NEAFS 42nd Annual Meeting

Atlantic City, NJ

Proceedings of the Northeastern Association Of Forensic Scientists – October 2016

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CRIM/CRIME SCENE SESSION ABSTRACTS
Thursday, October 13, 2016

MODERATOR: TRACY BRETON

"Dismantling a counterfeit currency ring – forensic examination from lab to crime scene" Irina Geiman, Unites States Secret Service

In 2014, the United States Secret Service (USSS), in collaboration with several law enforcement partners, completed an investigation of a prolific counterfeit $100 Federal Reserve Note. This counterfeit first appeared in 1999 in New York City and since then had predominantly circulated along the I-95 corridor. The note was believed to have been originally manufactured in Israel and distributed in the United States by two Russian-speaking brothers from Queens, NY. In January 2014, the counterfeiters established a printing plant in Cherry Hill, NJ and began to manufacture the $100 notes. By the time the search warrant was executed on May 28th, several million dollars had already been printed.

The USSS Treasury Obligations Section examined thousands of these counterfeit notes and linked them together based on printing processes and printing defects. The resulting "Russian-Israeli" family came to include over $86 million in counterfeit notes passed and seized globally. A Document Analyst was involved in every step of the investigation, from tracking development of the notes and progression of simulated security features, to identifying printing equipment imported by suspects, assisting with trash pulls during surveillance, and ultimately participating in the counterfeit printing plant suppression. As a result of this investigation, thirteen defendants were convicted on multiple charges and received sentences ranging from 2 to 15 years of jail time.

This presentation will discuss the forensic examination of counterfeit currency, linking documents through printing defects, and counterfeit printing plant suppression. Additionally, the presentation will demonstrate the importance of partnership between laboratory personnel and criminal investigators.

*** "Forensic Body Fluid Identification and Differentiation by Raman Spectroscopy and Chemometrics"** Claire K. Muro, Kyle C. Doty, Luciana de Souza Fernandes, Igor K. Lednev, University at Albany, State University of New York

The ability to identify body fluid traces at crime scenes, and preserve any DNA present, is critically important in forensic science. Identification can be difficult because many of the current techniques are specific to one body fluid, and typical biochemical methods are destructive- preventing any further analysis. To develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, we combined the specificity of Raman spectroscopy with the analytical power of statistical modeling. Raman spectra were collected from 75 body fluid samples, including peripheral blood, saliva, semen, sweat, and vaginal fluid. After preprocessing the experimental spectra, the samples were split into calibration and validation datasets. Several chemometric analysis techniques were trained and tested to find the best model. These included Partial Least Squares Discriminant Analysis (PLSDA) and Support Vector Machine Discriminant Analysis (SVMDA) modeling, and variable selection by interval PLSDA (iPLSDA) and Genetic Algorithm (GA). By exploring so many different combinations of classification algorithms and variable selection methods, we were able to study patterns in the data, the effects of various modeling parameters, and ultimately determine the most robust method for differentiation. The final model was an SVMDA model built on a dataset rendered by GA. This model accurately predicted the identity of 99.9% of the spectra from the calibration dataset, after cross-validation. More importantly, it correctly predicted the identity of 100% of the spectra in the external validation dataset. All five body fluids were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids.

*** "Shooting Distance Estimation using Gunshot Residue on Mammalian Pelts"**
Cory A. Weiss, B.S., Ralph R. Ristenbatt III, M.S., Pennsylvania State University; Jason W. Brooks, VMD, Ph.D., Animal Diagnostics Laboratory, Pennsylvania State University

Gunshot residue is produced from the discharge of a firearm, and for the purpose of this research GSR includes any residue originating from the propellant, primer, projectile, cartridge case, residues from previous shots, and cleaning agents or lubricants present in the barrel that travel with the bullet to create a pattern on the target. The patterns, both visible and enhanced, may permit an estimation of muzzle-to-target distance to aid in reconstruction of firearm-related events. In cases involving animals, visualization of GSR is complicated by fur color and length. This requires visual and chemical techniques to be adapted so that enough contrast is created to assist with muzzle-to-target distance estimation

Initial photography of the pattern permits visualization, but the periphery shows little contrast. The use of a high-intensity, tunable-wavelength light source may excite some GSR resulting in fluorescence, thereby increasing contrast between the fur and GSR. Infrared (IR) light can also be used to enhance GSR patterns using a specialized IR camera or an IR viewer. Radiography can be employed to detect the presence of radiopaque metallic particles surrounding the entrance hole. Once all methods of visual enhancement are complete, the Modified Griess test (MGT) can be used to detect the presence of nitrites.

Cow and rabbit hides were shot from a range of distances from contact to three feet with jacketed and unjacketed ammunition using handguns of various calibers, including .38 Special, 9 mm, and .45 ACP. Visualization with white light and IR light shows patterns increasing in size as muzzle-to-target distance increases. In some instances, the presence of a radiopaque ring, presumably metallic lead, around the entrance hole was detected with radiography. The ability to visualize this ring can be enhanced by adjusting characteristics of the image, allowing the radiopaque ring to be visualized at greater distances. As expected, both observable features begin to fade with increasing muzzle-to-target distance. The Modified Griess Test was conducted using filter paper rather than photographic paper and applying the heat to cheesecloth on the backside of the filter paper. Results of the MGT are consistent with the visual methods, as the patterns increase in size and become more dispersed with increased firing distance.

