20mg/dL; however, there are many factors that independently influence the concentration of creatinine in a urine sample. These factors include but are not limited to: gender, muscle mass, diet, fluid consumption, and several clinical conditions such as polyuria. With so many factors influencing the concentration of creatinine in the urine it is possible that many samples may

be determined to be dilute or substituted when, in fact, a value of less than 20mg/dL is normal for that donor. This presentation will discuss the use of creatinine concentration data from the aforementioned population of samples to address the issue of the 20mg/dL SAMHSA cutoff being too high as well as predispose the samples to falsely high level of positive diluted urine samples.

A simple method for the detection of creatinine in urine samples was used in this study to determine the creatinine concentration in 4,600 advanced toxicology urine samples. This method uses cation-pairing high pressure liquid chromatography with a ultra-violet detector (HPLC-UV). This HPLC-UV method allows for simple sample preparation and the automation of sample analysis. Using this method also allows for a large linear range of quantitation in the physiologically relevant range of 0.1-500 mg/dL and a limit of detection as low as 0.05mg/dL. For this study, 4,595 samples were tested for creatinine using the method described above. Out of these samples, 160 (3.48%) tested for a creatinine concentration of less than 20mg/dL. From these samples determined to be dilute or substituted, 140 (87.5%) samples were confirmed positive for one or more drugs. Altogether, 70% of the dilute samples were from women and 30% were from men. The average value for the dilute samples was 12.3 and the median value was 14. Twenty-one of these low creatinine samples were tested for pH, specific gravity and creatinine using urine drug adulteration test strips. Out of these 21 samples, 20 of them were suspected of adulteration based off the results of creatinine or specific gravity.

In conclusion, this presentation addresses the concerns of the SAMHSA guidelines for urine adulteration by reviewing the creatinine concentration of a population of unknown advanced toxicology urine samples and noting potential issues with the current cutoff limit. As many factors can influence the creatinine concentration this newly proposed value will help reflect this and help to reduce the number of samples reported to be falsely diluted.

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#### **Creatinine, Adulteration, Toxicology**

### **K24 Method Development and Validation for the Detection of Cannabinoids in Blood Using LC/MS/MS**

*Eduardo Padilla, BS, Texas Department of Public Safety Crime Laboratory, 5806 Guadalupe Street, Austin, TX 78752; and Sarah E. Martin, BS\*, 2435 Montgomery Road, Apartment 133, Huntsville, TX 77340*

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After attending this presentation, attendees will understand how a method was developed and validated for Texas Department of Public Safety (DPS) Crime Laboratory to detect cannabinoids, specifically,  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC, in blood using LC/MS/MS.

This presentation will impact the forensic science community by allowing for the detection of cannabinoids,  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC, in blood samples submitted to the DPS Crime Laboratory.

The objective of this research was to develop and validate a method for the detection of cannabinoids in blood using LC/MS/MS.<sup>1</sup> This project is beneficial to the toxicological field in that the compounds of interest,  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC, would be able to be detected in blood, which is the biological sample that is most often submitted to the Texas DPS Austin Crime Laboratory. Using LC/MS/MS, the cannabinoids would not need to be derivatized, while GC/MS requires it, saving time and therefore money. Methods were developed for detection of cannabinoids in blood on the LC/MS/MS by other agencies and manufacturers, but a cannabinoid method for the DPS laboratory will benefit the entire state by being free of charge to law enforcement agencies. The developed method for DPS was similar to the Dallas County Institute of Forensic Sciences method, but also took into account the methods in published scientific articles. This project required testing the different procedures obtained, and evaluating and optimizing each step performed, including the extraction technique, LLE or

SPE. Based on the results, a method for the DPS Crime Laboratory was created.

This method was validated through different parameters that tested selectivity, recovery, linearity, limit of detection, limit of quantitation, carryover, reproducibility, stability, and competency. The method showed no carryover at concentrations four times the highest calibers, 100 ng/mL for  $\Delta^9$ -THC and 250ng/mL for carboxy-  $\Delta^9$ -THC, was only selective for  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC, and showed that both  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC are stable reconstituted after twenty-four hours. The recovery efficiency for  $\Delta^9$ -THC was found to be 68.1% for the low concentration, 2ng/mL, and 56.8% for a mid concentration, 25 ng/mL. The recovery efficiency for carboxy-  $\Delta^9$ -THC was 23.7% for the low concentration, 2 ng/mL, and 18.6% for a mid concentration, 25ng/mL. These results are comparable to other agencies' results. It was also determined that the LOQ for  $\Delta^9$ -THC was  $\frac{1}{4}$  of the low caliber (0.5 ng/mL) and the LOQ for carboxy-  $\Delta^9$ -THC was  $\frac{1}{2}$  of the low caliber (2.5 ng/mL). The LOD for  $\Delta^9$ -THC was  $\frac{1}{32}$  of the low caliber (0.0625ng/mL) while the LOD for carboxy-  $\Delta^9$ -THC was  $\frac{1}{4}$  of the low caliber (1.25ng/mL). Regarding linearity, both  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC produced an acceptable curve with a quadratic inverse squared plot. The outside controls were all reproducible and fell well below +/- 20% bias and precision. Therefore, this method can now be used for Texas DPS casework in the future to test for the presence and the concentration of  $\Delta^9$ -THC and carboxy-  $\Delta^9$ -THC.

#### **Reference:**

- <sup>1</sup> Dallas County Institute of Forensic Sciences Toxicology Laboratory. Cannabinoids in blood by LC/MS/MS, Version 2.0.

#### **$\Delta^9$ -Tetrahydrocannabinol, Carboxy- $\Delta^9$ -Tetrahydrocannabinol, Liquid Chromatography Tandem Mass Spectrometry**

### **K25 An LC/MS/MS Analytical Method for Mephedrone and Naphyrone Metabolite Analysis in Urine**

*Cicely Berg, BS\*, Brent Dawson, PhD, and Hua-Fen Liu, PhD, AB Sciex, 353 Hatch Drive, Foster City, CA 94404*

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The goal of this presentation is to show a strategy for detecting metabolites of mephedrone and naphyrone in a urine matrix with tandem MS technology. Attendees will learn the identity of some of the prominent metabolites and their characteristic ions.

This presentation will impact the forensic science community by showing how mephedrone and naphyrone and their metabolites can be detected with tandem mass spectroscopy. Additionally the work will give forensic scientists confidence in the analysis of such compounds with qualifying and quantifying ion transitions.

Designer drug chemistry has been dominated by substituted phenylethylamines and tryptamines. The production, availability, and use of cathinone as a framework drug have been perceived as the next big thing. They include the beta-keto version of amphetamines which includes mephedrone. In 2010, mephedrone was made illegal in many countries. Naphyrone (also known as NRG-1, Energy-1, or O-2482) is a cathinone derivative that emerged in late 2010 as a new legal high in the UK after the banning of mephedrone. Naphyrone is a new designer drug and stimulant with many cases of abuse reported in the UK.

Until July 2010, naphyrone was not controlled by the misuse of Drug Act 1971 and was therefore not illegal for someone to possess. Since the Medicines Act prevented naphyrone for being sold for human consumption, it was often sold as a "pond cleaner." Currently very little safety or toxicity data are available for naphyrone, but its high potency by comparison with previous cathinones or MDMA (ecstasy) suggests that its use is likely to be associated with a higher risk of accidental overdose.

LC/MS/MS analysis can provide a fast analysis time as well as accurate, precise, and reproducible results. Here we present a method for the analysis of mephedrone and naphyrone metabolites in urine using a triple-quadrupole-ion trap mass spectrometer.

The method development process evaluated different sample preparation procedures, calibration curve construction, column selection, mobile phase selection, and ion suppression.

This analysis was performed on a reversed phase column analyzed by LC/MS/MS with a run time under five minutes. The mass spectrometer was operated in multiple reaction monitoring mode (MRM) in positive ion mode. The assay was shown to be accurate and precise with %CV and % accuracy within  $\pm 15\%$  of nominal across the full linear range.

**Bath Salts, Synthetic Drugs, Mass Spectrometry**

## **K26 Screening for K2: Monitoring JWH-018, 073, 081, and 250 and Some Prominent Metabolites by HPLC-MS/MS**

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The goal of this presentation is to show a strategy for detecting synthetic cannabinoids in a urine matrix with quadrupole-linear ion trap technology. Attendees will learn the identity of some of the prominent metabolites and their characteristic ions.

This presentation will impact the forensic science community by showing how synthetic cannabinoids and their metabolites can be detected with a hybrid quadrupole-linear ion trap mass spectrometer even in the absence of standards. Additionally, the work will give forensic scientists confidence in the analysis of such compounds with mass spectral library search techniques.

**Objectives:** To expand K2/spice screening method to include JWH-81 and JWH-250 metabolites using a quadrupole-linear ion trap, to identify the metabolites and generate a spectral library. Human liver microsomes and hepatocytes were incubated with the parent drugs (JWH-018, 073, 081, 250) to generate phase I and phase II metabolites. After predicting the precursor and product ions using a metabolite identification software package the incubated samples were analyzed using the multiple reaction monitoring to trigger acquisition of product ion spectra. Spectral comparison of the metabolite spectra to those of the parent drugs allowed for the identification of the metabolites. The objective of the work was to produce a highly sensitive screening method for JWH-81 and JWH-250 metabolites and parent drugs to augment our current method that screens for JWH-73 and JWH-18 metabolites and parent drugs.

**Materials and Methods:** A state of the art quadrupole linear ion trap and High Performance Liquid Chromatography (HPLC) were used to acquire the metabolite mass spectra. The initial multiple reaction-monitoring list was generated from a metabolite identification software package. Samples were analyzed on a biphenyl hplc column using an acetonitrile gradient with 0.1% formic acid to aid ionization. Standards were prepared in synthetic urine matrix and samples were prepared by diluting them with acetonitrile prior to centrifugation at 21,000 rcf. When the multiple reactions monitoring transition generated a signal above a set threshold, the linear ion trap obtained the product ion spectrum on the metabolite precursor. Once the metabolite identification was confirmed through spectral analysis, the product ion spectra were added to a spectral library.

**Result:** Multiple metabolites of each parent drug were found including alkyl chain hydroxylations, demethylations, indole ring hydroxylations, carboxylations and hydrogenations. The glucuronide conjugates of these phase I metabolites were identified in the hepatocyte incubation of JWH-250. The mass spectral analysis allowed for the identification of the prominent metabolites as hydroxylations on the aromatic rings or the aliphatic chain portions of the molecules. The

addition of 17 daltons to the recorded  $m/z$  values for the metabolite parent ion and the fragment ions versus those of the drug as well as the absence of other changes in the  $m/z$  values led to this conclusion. Representative product ion spectra were added to a compound database against which spectra from physiological samples could be searched using mass spectral software. Using dilute and shoot sample preparation allowed for rapid and sensitive sample analysis with good purity scores on spectral matches though sensitivity could be increased with enzyme hydrolysis of the glucuronide metabolites. When the microsome incubation solutions were spiked into urine samples, metabolites of JWH-018, JWH-73, JWH-081, and JWH-250 could be uniquely identified through a library search algorithm.

**Conclusion:** Many common metabolites of JWH-250 and JWH-081 were identified using the quadrupole linear ion trap. A spectral library of the most common metabolites was generated, and this library was used in a screening method to detect metabolites in urine samples. The method provides for the high sensitivity screening of physiological samples for JWH-018, JWH-073, JWH-081, and JWH-250 metabolites as well as the parent drugs.

**K2, Spice, Mass Spectrometry**

## **K27 Drug Screening Using a High Resolution Accurate Mass System**

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The goal of this presentation is to show the use of fast, high resolution, accurate mass system capable of generating TOF-MS data with up to 20 product ion spectra in under a second.

This presentation will impact the forensic science community by giving attendees a great option for generating high resolution, accurate mass data for use in forensics and toxicology.

**Objectives:** This research evaluates the use of a fast, high resolution, accurate mass system capable of generating TOF-MS data with information dependent acquisition (IDA) of up to 20 TOF-MS/MS in one second for drug screening purposes. Initial drug identification was accomplished by accurate mass MS and confirmation by accurate mass product ion spectrum which was searched against a library. The use of an existing product ion spectra library generated on a hybrid triple quadrupole ion trap system with the data generated from high resolution, accurate mass system is also evaluated.

**Methods and Sample Preparation:** Blood samples (250 $\mu$ L) were precipitated with the addition of 750 $\mu$ L of acetonitrile. Samples were then vortexed, centrifuged and the supernatant evaporated, before reconstitution with 500 $\mu$ L of 10/90 acetonitrile/water. Urine samples were diluted with five volumes of 10/90 acetonitrile/water.

**Chromatography:** The separation was carried out on a PFP Propyl 50mm x 2.1mm x 5 $\mu$  column. Mobile phase A consisted of 0.2% formic acid and 2mM ammonium formate in water and mobile phase B consisted of 0.2% formic acid and 2mM ammonium formate in acetonitrile. A linear gradient starting at 10% organic and ending at 90% organic was used with a total flow rate of 0.7mL/min and a total run time of 17.5 min.

**Mass Spectrometer:** The drug screening method consisted of a full range TOF-MS survey scan with IDA-triggering of up to 20 accurate mass product ion scans. Total cycle time for the TOF-MS – IDA – TOF-MS/MS (20) was approximately one second. The product ions were automatically searched against an existing library containing spectra for over 1200 compounds. The library was previously generated on a hybrid triple quadrupole ion trap system and was evaluated for compatibility with the accurate mass system.