"Accurately estimating the time since deposition of bloodstains aged for more than two years" Kyle C. Doty, Igor K. Lednev, University at Albany, State University of New York

Bloodstains found at crime scenes contain a plethora of information, which is currently underutilized. Specifically, knowing the age, or time since deposition (TSD), of bloodstains could add significant value to the investigation. This information would help with crime scene reconstruction by providing more clues about the time of a crime, or order of events, particularly when a human body is not available for physical examination. Here, a unique Raman spectroscopic approach for the confirmatory identification of blood and accurate TSD estimation was used for bloodstains aged for more than two years (ambient conditions). Support vector machines discriminant analysis (SVMDA) was used for blood identification. The SVMDA model allowed for confirming bloodstains' identity as blood through differentiation of blood from four other body fluids (i.e. saliva, semen, sweat, and vaginal fluid). To provide quantitative predictions of the TSD, partial least squares regression (PLSR) and principal component regression (PCR) models were built. Both models were internally cross-validated (CV) and externally validated. The PLSR and PCR models had a CV root mean squared error (RMSE) of 0.17 and 0.18, respectively. They both showed a high degree of linearity with an R^2 of 0.98. Also, both models demonstrated similar external TSD prediction abilities with an RMSE of prediction of 0.29 and 0.31 for PLSR and PCR, respectively. These results demonstrate that Raman spectroscopy can be used as a non-destructive analytical tool for confirming the identity of blood and discriminating between bloodstains on the scale of hours to days to years.

*** "An Assessment of the Model used to Estimate the Origin of Radial Spatter Patterns"** Yu Chen Lim, Ralph Ristenbatt III, Rachel Chesser, Pennsylvania State University

The bloodstain pattern analysis (BPA) model used to estimate the incident angle at impact of an airborne blood drop to a flat surface is based on the oblique projection of a sphere. In practice, the inverse cosine of the ratio of the width-to-length of the resultant stain is used to estimate the so-called impact angle, the angle between the drop trajectory and target surface at impact. Coupled with the directional vector indicated by the longitudinal axis of an elliptical stain, the drop trajectory can be approximated and visualized with string, if desirable. When several stains in a radial spatter pattern are examined collectively, their retrograde projections can be used to localize the area of origin or location of the source of the spattered blood. Despite using various geometric and mathematical principles, inaccuracies in the calculated result arise due to a number of assumptions. As such, this model should only be used as a gross estimation for the origin of a radial spatter pattern.

Modern computer software packages designed for BPA, which often boast higher accuracy, typically rely on the same mathematical principles; thus, it is important to determine if investments in such time and technology are justified. This project

aims to assess the accuracy and precision of the BPA model under various conditions and parameters to assess deviance (predicted v. measured). Additionally, it seeks to validate the claim that better measurements produce more accurate results. Bloodstains of fresh, defibrinated ovine blood were created on white, letter-size copy paper as well as various glass plates using varying heights, gauge sizes, and incidence angles. Resultant stains were measured using two methods: manually with a finely graduated scale and using Microsoft® Excel to estimate the best-fit ellipse. All measurements were used to calculate the incident angle. A one sample t-test was used to evaluate the accuracy of each method and results of both measurement methods were compared using a two sample t-test to assess if observed differences were statistically significant.

"Novel sensing approaches in forensic analysis" Jan Halámek, Juliana Agudelo, Erica Brunelle, Crystal Huynh, Lenka Halámková, Leif McGoldrick, University at Albany, State University of New York

Biomarker analysis is a long standing discipline in forensic science, for instance, the analysis of blood for the presence of various substances can be performed along with DNA for identification purposes. The forensic science field has developed rapidly over the years, however, the methods that are currently implemented still need improvement. Most of the modern and routinely used forensic science techniques require the collection of the sample at the crime scene, followed by transportation to a laboratory facility for its analysis. Crimes are usually committed in high volumes, thus, improvement to these analyses is highly needed. The backlog in the analysis of serology samples has been recognized and addressed by the NIJ, but major improvements are still required, as full-scale investigations can take weeks or even months. The process of matching DNA samples, however, is very time consuming and have caused backlogs in many states.

While this DNA analysis is, without a doubt, useful, it may not be the best method of analysis during the pertinent time frame of the first few hours of an active criminal investigation. There are many other biomarkers present in blood that can be analyzed in a much shorter amount of time by utilizing bioaffinity-based cascades. Our lab has developed and is in the process of developing more cascades for the purpose of identifying personal attributes from individuals such as age, biological sex, and general health conditions. These cascades have been developed for both blood and fingerprint analysis. The cascades created for blood analysis have focused on the determination of the age of the originator and the time since deposition of the sample.

Fingerprint analysis has been focused on pictorial comparisons since the process was adapted for forensics. Advances in this area has only progressed insofar that automated fingerprint identification systems can be used in certain cases (with an expert checking the results). Because of this, a fingerprint may be determined to be too smudged or smeared to be of use. What is often overlooked, however, is that the patterns used to match fingerprint samples are created by sweat/sebum emulsions excreted from the fingertips. Like all bodily excretions, the emulsions have their own unique chemical composition meaning there are biomarkers present for analysis. One of the cascades developed in this lab has focused on the analysis of amino acids in the samples. The cascades developed for fingerprint analysis have focused on the determination of biological sex. There is also ongoing research aimed at the development of a larger variety of cascades able to determine other attributes from blood and fingerprint samples.

"Differentiating Menstrual Blood from Peripheral Blood"
Nidhi Sheth, Pace University, NYC Office of the Chief Medical Examiner

Menstrual blood is a consequence of the shedding of the endometrium lining during menses. The differentiation of menstrual blood from other bodily fluids, especially peripheral blood, is of importance in sexual assault cases to determine if trauma has occurred, or if a disputed satin is blood or menstrual blood. With the advent of new technologies multiple methods have been reported for differentiating menstrual blood from peripheral blood. As of yet, however, no single, robust, confirmatory technique has been developed. To distinguish these body fluids, scientists are searching for specific or enriched makers that are differentially expressed between them. Candidate markers include proteins and nucleic acids found in the endometrium including those in STEM cells as well as in the immune and clotting systems. Techniques include ELISA and other immunochemistries, immunophenotypic analyses, RT-PCR and others. Tissue expressions as well as functional properties of markers were taken into consideration when evaluating them. Protein candidates include ER alpha, MMP14, MMP 7, MMP 11, desmoplakin, catenin, CD 73, CD 105 and others.

Samples obtained from crime scene can be degraded or may be present in small amounts. The objective of this review presentation is to present several of the approaches that have been explored for differentiating menstrual blood from other bodily fluids and to determine the most robust techniques that offer the most reliable results with real world samples.

"A Noah's Ark of Sperm Cells" Beth Saucier Goodspeed, Massachusetts State Police Crime Laboratory

Animal sperm cells can and have been encountered in evidence submitted from cases of sexual assault. For the analysts who process this type of evidence, it is important to be able to distinguish between human and animal sperm cells for several reasons such as determining the presence of animal versus human sperm cells may be important to corroborate certain elements of the case and the ability to differentiate animal from human sperm cells enables analysts to select the most forensically relevant sample for DNA analysis

Cases from the MA State Police Crime Laboratory will be presented. In two of these cases, animal sperm cells, specifically dog, were identified in evidence samples being processed for sexual assault cases. The third case was a case of animal cruelty in which it was important to say that the sperm cells were indeed human and not animal. The case scenarios, results, and standard statements used in the reports will be presented. In addition, a review of the characteristics of human sperm cells and the staining procedure currently practiced in the MA State Police Crime Laboratory will be discussed. Characteristics and photographs of sperm cells from several different species of animals will also be presented, as well as some interesting facts. The photographs will include microscopic pictures taken at the MA State Police Crime Laboratory as well as others obtained from literature. The recent training of the analysts in the Criminalistics Unit will also be discussed as well as planned changes to the existing MA State Police Crime Lab Crim Unit protocol.

"Determination of critical angle in automobile windshield glass using 9mm Luger ammunition" Peter Diaczuk, Pennsylvania State University, D&H Criminalistics Agency; Tara Goldfrank, John Jay College of Criminal Justice, CUNY

This research dealt with bullet ricochet off windshield glass. All variables were held constant with the exception of incident angle, the angle at which a projectile impacts the target surface. When a bullet strikes a target, it may perforate or penetrate the substance, fragment into pieces or ricochet off of the surface. Each surface has an angle called the critical angle, below which a projectile will ricochet. There are many factors that affect the ricochet of a bullet, such as: incident angle, impact velocity, bullet shape, bullet weight, bullet hardness, bullet center of gravity, the hardness of the impact surface and the response of that surface to the bullet's impact. It is important to note that not only do the characteristics of the bullet have an effect on the ricochet, characteristics of the impact surface do as well.

Laminated glass, which is used on automobile windshields, is composed of a thin polyvinyl plastic layer sandwiched between two pieces of glass. The glass is bonded to the plastic by heat and pressure.

This study used 9mm Luger ammunition with a full metal jacket (FMJ) bullet. According to the manufacturer, the muzzle velocity is 1150 feet per second, which was confirmed using a chronograph to determine the velocity of the specific lot of ammunition used.

Initial test shots at 5°, 10°, and 20° incident angles were performed to determine the affect the bullet had on the windshield. Five degrees yielded a clean ricochet with minimal damage to the windshield and a fairly intact bullet. This was not the case with 20°, where the bullet perforated the windshield. High-speed photography elucidated the mechanism of the bullet-glass interaction. Shots fired at 10° resulted in a ricocheted bullet that retained a substantial portion of its initial mass. Ten degrees was then bracketed by shots at 9 and 11 degrees to compare the damage caused by the bullet and the associated damage done to the bullet. A system was devised to capture and recover bullet fragments from both the surface of the windshield and from underneath the windshield, which was used to determine the percent of post-ricochet weight loss. Witness papers were used downrange to determine the ricochet angle using trigonometry.

"Bloodstain Pattern Analysis OSAC Update" Peter Valentin, D-ABC CSCSA, University of New Haven

This presentation will report on the development of the Organization of Scientific Area Committees (OSAC) and particularly the work of the Bloodstain Pattern Analysis Subcommittee.

The OSAC was formed in the United States under the "umbrella" of the National Institute of Standards and Technology (NIST), in part, as a response to concerns presented in the National Academy of Sciences Report *Strengthening Forensic Sciences in the United States: A Path Forward.* The individual subcommittees of the OSAC have served to continue the work of the various Scientific Working Groups.

Since the inception of the committees in 2014, there have been three face to face meetings. The outcomes of the BPA subcommittee meetings along with on-going task group work will be discussed in this presentation. Future tasks and affiliate

selection will also be reviewed. Time will be set aside to answer audience questions and receive comments on this global effort to better the forensic sciences.

*** "A Comparative Analysis of Commercially Available Protein and Peroxidase Reagents for Blood Detection and Enhancement on Laundered Clothing of Various Fabric Types"** Gabrielle A. Hartley, Claire L. Glynn, Ph.D., University of New Haven

Human blood is commonly encountered in forensic investigations, particularly on clothing following a violent incident. In certain circumstances, clothing may have been laundered prior to seizing to eliminate any traces of human blood. A number of commercially available products are available for the detection and enhancement of dilute bloodstains, including both protein and peroxidase based reagents. While each product has been researched individually in the literature, to date, these products have not yet been simultaneously analyzed on a variety of laundered fabric types. The aim of this study was to investigate six commonly used protein and peroxidase reagents, which are commercially available from a crime scene supply company, and determine their sensitivities and most appropriate utilization on varying colored fabric types post laundering.