**Results and Conclusion:** Positive blood and urine samples, as well as spiked matrix samples, were used for the evaluation of the drug screening method and the library search tool along with the library previously generated on a different mass spectrometer. All compounds in the spiked

matrix samples were correctly identified and confirmed. High concentrations of antidepressants, pain medications, or drugs of abuse were observed in the positive samples. The method allows for the positive identification of over 1,200 drugs and metabolites by using accurate mass along with MS2 confirmation. With the fast, high resolution, accurate mass system, TOF-MS along with 20 product ion scans were collected in about one second allowing for more analytes to be confirmed even with multiple co-eluting analytes. TOF-MS scan allowed for identification of analytes by exact mass with mass error less than 5ppm. Confirmation was accomplished by searching all generated product ion scans against a library with more than 1,200 compounds generated on the hybrid triple quadrupole ion trap system. Library search scores ranged between 75% and 98% showing that even though the library was generated on a different mass spectrometer, the spectra can be used since similar fragmentation patterns are obtained in both instruments.

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**Accurate Mass, Mass Spectrometry, TOF-MS**

## **K28 Two Fatalities, Survival Driver, and Topiramate**

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After attending this presentation, attendees will have greater awareness of how the misuse of prescription drugs can result in cognitive impairment and judgment which could lead to serious motor vehicle accidents and possible death due to overdosing.

This presentation will impact the forensic science community by informing attendees of a potential problem with prescription drug abuse such as topiramate without any street drug or alcohol.

Topiramate (Topamax®) is FDA approved for initial monotherapy or adjunct treatment for partial, generalized tonic-clonic seizures, Lennox-Gastaut syndrome, and as a migraine prophylaxis. The common side effects seen with topiramate include ataxia, reduced concentration, confusion, dizziness, fatigue, impaired psychomotor performance, memory impairment, somnolence, and speech difficulty. Topiramate is categorized in the sugar sulfamate family and is available in a capsule, sprinkle, and tablet forms in varying dosage strengths.

A 28-year-old man was reported driving on the freeway at a high rate of speed and weaving in and out of traffic when he struck another vehicle on the back quarter of the vehicle. The rear ended vehicle lost control and rolled over several times and landing in a grassy area along the right side of the freeway. The passenger of the rolled over vehicle was ejected out of passenger front window. He was rushed to a nearby hospital where he was pronounced dead by major head trauma, collapsed lungs, and open fractures on both lower legs. The driver was properly restrained but died at the scene because the roof of his vehicle collapsed on top of him.

The man that caused the accident was not injured. The suspect was given a field sobriety test at the scene but failed. The suspect was then transported to a local hospital where he voluntarily gave a blood sample for DUI toxicology testing the shows topiramate was the sole drug identified and confirmed in the blood at a concentration of 13mg/L. A reported blood toxic concentration range is 8 to 17mg/L. The therapeutic concentration levels are recognized at a mean of 5 mg/L.

Prescription drug abuse for the purpose of “getting high” is emerging today because of the ease of access to this prescription drugs. The side effects of topiramate further highlight the dangers of potential drug abuse; however, this case of topiramate toxicity that lead to two motor vehicle related deaths was unusual in that no other drugs or alcohol were detected and that topiramate was contributory to impaired human performance in operating a motor vehicle.

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**Topiramate, DUI, Prescription Drug**

## **K29 Development of a New Gas Chromatographic Column Set for the Analysis of Blood Alcohol Concentration**

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After attending this presentation, attendees will understand the importance of GC column chemistry in the resulting separation, and how this may be optimized.

The presentation will impact the forensic science community by making attendees aware of improved methodology available for BAC analysis.

Blood alcohol concentration (BAC) analysis may be the most common analytical test performed by laboratories involved in forensic and medical testing. This analysis typically uses static headspace sampling, followed by dual-column gas chromatography (GC) separation followed by flame ionization detection (FID). Chromatographic separation of the target analytes, as well as the possible interfering compounds, is critical for this analysis, especially given that the FID is not selective. Incomplete chromatographic separation will cause a quantification bias, and possibly invalidate the results of the test. This condition makes a number of demands on laboratories performing this work. Laboratories are also under time pressure, and must continually balance the need for separation with fast sample turnaround and short analysis times.

The most important chromatographic variable in this separation is the selectivity of the GC stationary phase; however, most GC column stationary phases were not designed with a particular separation in mind. This may lead to compromises in either the separation, or the analytical run-time of the analysis. By using a GC stationary phase and column dimensions which are specifically tuned for a separation will result in analytical improvements relative to the use of columns and dimensions that are not optimized.

The methodology of how this optimization is performed will be covered in this presentation. In brief, the use of thermodynamic modeling has been demonstrated as successful for both the development of new stationary phase materials and also for the optimization of GC conditions using commercial software without the need for lengthy experimental work in the laboratory. While these technologies have been proven successful, most laboratory personnel do not consider them in their method development work, and therefore most GC methods are actually operated far from optimal conditions.

This presentation will address the theory and development of two GC columns that are specifically optimized for the BAC separation using current analytical instrumentation. Through the use of thermodynamic modeling two new GC columns have been developed which allow for improved separation of the target analytes. Known interfering compounds have also been addressed in this approach, so as to maintain complete separation between the blood alcohols and possible coeluting compounds which may also be present in these samples. Finally, total analysis time has been minimized so that the complete separation occurs in less than three minutes, allowing for greater laboratory throughput as compared to most laboratories current operating conditions. Modeling accuracy will be demonstrated by comparison of theoretical results to those obtained in the laboratory. Also, comparison to existing column chemistries used for these separations will be shown to demonstrate the improvements possible using this approach.

The impact of this work to the forensic community is improvement in data quality for this very common analysis. Through the use of optimized GC column dimensions and stationary phase chemistry this analysis will have improved chromatographic resolution in shorter analysis times than what is common in current laboratories. This will enable laboratories to

have greater sample throughput without sacrificing analytical quality, and will likely improve analytical quality for most testing laboratories performing BAC analysis.

**Blood Alcohols, GC-FID, Ethanol**

### **K30 Clinical Pharmacologic Factors in Interpretation of Serum Benzodiazepine or Opiate Concentrations in Automobile Drivers**

*Fran Gengo, PharmD\*, Michelle Rainka, PharmD, James R. Miller, and Horacio Capote, MD, DENT Neurologic Institute, 3980 Sheridan Drive, Amherst, NY 14226*

After attending this presentation, attendees will understand some principles of clinical pharmacology that influence the expected effects of benzodiazepines and opiates in individual drivers. Factors such as medical use versus recreational use, the medical condition being treated, the specific benzodiazepine, the duration of treatment, and the time since drug administration will markedly influence the likely effects produced by these drugs in individual drivers.

The presentation will impact the forensic science community by providing insight into important factors that can influence the effects produced by concentrations of benzodiazepines or opiates

Following automobile crashes, in the absence of measurable ethanol concentrations, law enforcement officers often require drivers to submit blood samples for determination of the presence and concentration of drugs. However, millions of drivers use opiates and benzodiazepines for legitimate medical indications. Published literature suggests that 2.4% of drivers are currently taking benzodiazepines, and 11.1% of drivers are taking opiates.

A review of published literature reveals that in patients using these medications chronically, measures of impairment are often no different from placebo. Much data reporting an increase in odds ratio for motor vehicle accidents in drivers taking opiates or benzodiazepines occur within the first one to two weeks of drug exposure, or in drivers using these drugs recreationally.

A review of the literature reveals the following published results. The effects of alprazolam 2mg per day was examined using the Hopkins verbal learning test and by measuring benzodiazepine receptor density using SPECT scans. Subjects treated for 24 days showed a steady decrease in impairment scores, with day 24 effects proving no different than placebo. This indication of tolerance correlated with steady decreases in benzodiazepine receptor density. A similar study, measuring psychomotor performance and memory in subjects treated with alprazolam (.25-2mg) daily for three weeks, showed that after two to three weeks of daily administration, no significant impairment remained. Another double-blind placebo controlled crossover trial in 16 normal male volunteers confirmed that sedative and psychomotor effects, following chronic alprazolam administration, were no different from placebo after ten and four days respectively. The effects of diazepam (.2mg/kg for 15 days followed by .3mg/kg for seven days) and oxazepam (.8mg/kg for 15 days followed by 1.2mg/kg for seven days) were also studied using a battery of psychometric tests in healthy subjects. Following the first dose there was marked impairment; however by the end of 15 days performance was similar to testing prior to drug administration. When the dose was then increased for the final week of treatment no further impairment was measured. Even when examining only the acute effects of benzodiazepines there are marked differences between agents. A study compared the skills related to driving after a single administration of diazepam 10mg and lorazepam 2.5mg. Lorazepam impaired all measured skills for 12 hours while diazepam impaired only perceptual speed and coordinative skills for five to seven hours. Nonetheless, there were measurable plasma concentrations of both drugs for at least 24 hours.

Much like benzodiazepines, there is an intuitive presumption that all individuals with measurable blood concentrations of an opiate will be

impaired. However, a substantial amount of literature suggests impairment from chronic therapeutic opiate use is no different from placebo. A study in Texas compared the driving records of 104 former heroin users during one year while they were maintained on methadone. No significant difference was found in convictions for accidents, negligent collisions, or other moving violations compared to the entire pool of Texas licensed drivers. Another study, which examined various forms of cognitive function in 17 opiate-dependent subjects, demonstrated an improvement two months after methadone maintenance when compared to baseline. Likewise, six patients with severe non-malignant pain demonstrated positive behavioral and neurophysiological changes after instituting a sustained daily morphine dosage. Patients' mood and clinical pain were rated on visual analog scales, while their reaction time was measured using a standard auditory task. The non-impairment of patients' reaction time and evaluation of mood both failed to indicate a sedative effect. Neurophysiologic measures such as late auditory evoked potentials (AEP) and a P300 component were used to measure vigilance and cognitive performance. Auditory P2 and P300 amplitude actually increased under morphine use showing improved vigilance

These and other studies by various authors which have appeared in peer reviewed publications and their own clinical experience from a pharmacokinetic and pharmacodynamic perspective will be discussed. In addition, there will be discussions about those patients treated with opiates or benzodiazepines whose medical diagnosis can produce symptoms easily misinterpreted as drug impairment. Examples include cancer pain treated with opiates and spasticity treated with benzodiazepines, both which can produce outward signs that can be confused with drug intoxication.

In summary, the impairing effects produced by specific concentrations of opiates or benzodiazepines will vary depending on whether these medications are being used to treat chronic medical conditions, being used acutely, or being used recreationally.

**Benzodiazepines, Opiates, Automobile Drivers**

### **K31 Comparison of Ethanol Elimination Rate of Human Females and Body-Paired Males**

*Michael R. Corbett, PhD\*, and Andy Huang, BSc, University of Ontario Institute of Technology, Faculty of Science, 7309 Sandhurst Drive, Mississauga, ON L5N 7G8, CANADA*

After attending this presentation, attendees will learn about the importance of the formulation of ethanol elimination rate and human gender difference.

This presentation will impact the forensic science community by revealing the prevalence of higher ethanol elimination rates in forensic (driver) female subjects that were investigated.

Subjects consumed commercial alcoholic beverages (beer, wine, liquor, coolers, and/or liqueur) up to 2.2g of ethanol per kg body mass (median=1.1g/kg) in up to 290 min. (median=151 min). Thereafter, they provided suitable breath samples into two different breath alcohol testing instruments about 20 minutes apart. Instrument calibration was confirmed using forensic ethanol standards from different manufacturers with differing concentrations and simulators. The ethanol elimination rate was the post-absorption/distribution linear decrease of alcohol with time.

Ethanol elimination rates for 102 females were paired with varied body criteria of ultimately 207 males from a larger database (109 females, 702 males) of post-offense forensic toxicology casework. Data from females were first paired with males of identical age (yrs), and then varied body criteria of: (i) body mass index (BMI) ( $\pm 3$ , n=98); (ii) body mass (BM) ( $\pm 4.5$  kg, n=70); (iii) estimated total body water (ETBW) ( $\pm 10$  L, n=83); and, (iv) estimated liver weight (ELW) ( $\pm 300$  g, n=84). ETBW was calculated from equations derived by Watson<sup>1</sup> and ELW from Chouker.<sup>2</sup> Ethanol elimination rates were formulated in concentration per time (mg/210 L/hr), mass per time (g/hr), and mass per body mass per time (g/kg/hr). No significant differences (albeit less paired subjects) were

found for smaller ranges of body criteria (e.g., BMI  $\pm 2$ ,  $\pm 1$ ; BM  $\pm 2.3$ kg,  $\pm 0.9$ kg; ETBW  $\pm 5$ L,  $\pm 2$ L; ELW  $\pm 200$ g,  $\pm 100$ g).

Breath ethanol elimination rates in concentration per time were significantly higher ( $p < 0.01$ ) in females than males for all body criteria considered (e.g., median of 20.3 vs. 17.1 mg/210 L/hr for BMI  $\pm 3$ ; 20.5 vs. 17.2 for BM  $\pm 4.5$ kg; 20.6 vs. 17.4 for ETBW  $\pm 10$ L; 20.2 vs. 17.4 for ELW  $\pm 300$ g). Females had a wider and higher range in elimination rate (concentration per time) for all body criteria considered (e.g., 13.7 to 29.0 mg/210 L/hr vs. 11.9 to 23.1 mg/210 L/hr for BMI  $\pm 3$ ; 13.6 to 29.0 vs. 11.9 to 24.3 for BM  $\pm 4.5$ kg; 13.6 to 29.0 vs. 11.9 to 24.3 for ETBW  $\pm 10$ L; 13.6 to 29.0 vs. 11.9 to 24.3 for ELW  $\pm 300$ g).

Breath ethanol elimination rates in mass of ethanol per time (g/hr) were statistically lower ( $p < 0.01$ ) in females than males when paired for BMI (e.g., median of 7.60 g/hr vs. 8.92 g/hr for BMI  $\pm 3$ ), but were not statistically different for other body criteria (e.g., median of 7.90 g/hr vs. 8.51 g/hr for BM  $\pm 4.5$ kg; median of 7.86 g/hr vs. 7.77 g/hr for ETBW  $\pm 10$  L; median of 7.80 g/hr vs. 8.26 g/hr for ELW  $\pm 300$ g).