Following informed consent, venous blood was obtained from volunteers into sterile vacutainer EDTA vials. Five fabric types were selected, namely; white cotton, black cotton, white polyester, black polyester, and blue denim. Three protein based reagents and three peroxidase based reagents were selected and purchased from Sirchie: Hungarian red, Coomassie blue, amido black, luminol, Leuco Crystal Violet (LCV), and Bluestar® Forensic Magnum. 100 L of human blood was deposited onto each fabric type in a range of seven dilutions from neat to 1 in 1 million. Each sample was done in triplicate and photographed prior to laundering and enhancement. Following laundering, each sample was photographed and subsequently enhanced following the manufacturer's instructions provided with each of the 6 reagents. The results of each reagent, dilution and fabric type were compared, using a scale from 0-4 (0 = no reaction; 4 = strong positive reaction).

The results of the post-laundering enhancement of neat blood and blood dilutions on the varying fabric types revealed the peroxidase based reagents (luminol, LCV and Bluestar® Forensic Magnum) to have the greatest sensitivities on the natural fabric types (white cotton, black cotton and denim) as they all reacted positively on these fabrics down to 1:1,000. However, when the protein reagents were tested on the dilutions and varying fabric types, they revealed the greatest sensitivities (1:10) on the white polyester when compared to the peroxidase reagents, which only produced positive reactions on the laundered neat blood. As the protein based reagents are color reactions and are not based on chemiluminescence, their use on dark fabrics revealed indeterminate results. The results of this study suggest peroxidase based reagents to be the superior method for use on natural fabrics and all dark fabrics. Protein based reagents have previously been shown to have merit for the enhancement on non-absorbent surfaces, while in this study, their use, albeit inferior in most cases to peroxidase based reagents, was shown on absorbent fabrics.

This study highlights the variety of commercially available blood detection and enhancement reagents offered and reveals their advantages and disadvantages in certain settings and on difficult types of evidence. The results of this research provide a much needed comparative analysis of these reagents and could be used in the decision making process for forensic investigators evaluating fabric evidence.

"Plutonium Age-Dating in Nuclear Forensic Investigations Using Thermal Ionization Mass Spectrometry" Ashton D. Lesiak, Jamie L. Doyle, Floyd E. Stanley, Lav Tandon, Los Alamos National Laboratory, Los Alamos, New Mexico

Since 1995, over 2500 nuclear trafficking cases have been reported around the world. Many of these instances involved hoax/surrogate substances being misrepresented during illicit exchanges, but several involved the diversion of special nuclear materials (SNM) from legitimate control. As in traditional forensics, such interdicted items are subjected to various analyses for comprehensive identification of exploitable signatures. In the Actinide Analytical Chemistry (AAC) group at Los Alamos National Laboratory (LANL), such work focuses on the investigation of pre-detonation samples, in cooperation with federal agencies. Analyses performed for characterization may include: non-destructive measurements (density, microscopy, x-ray diffraction and fluorescence, gamma spectrometry), actinide assay, alpha spectrometry (e.g. particle characterization), and various forms of mass spectrometry. Mass spectrometry techniques are particularly valuable due to the potential to: 1) detect signature elemental impurities; 2) determine isotopic enrichment and anthropogenic inputs; and 3) measure material "age" (time since last processing). These parameters can give a broad picture of a material's composition, intended use, history, and possibly even producers.

Here, we further introduce the concepts of nuclear forensics and present the findings of a recent, international case study interrogating the isotopic and chronometric parameters of the oldest plutonium reference materials available for use in modern U.S. nuclear forensic efforts. Investigated materials include New Brunswick Laboratory Certified Reference Material (CRM)-136, CRM-137, and CRM-138. These substances range widely in isotopic composition (e.g. nominally weapons grade to "high burn" fuel) but are all traceable to the early years of the nuclear era. Results are presented for both single ($^{240}Pu \rightarrow {}^{236}U$) and multi-

($^{241}Pu \rightarrow {}^{241}Am \rightarrow {}^{237}Np$) generational isotope decay relationships and are cross compared with the evaluation of newer materials (i.e. CRM-126a) to explore considerations of chronometric concordance and the impact of reduced in-growth times. Findings from this effort support the use of high performance thermal ionization mass spectrometry (TIMS), paired with coulometric Pu assay, to age-date even relatively old materials to within a tolerance of approximately ± 1 month (k=2) and with reduced uncertainties for primary chronometers in younger materials.

"Chemical Assays for Recognition of Originator Attributes from Fingerprints"
Erica Brunelle, Crystal Huynh, Anh (Ami) Minh Le, Lenka Halámková, Juliana Agudelo, Leif McGoldrick, Jan Halámek, University at Albany, State University of New York

The analysis of fingerprint samples via pictorial comparisons has been largely accepted by the scientific community as a dependable method of identification. While this method is fairly well established, it is not applicable for all situations. For example, when only partial or smudged fingerprints are collected, a match is unlikely to be found. In cases such as these, the chemical composition of the samples would be of more use than the image of the fingerprint. It has recently been demonstrated using bioaffinity-based enzyme cascades and chemical assays that the amino acid content in fingerprints can be used to differentiate between male and female fingerprints. The research displayed here further investigates the use of straightforward chemical assays instead of the more complex biochemical assays. Chemical tests are fairly well-known, especially in the field of forensic science, where there are field kits that are used for the on-site analysis of drug samples. The most common tests for illicit substances are Marquis, Simon's and Chen's test.

As with any multi-analyte system – enzymatic or chemical – it is possible for multiple amino acids to correspond to the same attribute and can, therefore, compromise the overall results. To eliminate this possibility, it is pertinent that there be systems developed that are restricted to one analyte (amino acid) or a specific combination of analytes that are correlated to the desired originator characteristics. To insure that the methods presented here are practical and can be used on samples left on more than one particular surface, research showing the performance of the system on samples collected from various surfaces is also provided.

The developed chemical assays also have the potential to be coupled with a portable apparatus for use directly onsite where the assay can subsequently be performed and the results interpreted by non-scientific personnel, unlike most currently available techniques. This can be done in a manner that is similar to water test kits and the VOCkit system which is a small strip that has a grid of several dozen indicator chemicals imprinted on it which is used by the Army for the detection of threat agents such as anthrax, sarin and mustard gas.

*** "Investigating the identification of vaginal material using histological, spectroscopic and molecular models"** Justine A. Kawa, Claire L. Glynn, Ph.D., University of New Haven

The goal of this presentation is to inform attendees of the recent advancements in three emerging techniques for the identification of vaginal material in a forensic context.

This presentation will provide the forensic community with insight into multiple techniques; histological, spectroscopic, and molecular, to identify and distinguish vaginal material from other biological fluids. Vaginal material has yet to have its own validated, specific, and reliable test for identification.

Body fluid identification is a crucial component in the forensic investigations of sexual assault. The recovery and identification of vaginal material has been a significant challenge for forensic scientists for decades. Forensic STR DNA profiles can be used to identify the donor, however a crucial facet is to identify the body fluid source of that DNA, thereby elucidating the circumstances of the case. The identification of vaginal material present on penile swabs, hand swabs or foreign objects can be used to confirm or refute a victim's or suspect's statement. Several methods have previously been proposed, however none have garnered widespread acceptance. Traditional methods include histological staining, however face challenges due to similarities in the cellular makeup of vaginal, buccal and skin epithelial cells. In recent years Raman Spectroscopy has gained much attention for its use in the identification of body fluids, however vaginal material has to do date been overlooked. Finally, microbial profiling of vaginal material has most recently been proposed as a potential molecular biomarker, however this work is still very much in its infancy. Further investigation of both traditional and emerging techniques to identify vaginal material is absolutely necessary to address the challenges faced in the industry today.

Following informed consent, this study investigated three techniques; histological, spectroscopic, and molecular methods. Histological methods involved staining skin (n=10), buccal (n=10), and vaginal (n=10) epithelial smears with six histological stains, namely: Hematoxylin and eosin, Crystal Violet, Lugol's Iodine Stain, Csaba's Stain, Dane's Stain and the Ayoub-Shklar Stain. While some of these stains are not considered emerging, the last three have recently been identified as having the potential

to identify vaginal epithelium. The Raman DXR microscope was used to analyze the spectra for vaginal material, buccal cells, skin epithelial cells, venous blood, menstrual blood, saliva, and semen (n=10 for each). Molecular methods involved relatively quantifying the expression of Lactobacillus gasseri and lactobacillus crispatus, two common vaginal bacterial species. Real-time PCR was used with TaqMan Universal PCR MasterMix, and a primer and probe pair was designed for each bacterial species. Cycle threshold values were used to determine expression levels.

In the histology, due to the contrast in morphology, skin cells were easily distinguished from vaginal and buccal cells once stained with any method. However, the Dane's stain was found to be the most superior stain for the differentiation between all three cell types, as each cell type resulted in different colors, reducing subjectivity. Using Raman spectroscopy, averaged spectra for each body fluid were taken and significant peaks were noted and identified. The resulting peaks from the previously researched body fluids such as venous/menstrual blood, semen and saliva, were as expected, while the resulting spectra from the vaginal, buccal and skin epithelial cells showed variation in peak presence and intensity. Preliminary results obtained from the molecular investigation of the vaginal bacteria indicate higher expression levels in vaginal material when compared to other body fluids, however further experimental investigation and analysis of data is required.

This research has highlighted the value of traditional methods and the merit of emerging methods for the identification of vaginal material in forensic investigations. Each method has been shown to have immense potential for use in casework, albeit each with their own shortcomings, thereby reinforcing the need for continued research and validation in this field.

"Crime Lite 82S Green Light Validation" Julianna Moge, Western New England University and Beth Saucier Goodspeed, MA State Police Crime Laboratory

Blood stains mask the results of the Criminalistics unit's Griess test for nitrites, which is a component in gunshot residue. The Crime lite 82s Green Light Validation was validated to enable criminalists to document the measurements of the residues to further determine the distance between the gun and the bullet hole in clothing as well as determine adequate locations to collect gunshot residue during primer residue collection. Different pieces of fabric were used with different variables to determine the effectiveness of the light. This piece of equipment would be used in the laboratory after the clothing is collected rather than at a crime scene. This piece of equipment is an alternative light source that is specific for 480-560 nm wavelengths. I worked with fourteen different samples of clothing, each piece having different variables. The difference between each sample was either the type of fabric or the distance between the end of the firearm and the article of clothing. Six of the samples were made to observe only gunshot residue while eight of the samples were made to observe both gunshot residue and primer residue by having each sample draped over the firearm. Pictures were taken using the equipment with both the lights on and off in the room. Observations were also made from each sample. Both dark and light fabrics showed particles more clearly when in a darkened room when using the camera for viewing as well as viewing with the goggles. The green light revealed more particles on the fabric than the naked eye could detect. The best height the light should be at when observing gunshot residue is about 6 inches. When observing primer residue, the best height for the light to be positioned was about 8 inches. Also, it was more beneficial to change the exposure setting of the camera to a higher exposure level in order to brighten the particles that fluorescent under the green light. The Crime lite 82s Green Light Validation has proven itself successful and allows for the analyst to be able to document the gunshot residue before testing the clothing. The green light provides a way for the technician to find gunshot residue and an appropriate area for primer residues.