Elimination rates in mass of ethanol per body mass per time (g/kg/hr) were not significantly different between gender for all body criteria (e.g., median of 0.119 vs. 0.119 g/kg/hr for BMI  $\pm 3$ ; 0.112 vs. 0.119 g/kg/hr for BM  $\pm 4.5$ kg; 0.116 vs. 0.119 g/kg/hr for ETBW  $\pm 10$ L; 0.114 vs. 0.120 g/kg/hr for ELW  $\pm 300$ g).

A higher proportion of females had elimination rates (concentration per time) greater than 20.0 mg/210 L/hr than males for all body criteria (e.g., 54.1% vs. 9.2% for BMI  $\pm 3$ ; 57.1% vs. 18.6% for BM  $\pm 4.5$  kg; 57.8% vs. 18.1% for ETBW  $\pm 10$ L; 53.6% vs. 16.7% for ELW  $\pm 300$ g).

Dettling et al. (2007) reported that different liver masses as calculated in relation to the distribution volume account for the differing ethanol elimination rates between men and women.<sup>3</sup> Dettling et al. (2008) reported that gender differences in the pharmacokinetics of ethanol can partly, but not completely, be explained by progesterone levels.<sup>4</sup> Dettling et al. (2009) reported the difference in average ethanol elimination rate between gender of 0.012 g/kg/h was statistically significant ( $p < 0.0001$ ).<sup>5</sup> We found the ethanol elimination rate is higher in human females than males when formulated in concentration per time, but no significant difference in concentration per body mass per time.

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#### Ethanol, Elimination, Female

## K32 Improved Blood Alcohol Concentration Analysis Utilizing Two Novel Chromatographic Stationary Phases

Jessica Westland, MPS\*, and Frank Dorman, PhD, Penn State University, 107 Whitmore Labs, University Park, PA 16802

Headspace gas chromatographic analysis of blood alcohol concentration is a routine analysis carried out in forensic laboratories. After attending this presentation, attendees will understand the principles of blood alcohol headspace analysis and why the forensic community is focused on optimizing this methodology.

This presentation will impact the forensic science community by providing an improved methodology for the analysis of blood alcohol concentration. The improvement focuses on the modification of the protocol, the automation of the analysis, and the separation and resolution of the alcohols.

In the United States, alcohol abuse is associated with automobile fatalities, industrial accidents, and numerous other incidents such as alcohol poisoning and drug facilitated sexual assault. Due to its enormous impact on society, a rapid and precise methodology for the determination of blood alcohol concentration is desired.

Blood alcohol concentration reflects the amount of ethyl alcohol in the body. The most significant blood alcohol compound is ethanol. After ethanol is absorbed through the stomach and small intestine, it is eliminated from the body via metabolism and excretion. To accurately determine a subject's blood alcohol concentration, numerous factors must be taken into account, including the qualitative and quantitative analysis of the alcohols and their metabolites. There are a number of other compounds present such as methanol, isopropanol, and acetone that can interfere with the identification and quantitation of ethanol. A common interference observed with these other alcohols is their possible co-elution with ethanol. By use of the application specific columns, an improved baseline resolution of ethanol from all other potential components can be achieved.

The presentation will focus on the improvement of the established methods for blood alcohol analysis. Current methods include both direct gas headspace and solid-phase microextraction (SPME) under either static or dynamic conditions. The direct injection methodology does not have optimal standards due to its sample introduction of biological samples onto the gas chromatographic column. This frequently leads to column contamination and decreases performance. The integration of head space sampling into the method prevents the buildup of non-volatile contaminants in the injector and on the column. It also helps to maintain consistent performance and extends the lifetime of the column.

The selected instrumental technique is gas chromatography combined with either a mass spectrometer (GC-MS) or a flame ionization detector (GC-FID). The main element of this improved methodology is the use of two new gas chromatographic column stationary phases. These stationary phases, which are application specific, show an improvement in the separation and resolution of the studied alcohols.

These two capillary column stationary phases were developed specifically for blood alcohol analysis. The new stationary phases focus on the same analysis, but will improve the baseline resolution of all low molecular weight alcohols and their metabolites in minimum needed analysis time.

The presented method includes the evaluation of blood alcohol concentration by both direct gas headspace and SPME. An investigation of the method detection limits (MDLs) was also conducted in order to report with greater confidence the degree of uncertainty than previous methods (0.025 – 0.100 %). The instrumental technique utilized was GC-FID. The technique was chosen because of its accessibility in forensic labs, low operating costs, reliability, as well as its specificity in analyzing these volatile compounds.

#### Blood Alcohol Concentration, Head-Space Analysis, SPME

## K33 New Criteria for Accepting Breath Alcohol Test Results Using Exhalation Profile Data

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After attending this presentation, attendees will understand new criteria for accepting breath alcohol concentration (BrAC) test results using exhalation data to determine breath alcohol sample irregularities.

This presentation will impact the forensic science community by providing changes to BrAC testing, through new parameters for sample acceptance, utilizing exhalation profile Phase III slope data.

A breath alcohol exhalation profile is the plot of BrAC as a function of time or volume during sample submission into a breath alcohol instrument utilizing infrared spectrometry for analysis. Breath alcohol exhalation profiles normally have three distinct phases.<sup>1</sup> Phase I is the initial emptying of the residual air from the instrument. Phase II is the rapid increase in BrAC as breath from the upper bronchial passages is analyzed. The nonlinear phase transitions into Phase III, with a linear increase of BrAC over time and volume of soluble gas exchange beginning with approximately 1.2 liters of lung air volume until expiration has ceased.

Older breath alcohol equipment do not retain all exhalation profile data and use moving point averages to minimize noise to detect mouth alcohol for a determination of BrAC. Recent breath alcohol instruments preserve all BrAC and flow rate data. This data may be used to redefine sample acceptance through additional criteria.

BrAC as a function of Phase III volume slope is a consideration. Phase III slope should correlate to the BrAC sample at a fixed volume under normal breathing conditions. In addition, subjects undergoing hypoventilation prior to breath alcohol testing achieve significantly higher BrAC test results and exhibit distinctive exhalation profiles.<sup>2</sup> Hypoventilation samples are characterized by a rapid Phase II rise in BrAC coupled with a lower Phase III slope. BrAC Phase III slope correlation redefines sample validity and identifies abnormal samples including hypoventilations.

The Missouri breath alcohol program conducted a small exhalation profile study between April 2009 and October 2010. It consisted of twenty-four subjects (20 men, 4 women) selected during law enforcement training programs. Each subject was dosed with ethyl-alcohol to achieve varying BrACs ranging from 0.03 to 0.15 g/210 L, mean and median 0.084 g/210 L. The subjects provided 69 breath samples including 10 hypoventilations. Samples were collected using a DataMaster DMT manufactured by National Patent Analytical Systems, Inc.(DMT).<sup>3</sup> The DMT was selected for retention of concentration data, mass flow rate at 0.25 second intervals per sample and ease of data exportation. Microsoft Excel<sup>®</sup> was used for data analysis.

A correlation between the Phase III linear slope as a function of volume and the BrAC at a fixed delivered volume (1.5 liters) was observed, although it exhibited inter-subject variability ( $R^2 = 0.70$ ). The ratio of the BrAC at 1.5 liters over the Phase III slope (BrAC/Phase III slope) was calculated for each sample (median 11.2 liters, s.d. 2.2 liters). No outliers appeared when the normal three standard deviation rule was applied; however, 8 of 10 hypoventilation tests were outliers using the normal sample data ratios as the standard.

Between October 2010, and May 2011, the BrAC/Phase III slope ratio was calculated for breath exhalation data collected from 504 DWI suspects using 27 DMTs. The DWI subject data was randomly selected from 11,834 breath samples and 152 instruments.<sup>4</sup> Subject BrACs ranged from 0.015 – 0.345 g/210 L, mean and median 0.14 g/210 L. The BrAC/Phase III slope ratios were significantly different than laboratory results (mean 13.5, median 13.2, s.d. 3.1,  $p < 0.0001$ ). The basis for this difference is unclear. The same  $\pm 3$  SD test was applied to the DWI subject data, and 17 of the 504 samples (3.4%) were determined to be statistical outliers. The outlying samples were plotted to assess causation and difference. The 17 samples yielded five hypoventilations with 12 samples exhibiting breath alcohol

sample characteristics inconsistent with current exhalation profile models.<sup>1</sup>

Application of BrAC/Phase III slope ratios to breath alcohol exhalation data functions as a method to identify hypoventilation and other anomalies during subject exhalation. Other approaches to BrAC data interpretation including computation of the averaging value, Fourier transforms and integration for area under the curve, may increase quality control and reduce uncertainty for BrAC testing. Redefining parameters for sample validity through the exhalation profile is warranted. Additional criteria for sample acceptance and reporting should be incorporated in breath alcohol instrumentation for reliability of results.

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### Breath Alcohol, Quality Control, Data Interpretation

## K34 Tools, Techniques, and Findings for the Qualitative Analysis of Delta-9-Tetrahydrocannabinol (THC) in Oral Fluids

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After attending this presentation, attendees will gain insight into scientific methods, applications, and technologies used to test oral fluids for drugs of abuse, particularly exposure to cannabinoids.

This presentation will impact the forensic science community by providing methods and data associated with the development and validation of THC in oral fluids for two separate collection devices.

Devices and techniques used for oral fluid collection and drug screening provide both advantages and new challenges to the field of forensic toxicology. When compared to conventional biological matrices, oral fluids are easier to collect, less prone to adulteration, offer on-site specimen screening, and reflect more recent drug use often associated with impairment.

For each device, the sample collection process involves placing an adsorbent pad in one's mouth until saturated with oral fluid. Following saturation the pad is placed into a pre-packaged tube filled with a diluent buffer, which helps preserve the matrix and prevent adsorption of THC to the pad. Next, the specimen can be analyzed on-site using an immunoassay, which identifies positive samples based on drug class. If an oral fluid specimen is found to be positive the collection process is repeated and the device will be sent for more a selective confirmation analysis.

The primary goals of this research consisted of developing and validating a more selective and sensitive confirmatory method complimenting immuno-assay field findings for the detection of cannabinoids in oral fluid. Challenges met during development included; the need to detect low concentrations with a cut-off concentration of 0.50 ng/mL (in oral fluid + diluent buffer), complexity and variability of the matrix and limited specimen volume for testing. To overcome such obstacles sample preparation involves acidic dilution of the specimen followed by a solid phase extraction (SPE) and derivatization. The derivatized extracts are then analyzed for THC by multi-dimensional GC/MS.

Validation studies involved matrix matching of synthetic oral fluid to human oral fluid, as well as, between the two collection device's diluent buffers. Each matrix was tested for and showed comparable recovery and precision and accuracy around the cutoff. All batches consisted of three spiked cutoff calibrators, three spiked positive controls (125% cutoff concentration), and three negative controls to be used for evaluating precision, accuracy, and reproducibility of the method. Stability and recovery of THC was performed by spiking positive control pools at three concentrations into each of the two collection devices containing the absorbent pad. Enough devices were spiked to compare and evaluate three concentrations, in triplicate, at nine time intervals over 21 days. Sensitivity and specificity of the assay was performed by analyzing a minimum of 40 blind controls at concentrations ranging from 0% - 1000% over four analytical batches. Testing for interfering substances involved spiking commonly encountered drugs, as well as, three possible adulterants including mouthwash, toothpaste, and denture adhesive into blank matrix for analysis.

Validation results and findings all met acceptance criteria provided by the standard operating procedure for qualitative method validation. The limit of detection was calculated to be approximately 0.01ng/mL using the signal to noise ratio of a cutoff calibrator. For precision data negative controls, cutoff calibrators and positive controls were evaluated in triplicate over ten batches. All negative controls were found to be negative and the %CV values for the cutoff calibrators and positive controls were 10.2% and 6.9% respectively. Due to no false positive or false negative findings both sensitivity and specificity were calculated to be 100%. Stability studies showed a decrease in THC values over time when positive controls (125% cutoff) were prepared in bulk and frozen. To accommodate for loss of THC over time, positive controls were hand spiked with each batch. Stability studies also showed one of the collection devices to be superior regarding recovery of THC. Although interference studies showed no interferences, certain toothpaste and mouthwash brands spiked into synthetic oral fluid did show a peak very close to the retention time of THC, with ion ratios being just outside that of the acceptable range. Further investigation determined the possible identity of the interfering peak and showed that the introduction of a "disqualifier" ion to the method clearly distinguished the peak from THC without affecting sensitivity. Also, human oral fluid collected following use of the toothpaste and/or mouthwash containing the interferer lacked concentrations required to impact qualitative results.

**THC, Oral Fluids, Multi-Dimensional GC/MS**

### **K35 A Rapid and Comprehensive Analysis of HFAA Derivatized "Bath Salts," Synthetic Cathinones, and Amphetamines in Postmortem Blood by Supported Liquid Extraction (SLE) With Gas Chromatography-Mass Spectrometry Detection**

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After attending this presentation, attendees will understand new trends in "Bath Salts," synthetic cathinones, and amphetamine abuse. Attendees will learn a unique approach to utilizing Supported Liquid Extraction (SLE), the challenges of derivatizing with Heptafluorobutyric Acid Anhydride (HFAA), and techniques to overcome analytical column contamination due to byproducts caused from HFAA derivitization.

This presentation will impact the forensic science and law enforcement communities by highlighting the abuse patterns for new

designer drugs. Abuse of synthetic cathinones and amphetamines, particularly in our youth, is on the rise. A comprehensive approach to extraction, detection, and quantification of "Baths Salts," synthetic cathinones, and amphetamines is required to fully assess the extent of this problem. Challenges related to identifying these compounds, including compound volatility and derivitization, will be discussed. The method presented suggests one possible approach that may be utilized to reliably characterize and identify these new compounds. Efficient testing procedures are a necessary first step in a comprehensive surveillance program.

3,4-Methylenedioxypropylvalerone (MDPV), is a new "designer" drug with observed toxicities including tachycardia, hypertension (vasoconstriction), insomnia, hyperthermia, mydriasis, panic attack seizures, and aggressive behavior. MDPV is a psychoactive, synthetic analog of the CNS stimulants cathinone and pyrovalerone. Because of their appearance, MDPV is often referred to as "bath salts."

A new procedure to facilitate a rapid, comprehensive, and simultaneous detection and analysis of amphetamine, phentermine, methamphetamine, amantadine, nicotine, pseudoephedrine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), phenylpropanolamine (PPA), MDPV, mephedrone, and 3,4-Methylenedioxy-N-ethylamphetamine (MDEA) was developed using Supported Liquid Extraction (SLE). Beta-Phenethylamine was also analyzed as it is a common byproduct which is often observed in postmortem blood specimens.

Ten postmortem samples that tested positive for the amphetamine drug class via ELISA screen were further evaluated using this new method. Extraction is rapid, utilizing a small sample volume and involving two steps. The two step sample preparation and extraction utilizes an ISOLUTE SLE cartridge (Biotage, Charlotte, NC) combined with a positive-pressure manifold. The SLE cartridge composition contains Diatomaceous earth, a porous structure. Using SLE, the aqueous sample solution that has been pH adjusted with alkali or NH<sub>4</sub>OH will penetrate into the pore of the Diatomaceous earth. When eluted with a hydrophobic solvent, an extraction interface occurs between the two liquid phases. The high surface area of the Diatomaceous earth provides for enhanced extraction efficiency while alleviating much of the technical expertise associated with traditional Liquid-Liquid Extraction (LLE). SLE is able to minimize the impact from hemolyzed specimens, a common concern when dealing with postmortem blood samples. In addition, costly disposal of organic waste is not required because of minimal extraction volumes. This extraction is followed by derivitization with HFAA and Gas Chromatography-Mass Spectrometry (GC/MS) detection. This method was challenged by analytical column contamination from the HFAA which was overcome by incorporating a post conversion/derivitization clean up procedure that uses a phosphate buffer. This clean up procedure helped to maintain chromatographic quality without adding a labor intensive step.

With this methodology, a single analytical protocol can be used to conduct the confirmation test for a comprehensive panel of sympathomimetic amines on postmortem blood samples that preliminarily tested positive by immunoassay for amphetamines. Criteria for method validation which include accuracy, inter-assay precision, intra-assay precision, and analytical measurement range studies will be reported. In comparison with traditional LLE, this method offers considerable savings in sample volume, extraction time, solvent usage, waste production and disposal.

**Bath Salts, Postmortem, Supported Liquid Extraction (SLE)**

## K36 Chromatographic Analysis of Synthetic Amphetamine Street Samples

Amanda Leffler, BS\*, 445 Waupelani Drive, Apartment M22, State College, PA 16801; and Frank Dorman, PhD, Penn State University, 107 Whitmore Labs, University Park, PA 16802

After attending this presentation, attendees will learn what to look for when analyzing synthetic amphetamine street samples. This presentation will discuss the development of the extraction of the drug compounds from various commercial media, followed by separation using gas chromatography with mass spectrometric detection (GC/MS). Quantification of the active compounds based on the use of appropriate internal standards will also be addressed

This presentation will impact the forensic science community by providing an efficient method for analyzing street amphetamine samples and hence increasing the amount of evidence that analysts can process in a given time. Identification of the active components and added constituents in the samples can be made. Additionally, if an individual is in a possible overdose situation, rapid analytical methodology is also important.

The production and marketing of synthetic amphetamines is an ever-increasing threat to society. In 2007 a new line of "legal highs," labeled as bath salts, plant food, and jewelry cleaner, have flooded the streets of the United States, as well as other countries. Three years later in 2011, over one thousand calls were made to poison control centers across the country in response to these drugs.<sup>1</sup> Most of these products are cathinone analogs that are currently popular due to their amphetamine-like effects and their ease of access online and in "headshops" across the country.

Cathinone and its derivatives are extracted from the khat plant and can cause rapid heart rate, chest pains, insomnia, depression, suicidal thoughts, or seizures. The analogs used vary from sample to sample, as well as their concentrations, and when used in conjunction with other drugs can prove to be fatal.<sup>2</sup> Pyrovalerone is a Schedule V stimulant originally synthesized in 1964 to suppress appetite or treat chronic fatigue and MDPV, or 3,4-methylenedioxypyrovalerone, is the methylenedioxy analog of pyrovalerone that is not currently scheduled in the United States. Both of these compounds have been found previously in "legal highs" along with cathinone analogs such as methylenedioxymethamphetamine (mephedrone). Plant feeder has also been found to contain 3-fluoromethcathinone and other isomers of the drug, and has become popular due to methcathinone's simple synthesis from ephedrone.<sup>3</sup>

The Drug and Chemical Evaluation Section is a section of the DEA Office of Diversion Control (ODE) that is gathering information on the abuse of synthetic amphetamines to support their possible scheduling. Such information includes identification to establish prevalence and trends.<sup>1</sup> Therefore, the analysis of such designer drugs and the determination of their individual compounds may help ban their production and legalize their use. This study presents the development of a sample preparation and chromatographic method for the chemical characterization of selected synthetic amphetamine samples for the purpose of identification of the active components and added constituents.

Synthetic amphetamines are entering the drug market faster than they can be restricted. A quick and efficient extraction method will allow for a more efficient analysis of such compounds. In regards to the forensic community, crime labs already have a large workload. Thus creating a more efficient methodology will benefit analysts by increasing the amount of evidence that they can process in a given time. Additionally, if an individual presents a possible overdose situation, rapid analytical methodology is also important. This presentation will discuss the development of the extraction of the drug compounds from various commercial media, followed by separation using gas chromatography with mass spectrometric detection (GC/MS). Quantification of the active compounds based on the use of appropriate internal standards will also be addressed.

The developed chromatographic method provides qualitative and quantitative analysis of synthetic amphetamines in samples seized on the

illegal drug market and in the compounds referred to as "legal highs." Potentially, this method could reduce the time a new drug is on the market. Future studies will also involve diluents used in the processing of these "legal highs."

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## Bath Salts, Chromatography, Amphetamines

## K37 A Novel LC/MS/MS Method for the Detection and Quantitation of GHB

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After attending this presentation, attendees will be introduced to a new LC/MS/MS method that can be used for the detection of gamma-hydroxybutyric acid (GHB) using Hydrophilic Interaction Liquid Chromatography (HILIC).

This presentation will impact the forensic science community by supplying an effective method of GHB analysis that is time efficient, involves very little sample preparation, and does not require sample derivatization.

GHB is a common Schedule I central nervous system depressant that is often implicated in the commission of many drug-facilitated sexual assaults because it leaves the victim confused, unable to resist, incapacitated, and promotes memory loss. GHB is also clinically used for the treatment of cataplexy. HILIC is a newer technology, and although there has not been extensive research done on this particular chromatography, it has been shown to be useful for the analysis and separation of polar compounds. Due to this property, it has been speculated that it would work very well for GHB, but has yet to be applied to biological samples, such as urine. HILIC works by applying a water-miscible mobile phase across a strongly hydrophilic stationary phase. This current research focused on the development of a new LC/MS/MS method that can be used in conjunction with HILIC to identify and quantify GHB in urine samples. This method included a Zwitterion Chromatography (ZIC<sup>®</sup>) HILIC column (3.5mm, 100 x 2.1mm) from SeQuant. The mobile phase consisted of 10mM ammonium formate in water, pH 6.38, for Solvent A, and 100% acetonitrile for Solvent B. The following gradient was employed: 90% organic to 40% organic in 20 minutes (~2.5% per minute), then back up to 90% organic at 10% per minute, maintaining a flow rate of 0.2 mL/min for the duration of the run. The gradient allowed sufficient time for the compound to come off the column, and also for the column to re-equilibrate, which is important due to the sensitivity of the column. Additionally, the gradient provided a wash step to clean the column of any impurities that had the potential to be carried over into subsequent samples. LC/MS/MS was performed using electrospray ionization with negative ion mode monitoring. Ions were examined in MRM mode, and the 103 to 85 and 103 to 101 transitions were used. GHB-d6 was used as the internal standard, and the 109 to 90 transition was used. A linear range of GHB standards were observed from 0.01ug/mL to 10 g/mL. GHB was also detected in synthetic urine in concentrations as low as 0.04mg/mL. It was found that injecting straight synthetic urine did result in some ion suppression; however, when the samples were diluted by 75% with acetonitrile, there was not as much ion suppression. Potential interferences, such as gamma-butyrolactone (GBL) and 1,4-butanediol, will also be tested to ensure that drugs with similar structures to GHB will not provide any interference with the results on this particular column. Blind studies will

also be performed.

GHB is often difficult to detect due to its increased rate of metabolism and excretion in the body; this new method will provide a simple, quick method for the detection of the drug in urine without extensive extraction or derivatization procedures using ZICÒ-HILIC and LC/MS/MS.

LC/MS/MS, ZIC-HILIC, GHB

### **K38 Maternal Death in a Young, Irish Primigravida (in the Seventh Month of Pregnancy) With Positive Postmortem Toxicological Analysis for Pentobarbital**

*Khalid Jaber, MD\*, Firebrigade Training Centre, Malahide Road, Marino, Dublin 3, IRELAND*

After attending this presentation, attendees will understand a unique case of a maternal death in a pregnant young female, whose postmortem toxicology analysis revealed pentobarbital. The decedent was a mature student enrolled in third-level education, with a history of bipolar disorder and previous episodes of self-harm.

This presentation will impact the forensic science community by presenting a case of a death of pregnant female identified with pentobarbital in her blood and urine at a level just outside the therapeutic range, and almost borderline toxic level. It is certainly the first such case in Ireland, and possibly the first case reported in English literature of a suspected suicidal death by the ingestion of pentobarbital by a pregnant female.

Maternal death is a rare phenomenon in Ireland and in other developed countries. This is the case of a young, unmarried, Irish primigravida found dead in her seventh month of pregnancy. She lived with her mother and her step-father in a middle class neighborhood. She was a mature student in a third level education. She was known to be a non-drug abuser, and an advocate for curtailing the abuse of drug culture. She first attended antenatal clinic in her second trimester. Her pregnancy was progressing well with no identifiable antenatal abnormality. According to her family, she was excited about her pregnancy and was looking forward to motherhood and the baby.

Prior to this pregnancy, she was diagnosed with bipolar disorder for which she was not taking medication. Episodes of self harm were documented, that never materialized into life threatening or incapacitating injuries. She was found by her step-father in a kneeling-like position in her tidy bedroom. She was placed into supine position on the bedroom floor. She was found holding a pen in her left hand. Her face and upper torso revealed diffuse marked, purple, postural hypostasis (*livor mortis*) discoloration. Prominent, bilateral, petechial hemorrhages were noted on the eyelids and conjunctiva. Mucosal surfaces of the lips revealed similar petechiae. Larger, punctate (ecchymotic) hemorrhagic spots were noted on the upper torso.

Non-indenting, pale, neck-markings were seen on the anterior and posterior aspects of the neck. Internal hemorrhages were identified in the soft tissues of the neck and involving focally some of the neurovascular structures. The lungs were mildly congested and mildly to moderately oedematous. The heart was mildly dilated, but not scarred. The coronary arteries were free of acute thrombi. The external genitalia revealed no evidence of external trauma. The vaginal canal contained a small amount of pinkish-white discolored fluid. The cervix appeared purple, but not associated with bleeding or mucosal disruption. There was no evidence of DIC or TTP. The rest of the maternal examination was unremarkable; especially as there was no evidence of unusual allergic skin manifestations. The placenta was posterior and fundal. There was no evidence of placental abruption or infarction. The placental surfaces appeared unremarkable.

The fetal membranes were intact. The amniotic fluid was of normal and not meconium stained. The fetus was well developed for his gestational age. There were no congenital abnormalities (externally or internally). Re-examination of the scene two days later recovered two capped brown glass

bottles, with Spanish language labels, bearing a canine picture and designation for veterinary medicine usage. Analysis of these bottles confirmed the presence of pentobarbital. This drug was identified in the postmortem blood and urine and the amniotic fluid. Phenobarbital level was 11ug/ml (therapeutic range is 2-10ug/ml, toxic levels start at 12ug/ml while lethal levels are much higher).

This drug is used in euthanasia in veterinary practices. In humans it has limited, therapeutic, medically-approved applications: treating intractable epilepsy, induction of neurological coma to protect the brain during certain procedures, and in the control of intra-cranial hypertension. It is likely the bottles were sourced out of country, possibly via the internet. A partially illegible short suicidal note recovered from the scene included, "I cannot continue anymore."

This drug is known to be a direct CNS and myocardial depressant. The decedent stomach contained evidence of a recently digested food and this drug is known to impair gastrointestinal motility and increases hepatic microsomal activity. It causes a decrease in sympathetic activity and thus increases venous capacitance.

In the United States, Andrew Grant DeYoung recently died in a Jackson prison following a three-drug protocol that included pentobarbital. Also executions in Alabama and Georgia, using pentobarbital, have raised concerns among human rights activists and opponents of the death penalty on the merit of employing this drug in inmate sanctioned executions. Roy Blankenship and Eddie Duval Powell, are inmates who apparently have exhibited signs indicating they might have been conscious or in pain during this chemical death process.

The literature review of suicidal maternal deaths in association with drug(s) overdose, especially barbiturates is discussed. Pertinent scene and autopsy photographs are presented to highlight the described circumstantial and anatomical findings.