*** "The effect of washing and blood enhancement reagents on the use of Raman spectroscopy for human blood identification"** Tyler J. Schlagetter, Claire L. Glynn, Ph.D., University of New Haven

In forensic investigations, determining the identity of an unknown biological stain can aid both in reconstruction and identification of an individual. Human blood is commonly found at crime scenes, which is first presumptively identified at the scene, then confirmed in a laboratory setting. However, many of the tests used, both presumptive and confirmatory, consume the sample in question, preventing further analysis, namely DNA profiling. Raman spectroscopy has been gaining interest as a new method of body fluid identification, partly due to its non-destructive nature. Prior research has demonstrated that Raman spectroscopy provides a unique spectrum for blood, while also preserving the sample for DNA analysis. The aim of this study was to further investigate the use of Raman spectroscopy for human blood identification in simulated crime scene samples to include bloodstains; on a variety of fabrics, at varying dilutions, following washing, and finally post-enhancement.

Following obtaining informed consent, venous blood was collected from volunteers in sterile vacutainer EDTA vials. Using a 780-nm wavelength laser and a controlled laboratory setting, Raman spectroscopy was performed on samples of blood under various conditions. These conditions included five fabrics (black and white cotton, black and white polyester, and denim), a series

of dilutions (1:10 to 1:10^6, both wet and dry), and after washing and treatment with three enhancement reagents (Leuco Crystal Violet (LCV), coomassie blue, and Luminol).
A method of extraction of the stain from the fabrics was also tested. Baseline corrections for fluorescence were performed as necessary.

The results obtained from the bloodstains on a variety of fabrics showed that by using spectral subtraction, a signal similar to blood could only be recovered from the white cotton and white polyester samples. The results obtained from the diluted bloodstains revealed that only the neat blood gave a signal while wet. When dried, the neat blood, as well as the 1:10 and 1:100 dilutions gave a signal with peak shapes similar to the blood reference. However, the peaks became significantly less intense after each successive dilution. The results obtained from the bloodstains that were washed and subsequently enhanced revealed Luminol to have no effect or interference on the ability to obtain a clear blood spectrum following spectral subtraction. However, LCV and Coomassie blue introduced interference giving indeterminate results. The results obtained by utilizing an extraction method of the stain from the fabric revealed a spectrum with similar peak shape and location, but lower intensity, resulting in a weak match to a library reference for blood, regardless of the substrate the stain was extracted from.

This study demonstrated the capabilities of Raman spectroscopy as a means of identifying human blood in a variety of situations common to forensic investigations. The impact of washing and blood enhancement reagents reveals the importance of choice of method and its bearing on subsequent Raman analysis.

"The simultaneous estimation of a blood spot's time since deposition and age of its originator via a bioaffinity assay"
Juliana Agudelo, Lenka Halámková, Erica Brunelle, Roselyn Rodrigues, Crystal Huynh, Jan Halámek, University at Albany, State University of New York

In the field of forensic science, blood is a major contributor of evidence in investigations involving violent crimes. However, blood is usually used only for its genetic content, overlooking its unique composition of proteins and low-molecular-weight compounds that often serve as biomarkers in clinical diagnostics. We have developed a biocatalytic assay for on-site forensic investigations to identify simultaneously the age range of the blood sample originator and the time since deposition (TSD) of the sample. Due to its age-dependent and enzymatic nature, we chose alkaline phosphatase (ALP), a biomarker commonly used in clinical diagnostics for bone growth and disease, for the assay. We describe the assay, with which we determined the age range of the originator using human serum samples. ALP levels corresponding to old and young originators were monitored after deposition for 48 hours in order to mimic a crime scene setting. We found that the stability of ALP in serum allows for the differentiation between old and young originators up to two days after the sample was left under these mimicked crime scene conditions. This system has the potential to become part of a forensic field kit and to be utilized by all personnel in law enforcement.

*** "Using SPME-GC/MS to Detect Volatile Compounds Remaining from the Storage of Dead Mice"** Angelica Wilz, B.S., Thomas Pritchett, M.S., Cedar Crest College

One of the main objectives in forensic science is to analyze evidence so that a linkage can be made. The linkage can be between a number of different elements such as the victim(s), suspect(s) and crime scene(s). These linkages help law enforcement officers reconstruct the events that took place during a crime, which aides their investigation. One possibility is linking a body to a certain area based on the VOCs detected in the surrounding area (soil, air etc.). Body odors are emitted from decaying bodies due to different taphonomic processes and therefore the VOCs released from the body could be different.

In this study, the headspace above decaying mice inside arson cans was analyzed by utilizing three different solid-phase microextraction (SPME) fibers; polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), and polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR). Data will be presented on the analysis and comparison of the VOCs collected using these fibers with a splitless method for Gas Chromatography/Mass Spectrometry (GC/MS). Cleaning and storage conditions in addition to instrument parameters were assessed to determine the optimal procedure. A diluted standard mixture, of similar compounds to those observed in previous literature, was prepared and run using a split method to confirm that the method was working properly.