Maternal, Death, Pentobarbital

### **K39 A Klimaxic Head Trip**

*Veronica Hargrove, MS\*, Randall Frost, MD, Kimberley Molina, MD, and Michael Neerman, PhD, Bexar County Medical Examiner's Office, 7337 Louis Pasteur Drive, San Antonio, TX 78229*

After attending this presentation, attendees will better understand synthetic cannabinoids; discussing its pharmacology and toxicity.

This presentation will impact the forensic science community by increasing awareness and understanding of the possible toxicity of synthetic cannabinoids. Synthetic cannabinoids are relatively new drugs in the United States and it is important to make this information known to any and all parties that may be impacted by its use and/or abuse. Therefore, this information can potentially be used to educate forensic toxicologists and medical examiners as well as the lay public as to the dangers of synthetic cannabinoid use.

Synthetic cannabinoids were originally produced as research tools in order to create drugs that could potentially be used in the treatment of nausea, glaucoma, and appetite stimulation. These compounds were designed to mimic marijuana by binding to the same cannabinoid receptors that THC, the active ingredient in marijuana, bind to in the brain. There are hundreds of these compounds available, all with varying potency and varying affinity to the cannabinoid receptors. Most of these compounds are much more potent than THC and have been shown to cause increased heart rate, increased blood pressure, agitation, hallucinations, and seizures. Emergency departments and poison control centers are both seeing a rise in visits and calls from people using these compounds. The American Association of Poison Control Centers estimates 2,915 calls about synthetic cannabinoids in 2010 and as of June 30, 2011, approximately 3,094 calls have so far been received. In November 2010, the Drug Enforcement Agency (DEA) moved to designate five of these synthetic cannabinoid compounds as Schedule I drugs. In this one-year period the DEA as well as the United States Department of Health and Human Services will study

these drugs further in order to evaluate whether it is necessary to permanently control these substances.

Three cases in which synthetic cannabinoids were found in blood will be discussed. Two of these cases are medical examiner cases and one is a driving while intoxicated case. A 58-year-old male with a history of heart disease, Parkinson's disease, and hypertension bought some synthetic marijuana, called "Head Trip," at a convenience store. After smoking the Head Trip, he began to complain of not feeling well. He was found unresponsive about an hour later by family members and taken to the hospital where he later died. A 29-year-old healthy female with no medical history or disease was discovered unresponsive in a hotel room by her boyfriend. The boyfriend called 911 and she was pronounced dead upon emergency personnel arrival. Possible marijuana and synthetic marijuana called "Klimax" were found in the room. A 23-year-old male driver was pulled over for drifting in and out of his lane. He was arrested for DWI after appearing intoxicated to the officer and during a search of his car two bags of "Head Trip" were found. Blood was sent to the Medical Examiner's office for testing. Results and implications of use of synthetic cannabinoids for all three of the previously described cases will be discussed.

**Synthetic Cannabinoid, Pharmacology, Toxicity**

## **K40 A Systematic Study of the Cellular Toxicity of Common Amphetamine and Ring-Substituted Drugs**

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After attending this presentation, attendees will have an understanding of the effects and comparative toxicity levels between a number of amphetamine type stimulant compounds and the need for such systematic studies

This presentation will impact the forensic science community by informing health care professionals, law enforcement agencies, and those involved in drug administration about the potential dangers of toxicity of a number of established and new stimulant drugs that are increasing in popularity. It will allow attendees to make an informed choice in drug administration and treatment of drug users.

There are now a wide range of amphetamine and 3,4-methylenedioxyamphetamine (MDA) drugs available in the illegal drug market. While amphetamine and methamphetamine were originally used within this context as stimulants, the fact that some users experienced unpleasant side effects led to the development of the ring substituted 3,4-methylenedioxy compounds. These latter compounds, in addition to stimulant effects, are entactogens and facilitate inter-personal communication.

The majority of amphetamine-type-stimulants currently found "on the street" contain one or more of these drugs. MDMA (3,4-methylenedioxymethamphetamine), a.k.a. ecstasy, remains a popular contender on the party scene. Globally, there are distinct differences in the usage between countries. In the United Kingdom, the incidence of ecstasy usage has fallen in recent years whilst there has been an explosion of use across Northern America. Its usage is concentrated in younger adults, and in Europe is more commonly abused in males with approximately 2.5 million people using ecstasy in 2009.<sup>1</sup> MDMA is now tightly controlled across the globe but other analogues, including MDA and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) have also been growing in popularity. The legislative control of these compounds, under the Misuse of Drugs Act and their related laws and legislations abroad, often leads to the synthesis of uncontrolled analogues and derivatives of the initial substance in search of similar desired effects.

The mixture of synthetic analogues commonly found in tablets sold as "ecstasy" frequently includes MDMA, MDA, and MDEA. Profiling and analysis of street ecstasy samples via chromatographic methods has often been used to illustrate complexity of the mixture of stimulant compounds present as well as the multiple chemical impurities produced in the synthesis of street samples.<sup>2</sup>

While evidence of the toxicity of these compounds has often been seen in patients admitted to hospital emergency departments, the effects of these compounds and their related analogues on vital organs at a cellular level, is not well defined. Damage to organs involved in the metabolism and excretion of these compounds and their by-products has been reported however, the extent to which toxicity occurs and the mechanisms of action by which this damage is induced are still poorly understood.<sup>3</sup>

Cells were exposed to the drugs at concentrations ranging from 1.1mg/ml to 11mg/ml, after which they were incubated and then assessed morphologically for evidence of cell death in the form of either programmed cell death (apoptosis), uncontrolled cell death (necrosis). Further assessment of cell death was carried out using Annexin V assays in which the presence of apoptosis and propidium iodide (PI) provided evidence of general cell membrane disruption. Samples were analyzed using a flow cytometer, after which results were given according to the expression of annexin V and PI labelling. Results were expressed as the percentage increase in the presence of non-viable cells (Annexin V +/PI +) after correcting for background cell death. This data was confirmed using fluorescent microscopy and immunolabelling of the Annexin V in-situ. The most toxic of the compounds to the liver was amphetamine, with an LD50 of 1.5mg/ml, whilst the least toxic was methamphetamine with an LD50 value of 2.9mg/ml. Conversely in the kidney, amphetamine appeared to be the least toxic compound, while methamphetamine was more toxic. Respective LD50 values were calculated at 4.9mg/ml and 3.9mg/ml.

MDA showed higher levels of toxicity in both the liver and the kidney than both MDMA and MA. LD50 values for this compound were calculated at 2.1mg/ml and 3.7mg/ml respectively, while values for MDMA were 2.5mg/ml and 3.8mg/ml. The effects of all compounds supported both dose and time dependant increases in toxicity. In the process of data analysis, comparisons between amphetamine and MA, and MDMA and MDA were made due to the related nature of their structures.

The data supports published literature that MA and amphetamine become rapidly toxic to the kidney, whilst MDMA and its metabolite MDA are more toxic to the liver. This may be due to rapid accumulation of amphetamine and MA in the kidney as previously described in studies of the pharmacokinetics and distribution of amphetamines in the human body.<sup>4</sup> MDMA is cleared by hepatic metabolism, but with dose dependant increases this rapidly leads to saturation of hepatic clearance and biological toxicity. The profile of ecstasy users however, is all too frequently one of poly-drug abuse, dosing repeatedly in a recreational environment. Therefore, it is essential to perform systematic studies and poly-drug investigations to understand the profile of the patient presenting to the doctor, and the cold case to the forensic pathologist.

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**MDMA, Ring-Substituted Analogues, Cellular Toxicity**

## **K41 Forensic Toxicology Fellowship Training Model**

*Anna Kelly, PhD\*, and Ashraf Mozayani, PhD, PharmD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will be informed about a forensic toxicology fellowship program and how the more widespread implementation of such a program would benefit the forensic toxicology community.

This presentation will impact the forensic science community by drawing attention to the lack of comprehensive on-the-job training in the field of forensic toxicology and the advantages of providing a formal training program.

Programs in which a broad exposure to the various aspects of forensic toxicology in a lab holding American Board of Forensic Toxicology (ABFT) as well as ISO/IEC 17025 accreditation is provided to recent graduates are few and far between. By developing a formal training program for emerging forensic toxicologists, trainees will develop a strong foundation in the application of the fundamental principles of toxicology to actual casework.

A recent chemistry doctoral graduate was hired and given hands-on experience in the forensic toxicology laboratory, from the acquisition and processing of samples to the analysis and reporting of analytical findings for medicolegal (ML), driving under the influence (DUI), and drug facilitated sexual assault (DFSA) cases. The fellow was exposed to techniques including gas chromatography/mass spectrometry (GC/MS), enzyme-linked immunoassay (ELISA), and liquid chromatography/mass spectrometry (LC/MS). In addition to learning procedures already in place, the fellow was involved in the development and validation of methods, providing invaluable exposure to the method development process as it applies to the field of forensic toxicology. The HCIFS Forensic Toxicology Fellow developed a method for the detection of helium in postmortem specimens.

The fellow attended daily medical examiner briefings in which the medical examiners and representatives from the toxicology, anthropology, and investigation departments discuss cases that have come into the office, providing information regarding scene investigation and the circumstances of the death. Autopsies performed the previous day are also discussed. In attending these meetings, the fellow was able to see how the testing in the laboratory helps in determining cause and manner of death. Additional continuing education courses and observation of court testimony provided the fellow with exposure to a variety of areas, including medical examiner law and jurisdiction, medicolegal death scene response, determination of cause and manner of death, pathological and anthropological findings and how they contribute to determining cause and manner of death, and the role of the crime laboratory in death investigations. Being able to learn how each of the forensic disciplines work together with medical examiners in order to formulate the whole story in determining the cause and manner of death, and how toxicology analysis helps these investigations provided the fellow with a strong foundation from which to start her career.

It is encouraged that a fellowship program for toxicologists be implemented in order to provide recent graduates with a strong background which will help them establish a career in the field of forensic toxicology.

**Toxicology, Training, Fellowship**

## **K42 Drug Overdose Fatality Due to an Herbal Blend Containing Mitragynine and O-Desmethyltramadol**

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After attending this presentation, attendees will understand the effects of an herbal blend containing Kratom.

This presentation will impact the forensic science community by becoming aware of the Kratom herbal drug mixture that is available over the internet and contains mitragynine, a Kratom alkaloid, as well as O-desmethyltramadol, and has been implicated in a drug overdose fatality.

A 19-year-old college student was found unresponsive in his bed by his roommate. The prior evening, the decedent was at a party drinking alcohol. After arriving to his residence, he reportedly consumed a mixture of a Kratom tincture with soda and went to bed intoxicated. There were two vials at the scene. One was labeled "Full Spectrum Tincture 10mL Not for Human Consumption" and the other vial was unlabeled. A receipt for "Full Spectrum Alkaloid Tincture 300 mg" from Speciosa Specialist, Chicago, IL was present. Duloxetine and citalopram, which were prescribed to the decedent, were also collected from the scene.

The femoral blood was subject to routine volatiles, acid/neutral, alkaline, and ELISA screening. Ethanol levels were 0.07g/dL and 0.09g/dL within the femoral blood and vitreous, respectively. 9-carboxy-tetrahydrocannabinol was confirmed in the urine by GC/MS. The only other finding per initial screening was O-desmethyltramadol in the alkaline drug screen. Ingestion of tramadol would typically result in the presence of tramadol, N-desmethyltramadol, and O-desmethyltramadol; the presence of only O-desmethyltramadol makes this an unusual finding. In the decedent's femoral blood, duloxetine (0.21mg/L) and O-desmethyltramadol (0.81mg/L) were identified by LC/MS/MS. Due to lack of an authentic standard and reference laboratory test, quantification of Kratom alkaloids was not pursued. However, the presence of Kratom alkaloids including mitragynine or its diastereomers in the urine of the decedent and the two vials collected at the scene was confirmed by full scan LC/MS/MS and comparison to the specific mass spectrometric fragmentation pattern of mitragynine. O-desmethyltramadol was confirmed in the two vials qualitatively by GC/MS.

There is an increasing popularity of "legal highs" which can be purchased over the internet and are not regulated by the Food and Drug Administration (FDA). Therefore, the contents of the products are not always known to the consumers. In this case, a product called "Full Spectrum Alkaloid Tincture" was bought over the internet from Speciosa Specialist. The product description on the website states that it has alkaloids from Kratom that have been "isolated, purified to the highest level, and captured ..."<sup>1</sup> Products on this website are specified as not intended for human consumption. The vials collected at the scene; however, not only contained mitragynine, an alkaloid from Kratom, but O-desmethyltramadol was present as well.

Mitragynine is the alkaloid found in highest concentration in Kratom, which is a leaf native to Southeast Asia and has traditionally been used for medicinal purposes. Kratom was used by the workers as a stimulant and more recently, has been used for pain management and opioid withdrawal. However, it is now considered a controlled dangerous substance in Thailand, Bhutan, Australia, Finland, Denmark, Poland, Lithuania, Malaysia, and Myanmar. There is no medicinal use for Kratom in the United States and it has not been approved by the FDA for any use; however, it is not classified as a controlled dangerous substance in the United States.<sup>2</sup>

At low doses, mitragynine binds to delta-opioid receptors, but as the dosage increases, it binds the mu-opioid receptors. This receptor binding profile corresponds to the physiologic effects of mitragynine, where it is a

stimulant at low doses and has sedative effects at high doses. Mitragynine appears to be a drug with abuse potential with rare toxic effects. No cases of death solely due to mitragynine have been reported in the U.S.; however, over the last six years, mitragynine has been combined with another drug, O-desmethyltramadol, to create a more potent herbal mixture that is often referred to as Krypton. Krypton is available through the internet and has been associated with fatalities as reported in Sweden.<sup>3</sup> O-desmethyltramadol is a metabolite of tramadol, which is an opioid agonist used to treat moderate to severe pain. Tramadol acts as a mu-opioid receptor agonist and is a synthetic analog to codeine.

With the growing popularity of Kratom and other opioids that can easily be purchased over the internet, the forensic community should be aware of these drugs. The contents of the herbal extracts are not necessarily listed or known to the consumer since these products are not regulated by the FDA. In this case, the O-desmethyltramadol was incidentally found within the Kratom tincture. Since mitragynine and O-desmethyltramadol are mu-opioid agonists, the combined use of the drugs especially with other drugs and ethanol can lead to sedation and respiratory depression, and can be fatal, as was in this case.

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3. Kronstrand, R, Roman, M, Thelander, G, Eriksson, A. "Unintentional Fatal Intoxications with Mitragynine and O-Desmethyltramadol from the Herbal Blend Krypton." *Journal of Analytical Toxicology* 2011; 35 (242 – 247).

#### Mitragynine, Krypton, O-Desmethyltramadol

## K43 The Hidden Dangers of Clandestine Methamphetamine: Synthesis to Cellular Toxicity

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After attending this presentation, attendees will have an understanding of the origins and occurrence of chemical impurities in methamphetamine and appreciation of the potential toxicity of these compounds in a street drug, both alone and in combination.

This presentation will impact the forensic science community by informing health care professionals, law enforcement agencies, and those involved in drug administration about the potential dangers of toxicity of pure stimulant drugs and common impurities. This will allow attendees to make an informed choice in drug administration and treatment of drug users.

Methamphetamine (MA) is a widely abused stimulant, which may be manufactured using a variety of different routes. While amphetamine is the most commonly occurring phenethylamine of choice in Europe, methamphetamine is more prevalent in North America and the Far East.<sup>1</sup> New data from the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) shows that the geographic concentration and availability of these compounds is responsible for shifts in global user trends.<sup>2</sup> Given the global popularity and accessibility of the Internet, synthetic routes were chosen from this medium for methamphetamine synthesis. One of the most popular and straight-forward routes of synthesis is the reductive amination of benzylmethylketone (BMK), which is commonly employed in Europe.<sup>2</sup> This route involves the reaction of

methylamine with BMK in the presence of mercuric chloride and aluminium followed by purification of the crude drug base and subsequent crystallisation of the hydrochloride salt.

Routes such as this lead to complex mixtures of reaction by-products and a significant number of impurities. These compounds may contribute to the toxicity profile of street samples of methamphetamine. To date there had been no systematic study of the drug or its impurities. The aim of such a study was to determine whether these impurities contributed to the overall toxicity of the street methamphetamine samples. Alongside MA's addictive properties, it produces widespread organ toxicity. The most commonly occurring impurities identified in the mixtures, including benzaldehyde, pethylacetone, 1-phenyl-2-propanol and the related compound ephedrine were subjected to cytotoxicity testing against immortalized human kidney (CAKI-2) and liver (HepG2) cell-line models.

According to the pharmacokinetics of MA, a dosing strategy was developed from the reported distribution of the compound in the body and the recently reported levels of purity in street samples. We focused on vital organs that are involved in the metabolism and excretion of the compound and its by-products. Given that, respectively, 22% and 7% of the ingested compounds are expected to reach the liver and the kidneys, it was possible to assess the cytotoxicity of each individual chemical based on their purity within the sample.<sup>3</sup>

Cells were exposed to the drugs for one hour at concentrations ranges from 1.1mg/ml-11mg/ml, following which they were assessed morphologically for evidence of cell death, either programmed cell death (apoptosis), uncontrolled cell death (necrosis) or no effect at all. To assess for cell death, cells were then labelled with annexin V to evidence the presence of apoptosis and propidium iodide (PI) for evidence of general cell membrane disruption. These samples were then analyzed using a flow cytometer. Results were given according to the expression of annexin V and PI labelling. Results were expressed as the percentage of viable cells (Annexin V -/PI -) versus the percentage of non-viable cells (Annexin V +/PI +). This data was confirmed using fluorescent microscopy and immunolabelling of the annexin V in-situ.

The most toxic of the compounds was MA, with an LD50 of 3.9mg/ml to the kidney and 8.2mg/ml to the liver. 1-phenyl-2-propanol conversely, produced minimal toxicity with respective LD50 values of 37.1mg/ml to the liver and 111.9mg/ml to the kidney. All other compounds displayed significant toxicity with LD50 values of less than 10mg/ml. Effects of the compounds supported both dose and time dependant increases in toxicity. By flow cytometry, it can be deduced that apoptosis and necrosis are both activated following exposure to these compounds. For further mechanistic confirmation, changes in expression levels of genes associated with these pathways are investigated. This will include Bax (an apoptosis related gene), Beclin (autophagy related) and the Caspase family (apoptosis) on both exposure to impurities and in both dose and time dependant assays.

These data provide evidence of the cellular cytotoxicity of MA and related impurities at the sites of biological purification and excretion. Overall, the liver is more sensitive to these compounds than the kidney, with MA exhibiting the most significant toxicity. This supports published research that there is a complex and highly damaging cellular response to MA and that this drug continues to pose a significant risk to those who fall in its path.

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3. Volkow, N.D, Fowler, J.S, Wang, G.J, Shumay, E, Telang, F, Thanos, P.K & Alexoff, D (2010) "Distribution and pharmacokinetics of methamphetamine in the human body: clinical implications." *PLoS One* 5(12): e1526

#### Methamphetamine, Impurities, Cellular Toxicity

## K44 Metaxalone Related Deaths in North Carolina (2002-2010)

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After attending this presentation, attendees will be able to describe the types of postmortem casework associated with metaxalone at various concentrations.

This presentation will impact the forensic science community by providing information regarding metaxalone as it relates to cause and manner of death determinations.

Metaxalone (Skelaxin®) is a central nervous system (CNS) depressant indicated for the treatment of acute, chronic, traumatic, and inflammatory musculoskeletal disorders. It is typically prescribed in doses ranging from 800 to 3,200 mg daily. Approved by the Food and Drug Administration in 1962, metaxalone given orally at a therapeutic doses typically produces peak plasma concentrations  $\leq 4.0$  mg/L. Adverse reactions associated with metaxalone are generally related to CNS depression including drowsiness, dizziness, headaches, as well as nausea and vomiting. Limited information has been published on metaxalone toxicity. A review of the literature indicates that there are presently five reported deaths in which the causative agents included metaxalone.

At the North Carolina Office of the Chief Medical Examiner, cases suspicious for toxicological cause or with essentially negative autopsy findings are routinely screened for common over-the-counter, prescription and illegal drugs via various laboratory techniques. This presentation will detail 44 cases where metaxalone was detected during routine postmortem drug screening in support of cause and manner of death determination. The laboratory methods used to detect and quantify metaxalone in this laboratory have been previously described.<sup>1</sup>

Decedents were divided into three groups according to manner of death for the purposes of studying metaxalone concentrations in overdose and non-overdose situations (Table). The accidental and suicidal overdoses were subsequently divided into subgroups for further study: those where metaxalone was determined to contribute to the cause of death (attributed) and those where it was not (unattributed). The deaths in which metaxalone was determined to contribute to the cause of death were further divided into those where metaxalone additively combined with other drugs to cause the death and those where the drug was present in sufficient amounts to have caused the death regardless of other drugs and their concentrations.

Manner	N	Mean (median)	Range
Natural (n=4)		5.4	1.2-8.6
Accidental (n=24)			
Unattributed	13	N/A	<2.0-6.9
Attributed	11	15.4 (10)	4.4-50
Suicidal (n=16)			
Unattributed	4	N/A	<2.0-5.0
Attributed	12	25(24)	8.4-63

N/A - not calculated due to the number of concentrations reported as less than the lower limit of quantification.

Total, there were nine cases (seven suicides, two accidents) where the pathologist ruled that metaxalone was present at sufficient concentrations to cause death had it been the only drug detected. The mean (median) concentration of metaxalone in these cases was 34 (32) mg/L and concentrations ranged from 23 to 63 mg/L. Of these nine cases there was

only one in which metaxalone was the only drug detected (suicide, 63 mg/L) and the remaining eight were ruled multiple drug intoxications. Co-intoxicants included antidepressants (five of eight), antihistamines (three of eight), and miscellaneous CNS depressants (four of eight). Notable was the absence of opiates/opioids in this sub-group. This was not the case in deaths where metaxalone was ruled as an additive agent in the death (nine accidents, five suicides). In these cases, metaxalone mean, median and range were 10.8, 9.5 and 4.4-18, respectively. Opiates/opioids were ruled as contributing to death in 86% (12 of 14) of these cases.

In conclusion, of the 44 cases studied, 21 (47.7%) metaxalone was ruled noncontributory to death, 22 (50%) were ruled multiple drug intoxication and 1 (2%) single agent intoxication. Concentrations of metaxalone in these groups were <2.0-8.6, 4.4-50 and 63 mg/L, respectively.

### Reference:

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### Metaxalone, Death Investigation, Toxicology

## K45 The Methylecgonine to Cocaine Ratio in Blood Samples and the Effectiveness of Preservation With Sodium Fluoride

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After attending this presentation, attendees will understand the use of the methylecgonine to cocaine concentration ratio in blood samples as an indicator of enzymatic cocaine hydrolysis and effectiveness of preservation with sodium fluoride.

This presentation will impact the forensic science community by providing data on the methylecgonine to cocaine concentration ratio in blood samples as an indicator of enzymatic hydrolysis and effectiveness of preservation with sodium fluoride.

The limited stability of cocaine in forensic samples has been a problem for several decades. In unpreserved whole blood, cocaine (COC) will hydrolyze to methylecgonine (ME) by the action of cholinesterase and to benzoylecgonine (BE) by pH-dependent chemical hydrolysis. ME and BE are further converted into ecgonine (ECG). Fluoride inhibits plasma cholinesterase and as a result the conversions of COC to ME and of BE to ECG are reduced. The conversions of COC to BE and ME to ECG can be inhibited by acidification. Moreover, cooling slows all conversions. Addition of fluoride is generally recommended to prevent cocaine hydrolysis. In postmortem blood, acidification may occur, which makes the conversions of COC to BE and ME to ECG less important. However, enzymatic conversions continue after death. As a result, a large part of ME measured in postmortem blood may originate from postmortem hydrolysis of cocaine.

In the Netherlands, whole blood samples in cases of driving under the influence (DUI) of alcohol and/or drugs are collected in glass tubes containing sodium heparin and sodium fluoride (NaF). The samples are then sent to the Netherlands Forensic Institute (NFI) by regular mail, which generally takes one to two days, without cooling. After receipt at the NFI, blood samples are kept at 4°C for a maximum of two weeks and at -18°C thereafter. In autopsy cases, the interval between the finding of the body and the autopsy is generally one to two days. Preservation of (femoral) blood samples takes place after the autopsy, by using the same tubes as in DUI cases and by freezing at -18°C. Until the end of 2005, blood tubes contained 0.8% NaF and 700 IU/mL sodium heparin. From 2006 until mid-2007, these tubes were gradually replaced by tubes containing 0.4% NaF and 143 IU/mL sodium heparin, for commercial reasons.

In this paper, the ME/COC and BE/COC concentration ratios in whole blood samples of DUI cases with 0.8% NaF, DUI cases with 0.4% NaF, and autopsy cases were compared. The goal of this study was to obtain more

insight in the role of NaF as preservative and to investigate if the ME/COC or the BE/COC concentration ratio is indicative for hydrolysis of cocaine.

Electronic data files of the NFI were searched for concentrations of COC, BE and ME in blood samples from 1999 through 2010. The ME/COC ratios and BE/COC were calculated and statistically evaluated. Cases of DUI as well as autopsy cases were investigated. COC, BE, and ME were analyzed by using GC-MS, after solid phase extraction and derivatization, or by LC/MS/MS, after protein precipitation.

The results show that the median ME/COC concentration ratio increased over the years after 2006 in cases of DUI. This increase coincided with a gradual change from blood tubes containing 0.8% NaF to tubes containing 0.4% NaF from 2006 until mid-2007. A trend was not observed in the BE/COC concentration ratios or in autopsy cases over the years. The median ME/COC concentration ratios (and 95% range) were respectively 0.8 (0-2.1) for cases of DUI (0.8% NaF), 1.5 (0-4.6) for cases of DUI (0.4% NaF), and 1.9 (0-5.5) for autopsy cases. The observed increase in ME/COC concentration ratio is in line with the order of decreasing preservation in DUI cases. In autopsy cases, the median BE/COC concentration ratio was significantly lower than in DUI cases.

In conclusion, the results show that the ME/COC concentration ratio is probably a useful indicator of enzymatic cocaine hydrolysis in cases of DUI as well as in autopsy cases. A ME/COC ratio greater than 2.1 is indicative of (enzymatic) cocaine hydrolysis. This information will improve the interpretation of forensic results. Furthermore, the data show that a concentration of 0.4% NaF was insufficient to prevent decomposition of cocaine in blood during more than one or two days under the practical circumstances in DUI cases in the Netherlands. The relatively low BE/COC ratio in autopsy cases points to the protective role of postmortem acidification in the chemical hydrolysis of cocaine.

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**Cocaine, Hydrolysis, Sodium Fluoride**

## **K46 Postmortem Pediatric Toxicology**

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After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 13th 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 pharmacokinetic data and other relevant ancillary information followed by audience participation to provide interpretive clarity around the case-specific impact of the toxicological findings. This presentations allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Dr. Jack Kalin will review data related to toxicological findings in stillbirths. This specific population is somewhat unique given its direct dependence on the mother, her exposures and the pharmacokinetic and toxicokinetic changes that occur during pregnancy. These changes include altered maternal hepatic biotransformation, increased gastric emptying,

decreased gastric motility and altered renal blood flow. As an additional factor, placental transfer, biotransformation, etc. affect the fetal insult due to maternal exposure to potentially harmful agents. Coupled with maternal changes are the developing characteristics of the fetus, its metabolic capabilities, and the toxicodynamics effects of specific agents.