VOCs associated with decomposition stages of the mice were collected once a week for each can using a different fiber. The most abundant VOCs present within the first week of decomposition consisted of dimethyldisulfide, dimethyl trisulfide, dimethyl tetrasulfide, phenol and indole for all three cans and all fibers. These results support previous studies that indicate that sulfide compounds are among the first to be released during decomposition. By day 13 of decomposition, the PDMS fiber only detected five compounds (abundances of $2x10^5$ or greater). By day 16 of decomposition, the PDMS/DVB fiber detected over ten compounds and the PDMS/DVB/CAR fiber detected over thirteen compounds (both with abundances of $2x10^6$ or greater). In

addition to the compounds listed above, additional compounds included: hydrocarbons, esters, amines, alcohols, ketones and furans. Based on these results the PDMS/DVB/CAR fiber was chosen as the optimal fiber. This fiber was then utilized to determine the VOC signature that remained after the mice were removed from the cans.

Mice were allowed to decay over various time periods and then removed. After removal, the cans were sampled via the SPME fiber to determine how long the VOCs from decay persisted. It was found that as long as decomposition fluids remained in the can, the decomposition volatile organic compounds could be detected.

"Suitability of Condoms as a Substrate for Latent Print Development"
Amanda Minnock-White, Jessica Tarry, New York City Police Department Police Laboratory

Condoms can have significant value to forensic investigations and are commonly submitted for DNA analysis. A used condom has the potential to place both a suspect and the victim together if victim DNA is found on the outside of the condom and suspect DNA is found on the inside of the condom. However, DNA databases are currently not as robust as fingerprint databases; therefore fingerprint evidence can potentially provide the identity of a suspect if their DNA is not yet in the database. This study aims to determine if condoms are suitable for latent print development. Both lubricated and non-lubricated condoms were tested with both fresh fingerprints and aged fingerprints using various latent print development methods. These methods included visual and alternate light source examinations, cyanoacrylate fuming, various dye stains (MBD, Ardrox and RAM), Gentian Violet and Sudan Black. Areas of ridge detail were observed with all methods with the exception of Non-lubricated condoms with alternate light source examination. Based upon results it is recommend to use cyanoacrylate followed by a dye stain. The study concludes that fingerprints can be developed on condoms under these ideal conditions, indicating that it may be beneficial to analyze condoms submitted as evidence for the presence of latent prints.

*** "Quantifying retention time and development effects of fluorescent fingerprint powder on the green bottle fly, *Lucilia sericata* (Meigen)"** Veena Mehta, Jennifer Rosati, John Jay College of Criminal Justice, CUNY

Understanding blow fly behavior (Family: Calliphoridae) has become an increasingly important area of research, particularly due to the forensic implications. In forensic entomology, the developmental stages of blow fly larvae can be analyzed to estimate the minimum time of colonization. Many factors, such as competition, priority effects, and inhibition, play a significant role in larval behavior and development. However, due to the inability to distinguish blow fly larvae from each other, these factors remain largely unknown. Fluorescent fingerprint powders can be utilized to mark individuals, groups of larvae, or different species, which can then be incorporated into ecological studies. This experiment investigated the retention time of four fluorescent fingerprint powders (Evident™ Red, Green, Orange, and Yellow) as biomarkers using *Lucilia sericata* larvae. Eggs were collected from colony cages, and upon hatching, larvae were placed on 35 g of pork liver blended with 0.40 g of fluorescent powder. In order to observe retention time, each treatment condition (red, green, orange, or yellow dye) consisted of 4 experimental groups. Larvae were exposed to the labeled liver for 48, 72, 96, or 120 hours, after which they were placed on unlabeled pork liver. Control larvae were placed on pork liver without any powder. Three larvae from each experimental and control group were randomly chosen and observed for fluorescence every 24 hours throughout the experiment and for development every 12 hours until pupation. After the minimum time of pupation was recorded, development was observed every 24 hours. Fluorescence was recorded by photographing larvae under an alternative light source (ALS) at 450nm. Images were analyzed using ImageJ in order to quantify the fluorescence. Statistical analysis was performed in order to see if the treatment conditions significantly impacted the larval development as compared to the control group. The results from this study will be used to improve on a methodology to easily differentiate dipteran larvae. Tracking immature blow flies using fluorescence can be used to understand the many intraspecific and interspecific interactions that occur during larval development. This will ultimately further our understandings of coexistence mechanisms in carrion insects and improve our reliability of using blow flies in PMI estimations.

DRUG CHEMISTRY SESSION ABSTRACTS
Thursday, October 13, 2016

MODERATOR: SABRA BOTCH-JONES

"Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Update" Tiffany Ribadeneyra, M.S., F-ABC, Nassau County Office of the Medical Examiner/Division of Forensic Services, Sandra E. Rodriguez-Cruz, Ph.D., DEA Southwest Laboratory

The Scientific Working Group for the Analysis of Seized Drugs (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). Historically, SWGDRUG recommended minimum standards for the forensic examination of seized drugs and to seek their international acceptance. There have been numerous inquiries as to how the formation of the Organization of Scientific Area Committees (OSAC) will affect SWGDRUG's future. SWGDRUG will continue to work as part of the international community to improve the quality of the forensic examination of seized drugs. In addition, the resources provided on the SWGDRUG website will continue to be updated and available.

This presentation will provide attendees with an update on SWGDRUG activities during the year 2015 and currently in 2016. Recent activities include public comment adjudications for Supplemental Document 6 (SD-6) addressing measurement uncertainty for cases of weight or count extrapolations as well as a National Institute for Standards and Technology review of the SWGDRUG mass spectral library. Current issues being addressed are validation of qualitative methods by way of revising the SWGDRUG Recommendations Ver. 7.1 Parts IIIB and IVB. The SWGDRUG mass spectral library remains an extensively utilized resource within the forensic community and current status as well as future plans will be reviewed.