Dr. Marina Stajic will present a case involving lithium. Lithium, a classic antipsychotic agent, is a potentially teratogenic substance and is one of the few agents that can result in hypercalcemia in children. Pharmacokinetics of lithium in children varies from that in adults with a shorter half-life and faster renal clearance. Classically, lithium has a narrow therapeutic index leading to potential severe toxicity at low doses. There is debate as to the clinical utility of lithium in younger children.

Dr. David Benjamin will address an extremely difficult interpretation of clonidine and other toxicological findings in a suspected filicide. Clonidine, a centrally-acting alpha-2 receptor agonist used to control blood pressure in adults, is used in children to help control attention deficit hyperactivity disorder (ADHD) and other behavioral deficits. The use of this drug in children can induce sedation and a hypertensive crisis upon abrupt withdrawal.

Dr. Michael Heninger will discuss findings involving methadone and fentanyl and the problems associated such agents in children. Most childhood exposures to these compounds are either accidental or intentional for purposes of sedation, but not homicidal. Both compounds are classified pharmacologically as opioids and have the associated sequelae of these agents, including CNS-depression capable of producing death. Methadone has recently been tagged as a compound capable of producing death even with therapeutic use based on adverse cardiovascular events. Fentanyl on the other hand, has strict black box warnings due to potency (80-100 times that of morphine).

The case studies presented reflect current day findings in medicolegal investigations of childhood deaths. In years past, discussions of these type cases have been educational and demonstrative of the issues in this special population. Only through these continued case studies and audience participation can there be shared perspectives on the meaning of the toxicological findings.

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**Pediatric, Toxicology, Postmortem**



ATLANTA 2012

# LAST WORD SOCIETY



ATLANTA 2012

## LW1 Two Congressmen, Two Killings, and a Cannonball

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After attending this presentation, attendees will understand the facts associated with the deadly shooting in 1856 by California Congressman Philemon Herbert of a waiter who did not satisfactorily serve breakfast; the subsequent failure prosecutor Philip Barton Key to gain a conviction; Key's death by gunshot at the hands of cuckolded New York Congressman Daniel Sickles; the first (and successful) use of a temporary insanity defense against murder charges; and the ironic circumstances on the Gettysburg battlefield that transformed Sickles' right leg into a popular museum exhibit.

This presentation will impact the forensic science community by providing an entertaining summary of facts involving mid-19th century elected officials, murder, and maiming by firearms.

The history of the U.S. Congress includes criminals of a vast and fascinating variety. In the mid-19th century two particularly colorful Representatives used handguns in killings — one in a swank Washington dining room, the other in Lafayette Park adjacent to the White House — both of which resulted in murder trials. Neither Congressman was convicted, but their stories are linked by the prosecutor who failed to convict one and was killed by the other. On May 8, 1856, Representative Philemon T. Herbert, a California Democrat, walked into the dining room of Washington D.C.'s Willard Hotel. It was late morning — close to the hotel's 11:00 a.m. deadline for breakfast. Perhaps Herbert was late, perhaps he was drunk. Witnesses disagreed. When waiter Thomas Keating refused to speed up his service, Herbert complained in an argument that escalated until Herbert took a pistol from his pocket and shot Keating in the chest. Herbert was charged with murder. Prosecutor Philip Barton Key, son of Francis Scott Key, was unsuccessful in persuading the Dutch Minister — the most credible witness — to testify, and the jury could not reach a verdict. Key also prosecuted a second trial, which ended in Herbert's outright acquittal. Unlucky at law, Key was apparently lucky at love, of a sort. Soon after the Herbert trials, Key met Teresa, the wife of Representative Daniel Sickles, a Democratic Representative from New York and powerhouse member of the Tammany Hall political machine. In 1859, Sickles lay in wait in Lafayette Park, confronted and killed Key, then walked to the home of the Attorney General and confessed the murder. At trial, Sickles' defense team, led by lawyer Edwin Stanton (later the Secretary of War for Abraham Lincoln), proposed a defense never before entered in a U.S. court: Sickles, Stanton claimed, was driven mad with rage at his wife's flagrant infidelity and was innocent because the killing was an uncontrollable manifestation of temporary insanity. It worked. Sickles was acquitted, announced that he and Teresa had reconciled, and withdrew from public life without resigning his seat in Congress. When the Civil War began Sickles used his Tammany Hall connections to be commissioned as a colonel under another political clout-wielder, Major General Joseph Hooker, whose headquarters was compared to a frontier saloon and brothel. At the battle of Gettysburg, Union commander George Meade ordered Sickles to take his troops to a defensive position on Cemetery Ridge. Sickles thought otherwise and positioned his men more than a mile away. The insubordinate maneuver likely would have earned Sickles a court-martial had he not garnered sympathetic forgiveness as a result of his right leg being shattered by a cannonball while Confederate troops smashed through Meade's ill-positioned corps. The limb was amputated within the hour and Sickles ceremoniously donated the splintered bones — delivered in a small coffin — to the Army's Surgeon General, who had announced

interest in establishing a collection of "specimens of morbid anatomy." Sickles was said to have tried to visit his leg each year on the anniversary of their separation. Most of Daniel Sickles is now buried in Arlington National Cemetery; his leg sits in a glass display case at the National Museum of Health and Medicine.

**Murder, Insanity Defense, Adultery**

## LW2 Ötzi the Iceman – Austrian, Italian, or Other?

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After attending this presentation, attendees will better understand how the Copper Age influenced the Modern Age and what scientific methods can be used in that effort.

This presentation will impact the forensic science community by describing the use of various scientific methods in understanding the world's most studied 5,300-year-old mummy. This presentation will shed a contrarian light on the discovery of Ötzi the Iceman by using the archaeological, medical, toxicological, and engineering sciences which, when combined, lead to astounding opinions. This presentation will also shed light on early tattooing practices and artifacts as they might relate to today.

A German husband and wife, hiking along the Tisenjoch border between Austria and South Tyrol, Italy on September 19, 1991, near the Ötztal Valley, discovered a body sticking out of the ice — hence named Ötzi the Iceman — along with his equipment and clothing. No form of ID, such as credit cards, hunting license, passport, bar bill receipt, etc., was found upon his partially clothed body. At first, the body was believed to have been a hiker who inexplicably perished not long before the discovery. Others believed the body to have been only 500-years-old. For these reasons, the recovery was performed with great care.

Amateur recovery attempts to free the mummified, dried, jerky-like body, half frozen in ice, involved ski poles, picks, and hair dryers which caused some damage to the body and artifacts.

The artifacts found give us a fascinating picture of life in the late Neolithic period or the beginning of the Copper Age and represent the unique features of the discovery of the Iceman, Ötzi.

The tool artifacts claimed to have been associated with "Ötzi" include a copper axe, half-finished bow and arrows, quiver flint bladed "dagger" and sheath, retouching tool of stag antler, marble stone with leather cord, einkorn wheat kernel, hophornbeam tree pollen, flint drills, birch fire container, and bone needle fire starter. All of his belongings have undergone meticulous examination.

Associated with the body, the fragments of restorable clothing artifacts include goat skin leggings, belt pouch, hide coat, bear skin hat, deer skin shoes, backpack, and a grass mat overcoat.

Ötzi has been studied, analyzed, x-rayed and has had his personal space, innards and orifices invaded by over 100 scientists and researchers. It is now known that his spine, ankle, hip, and knee joints were strained and his teeth worn down. Other medical conditions discovered included a hardened aorta, a broken nasal bone, parasitic worms, and cysts. The oldest tattoos ever seen have been documented on his body. From Ötzi's preserved stomach, intestinal contents and fingernails have been analyzed. "Ötzi hurried through a forest he knew well, wincing from the pain in his injured right hand and pausing occasionally to listen for sounds that he was being pursued..." as Stephen S. Hall writes for National Geographic magazine.

Ötzi's origins, destination, and who he has have been debated. Ötzi's current resting place is in Bolzano, Italy. After almost 20 years of international analysis and speculation by others, it is time for forensic experts to enter the debate and present the possible politically correct or incorrect opinions and fan the contrarian notions as to Ötzi being Italian, Austrian, or other; and his effect on today's society.

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**Iceman, Ötzi, Anthropology**

### **LW3 More Than a Cold Case: The Frozen Murder of Iceman — A Heinous or a Merciful Crime Scene?**

*Matteo Borrini, PhD\*, Via del Mattone 17, Cadimare, La Spezia 19131, ITALY*

After attending this presentation, attendees will understand how it is possible to investigate an ancient death scene with a criminalist's point of view to reconstruct historical and cultural events.

This presentation will impact the forensic science community by suggesting that the area where the Iceman mummy was found can be interpreted differently from what has been done so far.

In September 1991 in the Ötztal Alps, on the border between Austria and Italy, a natural mummy radiocarbon dated to the Calcolithic Age (about 3300 BC) and consequently named Ötzi or Iceman, was found.

A CT scan and forensic light examination of the corpse pointed to the cause of death: an arrow point in the left shoulder, which cut the subclavian artery. Other pieces of evidence such as peri-mortem facial trauma and a deep defense cut on the right hand as a result of hand-to-hand fighting indicates a double assault. It is clear that the Iceman was killed, though some doubts may be raised looking at the recovery scene.

The body was found with a complete set of clothes: a coat and a cloak made of woven grass, a bearskin cap, a belt, a pair of goat skin leggings, a loincloth, and shoes. If one can reasonably explain a dressed man killed during a fight, it is much more difficult to explain the large number of artifacts and tools found with the body. Iceman was found, in fact, with a copper axe, a flint-bladed knife, a quiver of 14 arrows, an unfinished yew longbow, a wood backpack, two birch bark baskets, some small stone, and bone tools.

The first inconsistency with the primary crime scene are the numerous materials found with the body; hard to carry for a fugitive especially if he has a deep bleeding wound and if his unfinished bow is 72 inches long and his body statue only being 5 feet, 5 inches tall.

Other evidence is the absence of the shaft of the killer arrow. No "chipping" around the wound seems to indicate that Iceman did not extract the arrow. The aggressor may have taken the arrow to reuse it, but it is strange that he did not steal the complete quiver of the victim as this kind of object was very important. The same consideration should be given in regards the copper axe, a very expensive status symbol that was left with the corpse.

Iceman was quite old for his time and sick, as demonstrated by the Beau's lines on the fingernail and CT analysis. He was also a man of high social standing in his group as indicated by the axe. Peri-mortem lesions may indicate a fight for the village leadership; failing the competition, Ötzi would have demonstrated that he was not able to defend himself anymore or to be able to care of his village. In ancient communities, a leader must be dead in order to be replaced. The entrance angle of the arrow in the back of Iceman indicates that he was killed from behind as was the ancient ritual of expiation, where the victim is shot while he is running away from the village.

After the death, Ötzi's corpse was prepared for burial — the arrow was pulled out, he was dressed with all his clothes, and laid with all his objects surrounding him.

Some characteristics of the clothing and the location of the recovery scene can justify this conclusion. First of all, the weapons found with Ötzi are ineffective because they are unfinished. It is not likely that a fugitive

would run away with a lot of useless stuff. Typically in protohistoric graves, broken or fake weapons were usually buried to connect with the soul of the deceased and to prevent the ghost he fought against in the living to return back from the afterlife.

Further contradictory evidence is the improbability that Iceman would have chosen a snow-clad and a very difficult route for his escape, especially if wounded. The high location of the burial in a mountain pass is very similar to other contemporaneous graves generally interpreted as a spatial signal used by ancient transhumance communities.

In addition to this consideration, at the slopes of the mountain where Ötzi was found in recent centuries, a stele or monument, engraved with a bowman striking the back of another man with an arrow was discovered indicating a possible memorial of the ritual sacrifice of an important village chief.

How the recovery scene of Iceman believed to be the crime scene of an inexplicable murder can be reinterpreted as the burial site of a sacrifice victim will be presented.

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**Crime Scene, Cold Case, Iceman**

### **LW4 The Execution of a Pregnant Murderess: Global Views**

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After attending this presentation, attendees will gain insights into the correctional difficulties encountered by pregnant inmates.

This presentation will impact the forensic science community by studying the problems of pregnancy during imprisonment.

Bathsheba Spooner, 32, lived with her husband, Joshua Spooner, and her three pre-teen children in Brookfield, Massachusetts on March 1, 1778. Bathsheba Spooner was well named – for like her biblical namesake – death followed in her wake. It is indisputable that both the Bathsheba of the Bible and the Bathsheba of the 18<sup>th</sup> Century Massachusetts were femme fatales. Like King David, 17-year-old Ezra Ross, a private in the Revolutionary Army of George Washington, looked with covetous eyes on Bathsheba Spooner with whom he and two escapees from General Burgoyne's failed Saratoga campaign, hatched a plan to murder Joshua Spooner. The three soldiers were prevailed upon by Bathsheba Spooner to waylay and kill her husband as he returned from a visit to Cooley's tavern on the Old Boston Post Road on the night of March 1, 1778. The murder occurred as planned with Joshua Spooner having been assaulted by the three male perpetrators who, according to the death warrant, "did strike, beat, and kick" the victim with the body being dumped into a well fronting on the Spooner property in Brookfield. While the murder was in progress and the disposal of the remains was under way, Bathsheba was in her home performing housekeeping chores in the kitchen and not participating directly in her husband's murder nor giving any sign of being distressed by what was going on outdoors. The murder was ill-concealed and quickly discovered with Mr. Spooner's body being removed from the well and laid out in the sitting room of his home, as per the custom in those days. The Brookfield coroner, Thomas Gilbert, was summoned and arrived at the Spooner's house where Bathsheba was seen reluctant to place her hand on her deceased husband's forehead while saying "Poor Little Man." This touching was pursuant to the then prevailing medical myth called the "ordeal by touch." According to this old wives' tale, the skin of a murder victim would display color when touched by the murderer. Bathsheba's touching the forehead of the deceased left no imprint and had no discernible effect. At the Worcester Superior Court on April 24, 1778, the four murder conspirators, with Mrs. Spooner charged as an accessory before the fact, were tried, convicted, and sentenced to be hanged on June 4, 1778. Mrs. Spooner was represented by attorney Levi Lincoln who pleaded insanity on her behalf but the execution of the four was delayed until July 2, 1778 by a petition filed with the Massachusetts Council by Bathsheba in which she claimed to be some five months pregnant and requesting a delay of her

execution until the birth of her child. In the intervening wrangling over whether she was “quick with child” or not, it was first said by the male and female “experts” summoned on the question that she was not pregnant but even though a later opinion found her to be quick with child, her seeking a postponement of her hanging was denied. On July 2, 1778 all four of the conspirators were hanged with Bathsheba Spooner continuing to maintain that she was pregnant and requesting a post-hanging dissection to prove it. She was granted that request and a five month old male child was found to have died with its mother’s execution. This outcome reflects the first time in the United States that a state-sanctioned execution resulted in the death of an innocent child. In other countries than the United States, as well as under contemporary law in Federal and State jurisdictions, in today’s world the execution of a pregnant woman would be treated differently from the manner of its processing in Massachusetts in 1778. The alternative approaches taken in foreign countries will be recounted and discussed.

**Pregnancy, Execution, Penology**

## **LW5 Hoarders: A Postmortem Analysis**

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After attending this presentation, attendees will learn about cases of deceased hoarders brought before the Identification Review Committee of the New York City Office of Chief Medical Examiner and the processes involved in their identification.

The presentation will impact the forensic science community by giving insight into the complications and methods of identification even when it appears that a wealth of information is at their disposal.

The Identification Review Committee within the New York City Office of Chief Medical Examiner (NYC OCME) is a group assembled to discuss not-so straight forward cases for the identification of decedents within the five boroughs of New York City. The group is comprised of the collaborative effort of the disciplines that assist with identification: forensic anthropology, forensic biology (DNA), fingerprints, forensic odontology, x-ray, identification unit, and the medical examiner assigned the case. The Committee meets once a month and each discipline with information contributing to supporting or refuting the present identification results of their discipline’s testing or any additional scene and/or family information. Identification is not purely visual and relies heavily upon the combination of scene information, family, and science.

With the success of the reality TV series Hoarders™, the Identification Review Committee at the NYC OCME has also been witness to the reality of the many cases involving hoarders. Hoarding, also known as compulsive hoarding, is the excessive acquisition of possessions and the inability to dispose of them. The possessions may be of little or no value (i.e., trash) but the hoarder cannot bear to part with it. Unfortunately unlike the show where there is usually an intervention, massive house cleaning, reveal, and follow-up, the intervention on NYC OCME’s part is to an individual who has perished as a direct result of their hoarding habit. The additional challenge is that the individual is usually found long after they have died and is not visually recognizable due to advanced decomposition or skeletonization.

Forensic anthropological analysis is routinely performed on cases of decomposed and/or skeletonized individuals in addition to when the medical examiner requests assistance with aging and trauma analysis such blunt force or toolmark analysis. The forensic anthropology report is extremely valuable in providing sex, age, race/ancestry, stature, trauma, and pathological and general observations. Ironically, despite the amount of debris and mail which provides a timeline and possible identification, many of these decedents are still difficult to identify because no antemortem medical or dental records can be found. Many of the deceased hoarders are

older individuals who have led hermit lifestyles. Medical records and insurance cards provide some veracity to the name of the deceased individual found in the locked home but many times that individual has not seen a doctor in years and their medical records have been purged or destroyed.

Due to their isolated lifestyle, many have not had contact with their immediate families and only distant relatives are alive. If any family is located, many of them haven’t seen their potential relative in years to be able to do a visual identification even if it were an option. DNA testing in these cases does not add additional information as the relative is not only separated by distance but also by generations. Many relatives of deceased hoarders are nieces and nephews or even great nieces and nephews. If other desirable relatives for DNA testing are located, usually only one is found (such as a sibling) or only one cooperates and gives a DNA sample.

This presentation will touch upon the disease of hoarding and give an overview of just a few of the many cases of hoarding from the NYC OCME. These cases will be discussed in detail from their unusual causes of death such as “asphyxia due to fallen bookshelf” to the complicated process of identifying the individual even when investigators are literally wading through abundant amounts of information from within the home.

**Hoarding, Identification, Postmortem**



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Forensic Sciences Foundation, Inc., (Grant Support) - W16  
Midwest Forensic Resource Center (Grant Support) - A61

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## E

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GPM, OsiriX, Philips, UWA (Discussion of Commercial Products or  
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## G

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Netherlands Forensic Institute (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device) – B8, B10, W21

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James M. Robertson, PhD - A10  
Primer-E, Ltd., MIDI, Inc. (Discussion of Commercial Products or Services)  
Federal Bureau of Investigation (Employee)

James M. Robertson, PhD - A11  
Applied Biosystems, Inc., New England BioLabs (Discussion of Commercial Products or Services)  
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John B. Robertson, BS - C17  
Discloses no financial relationships with commercial entities.

Anthony M. Robinson - E35  
Discloses no financial relationships with commercial entities.

Corissa L. Rodgers, BA - A194  
Discloses no financial relationships with commercial entities.

Ryan P. Rodgers, PhD - C18  
National Science Foundation, State of Florida (Grant Support)

Nina B. Rodriguez, BS - D61  
Discloses no financial relationships with commercial entities.

Sandra E. Rodriguez-Cruz, PhD - A146  
SWGDRUG (Other Financial/Material Support)

Sandra E. Rodriguez-Cruz, PhD - W13  
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Scott D. Roffman, MS - W10  
Apple, Inc. (Discussion of Commercial Products or Services)  
Drug Enforcement Administration (Employee)

Christopher B. Rogers, MD - W1  
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Marcus Rogers, PhD  
Discloses no financial relationships with commercial entities. – B17  
Purdue University, Department of Homeland Security (Grant Support) – B16

Douglas E. Rohde, MS - BS2  
Discloses no financial relationships with commercial entities.

Maria Carolina Romanelli, MD - G71  
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Sara Roper, BS - A186  
Caymen Chemical Company (Discussion of Commercial Products or Services)

Roberto Rosa, PhD - A7  
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Karen B. Rosenbaum, MD - I5  
Italian VESPY (Discussion of Commercial Products or Services)

Melina D. Rosenberg, MS - I5, I16  
Discloses no financial relationships with commercial entities.

Adam R. Rosenblatt, PhD - D69  
Stanford University (Grant Support)

Alastair Ross - C25  
Discloses no financial relationships with commercial entities.

Tara N. Rossy, BS - A177  
Drug Enforcement Administration (Employee)

Amanda Roth, BA - D29  
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Walter F. Rowe, PhD - A33  
Discloses no financial relationships with commercial entities.

David Ruddell, PhD - A197  
New Wave Research, Thermo, Foster & Freeman (Discussion of Commercial Products or Services)

Leonid I. Rudin, PhD - W21  
Cognitech (Employee)

Donald J. Rudy, PhD - C2  
The Aerospace Corporation (Employee)

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## S

Kenneth J. Saczalski, PhD - C32, C37  
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Pauline Saint-Martin, MD - G68  
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Discloses no financial relationships with commercial entities.

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Claudia L. Sanchez, BA - A131  
Intelligence Community Postdoctoral Research Fellowship (Grant Support)

Yvette Sanchez-Owens, BA - A161  
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Lakshmanan Sathyavagiswaran, MD - G121, W1  
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Hunter Laboratories (Discussion of Commercial Products or Services)  
Texas State University (Grant Support)

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Anny Sauvageau, MD – G119, W4, W17  
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Forensic Art (Discussion of Unlabeled/Investigational Use of Product/Device)

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Marvin Schechter, JD - W20  
Discloses no financial relationships with commercial entities.

Lisa A. Scheinin, MD - G99  
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James C. Schneider, DDS  
James C. Schneider (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device) – F34  
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Cuyahoga County Coroner's Office (Employee) – F47

Kassra L. Scholl, BSc - H10  
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Candace H. Schoppe, MD - W2  
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Jason L. Schroeder, MS, MBA - D57  
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University of Central Florida (Employee)

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Life Technologies Corporation (Discussion of Commercial Products or Services)

David Shadoin - E35  
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Stephen A. Shaffer, BS - A106  
Carl Zeiss MicroImaging, LLC, Leica Instruments, Olympus Corporation of America (Discussion of Commercial Products or Services)  
National Institute of Justice (Grant Support)

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National Institute of Standards and Technology (Other Financial/Material Support)

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Lonza, Inc. (Discussion of Commercial Products or Services)  
Boston University School of Medicine (Other Financial/Material Support)

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Freewarefreeware (Discussion of Commercial Products or Services) – F4  
Midwest Forensics Resource Center (Grant Support) - A174, W16

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Donald E. Shelton, JD, PhD - E22  
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Google, Inc. (Discussion of Commercial Products or Services)

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Oak Ridge Institute for Science and Education (Other Financial/Material Support)

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Sudhir K. Sinha, PhD - W7  
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Edward Sisco, BS - D26  
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Applied Biosystems, Inc., QIAGEN (Discussion of Commercial Products or Services)

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National Transportation Safety Board (Employee)

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Bebo, BingBlogs, Blackplanet, Classmates.com, Craigslist, Facebook®, Foursquare, Flickr, LinkedIn, Myspace, Inc., Tagged, Tumblr, Twitter® (Discussion of Commercial Products or Services)

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Satellite City, Inc. (Discussion of Commercial Products or Services)

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Burlington Northern, Dinoseb, Trillium, Inc., (Discussion of Commercial Products or Services) – C15

James S. Smith, PhD - C12  
Oak Creek, Inc. (Employee)

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American Honda Motor Company, Inc., Chrysler Group, LLC, Ford Motor Company, Inc., General Motors, Inc., Mazda North American Operations, Toyota Motor Sales, USA, Inc., Volvo Car Corporation (Discussion of Commercial Products or Services)  
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NuSil Technology, LLC (Discussion of Commercial Products or Services)

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Nichevision (Discussion of Commercial Products or Services)

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Elsevier (Discussion of Commercial Products or Services) - W6

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MegaVision (Discussion of Commercial Products or Services) – BS3  
United States Army Criminal Investigation Laboratory (Employee) – BS3

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Richard M. Thomas, PhD - H41  
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Applied Biosystems, Inc. (Discussion of Commercial Products or Services)

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Nanofocus (Discussion of Commercial Products or Services)

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International Committee of the Red Cross (Employee) – H54, W12

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National Institute of Justice (Grant Support)

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Beckman Coulter, Inc., Florida International University (Discussion of Commercial Products or Services)  
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## T

Corinne B. Tandy, MS - H47  
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Aphantree Tanittaisong, MS - H49  
QIAGEN, SYBR Green (Discussion of Commercial Products or Services)  
National Institute of Justice (Grant Support)

Aaron M. Tarone, PhD - G107  
454, Applied Biosystems, Inc., Illumina, QIAGEN (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device)  
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Christina J. Tatum, MD – G20, W2  
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## U

Douglas H. Ubelaker, PhD – S1, S2, C25  
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Tanya Uldin, MA - H109  
University Center of Legal Medicine (Employee)

Noelle J. Umback, PhD  
New York City Office of Chief Medical Examiner (Employee) – A163  
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Susan C. Underkoffler, MFS - D67  
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Yuriy Uvaydov, MS - A144  
ThermoFinnigan (Discussion of Commercial Products or Services)

## V

Clara I. Valderrama, DDS - F39  
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Oluseyi A. Vanderpuye, PhD - A24, G88  
Discloses no financial relationships with commercial entities.

Stefano Vanin, PhD - G73, G79  
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National Institute of Justice (Grant Support) – G36  
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National Institute of Justice (Grant Support)

Beverley R. Vaughan, PhD – K40, K43  
Anglia Ruskin University (Employee)

Andrea Velasco Ordonez, BA - H100  
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Elizabeth Ventura, MD - G2  
Keppra (Discussion of Commercial Products or Services)

Karl G. Verebey, PhD - E19  
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Tiffany A. Vickman, BS - A42  
Applied Biosystems, Inc., Biomatrix, Mo Bio Laboratories (Discussion of Commercial Products or Services)  
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National Institute of Forensic Medicine (Employee) - W12  
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Carlos A. Villalobos, BA - H101  
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Jaclyn Vincent, BS - K37  
SeQuant (Discussion of Commercial Products or Services)

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## W

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National Institute of Justice (Grant Support)

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Materials & Chemistry Laboratory, Inc., Inciconel (Discussion of Commercial Products or Services)  
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Trimble, ESRI (Discussion of Commercial Products or Services)  
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Mitchell L. Weinberg, MD - G114  
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Chrysler Group, LLC, Ford Motor Company, Inc., General Motors, Inc., Nissan North America, Inc. (Discussion of Commercial Products or Services)

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Restek (Discussion of Commercial Products or Services)  
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John A. Williams, PhD - H40  
Innov-X Systems (Grant Support and Discussion of Unlabeled/Investigational Use of Product/Device)

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Bode Technology Group, Inc., QIAGEN (Discussion of Commercial Products or Services)

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Immunalysis, Microgenics (Discussion of Commercial Products or Services)  
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Pixmeo (Discussion of Commercial Products or Services)

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Brown & Bryant, Burlington Northern BNSF Railway Company, Dow  
Chemical, Shell Oil Company, Union Pacific Railroad Company (Discussion of  
Commercial Products or Services)

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WITec GmbH (Discussion of Commercial Products or Services)

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## **Y**

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## **Z**

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Sara C. Zapico, MSc - A2  
Thermo Scientific, NanoDrop Products, QIAGEN (Discussion of Commercial  
Products or Services)



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