"OSAC Seized Drug Committee Update" Thomas A. Brettell, PhD, D-ABC, Cedar Crest College

An update will be given on the activities of the Organization of Scientific Area Committees (OSAC) Seized Drug Committee including the status of standards and guidelines under consideration for inclusion into the National Institute for Standards and Technology Registry of Forensic Science Standards. Attendees will have an opportunity to ask questions.

"Direct Analysis in Real Time-High Resolution Mass Spectrometry as a Tool for Structural Determination of Psychotropic Unknowns" Rabi Ann Musah, PhD, Kristen Fowble, B.S., and Jason Shepard, PhD, State University of New York at Albany

Recent years have witnessed a dramatic increase in the abuse of synthetic cathinones. Novel structural variants continue to appear, making it difficult for law enforcement agencies to rapidly determine the structures of these unknowns. Challenges include the acquisition of large enough quantities of the purified materials required to conduct structural characterization studies, and the time consuming nature of these investigations.

Here, in-source collision induced dissociation (CID) accomplished by direct analysis in real time-time of flight-high resolution mass spectrometry (DART-TOF-HRMS) is used to show that cathinones fragment with certain characteristic neutral mass losses. These losses correspond to diagnostic structural features within cathinone subfamilies. Furthermore, we demonstrate that statistical analysis processing of the neutral mass loss data can be used to identify cathinones, distinguish cathinones from non-cathinones, and deduce important structural features of "unknowns" in a manner that facilitates structure determination of novel variants.

"Mass spectrometric signatures for forensic investigations: Direct analysis in real time-high resolution mass spectrometry (DART-HRMS) analysis for plant-based drugs of abuse" Ashton D. Lesiak, PhD, Los Alamos National Laboratory, Rabi Ann Musah, PhD, State University of New York at Albany

Mass spectrometric signatures using direct analysis in real time-high resolution mass spectrometry were used for identification of forensically-relevant plant materials. Law enforcement is increasingly concerned with the abuse of psychoactive plants for a host of reasons, including the lack of standard operating protocols for identification of plant-based drugs of abuse other than marijuana. Many of these botanicals contain Schedule I substances within their complex matrices. However, the plants themselves are unregulated and their purchase remains legal. Identification of plant-based drugs and products made from these psychoactive

botanicals by traditional methods is difficult due to the complex sample preparation needed prior to analysis. The use of an ambient ionization mass spectrometric technique, direct analysis in real time-high resolution mass spectrometry (DART-HRMS) provides an alternative means to conventional methodologies for the analysis of plant materials. Identification of their psychoactive components can be accomplished without the need for extractions, derivatizations or other complex sample preparation steps. Dimethyltryptamine, a Schedule I substance, was confirmed to be present in three plant materials (Mimosa hostilis, Diplopterys cabrerana, and Psychotria viridis) and both Banisteriopsis caapi and Peganum harmala were found to contain harmala alkaloids, including harmine and harmaline. Another hallucinogen, mescaline, was determined to be present in the flesh of the San Pedro cactus, which was legally purchased despite mescaline being a Schedule I substance. Unique chemical signatures for other abused plants, including Datura spp., kava, kanna, kratom, yohimbe and black cohosh also were acquired using DART-HRMS.

In addition to the rapid identification of psychoactive compounds in complex botanical matrices, the application of statistical analysis tools to the DART-HRMS-generated chemical signatures enabled discrimination between various species of mind-altering plants, regardless of whether extraction or other pre-processing steps had been applied to the products. The individual botanical constituents used in a mixture to make six hallucinogenic Ayahuasca beverages were differentiated from one other using statistical analysis tools, and a classification system for identification of a wide variety of plants abused for their psychoactive purposes can be made through the application of statistical analysis methods to DART-HRMS-derived chemical signatures.

"Validation of Mettler Toledo Quantos Dosing System for use in Quantitative Analysis of Seized Drugs" Eric P. Sorrentino, MS, Matthew Dinizio, Suffolk County Crime Laboratory

Quantitative analysis of seized drugs is currently a necessary evil in New York. Ten different classifications of controlled substances require quantitation with each classification having numerous controlled substances in them. This puts a heavy strain on crime laboratories in New York to have validated quantitative methods and instrumentation to use those methods ready to go if a quantitation is necessary. In addition, laboratories also need appropriate standard and control solutions depending on the instrument employed to quantitate with, as well as, the substance being quantitated.

Solutions prepared in most laboratories today are done volumetrically (mg/ml) not gravimetrically (mg/g). In volumetric preparation a powder sample is weighed, placed in a volumetric flask, and brought to a final volume using a solvent. In gravimetric preparation, a powder sample is weighed, placed in a container, and a solvent is used to bring the solution to a final weight. Conversion between mg/ml and mg/g occurs easily by using the density of the solvent used.

In order to decrease laboratory error from sample preparation, time for preparation, and cost of purchasing primary standards while increasing efficiency and case throughput the Quantos Dosing System from Mettler-Toledo was purchased by the Suffolk County Crime Laboratory (SCCL). It is a two-part instrument: the first being a balance (semi-micro or micro) and the second an automated dosing system that rests on top of the balance. Communication between the balance and dosing system occurs through the software. It accurately weighs out powder sample and immediately does the same with a liquid to prepare the solution.

This presentation will show the validation used to bring the Quantos Dosing System online at the SCCL. Multiple factors were assessed in the validation that include but are not limited to: software glitches and bugs, glassware, balance measurement uncertainty, linearity of the calibration line, repeatability, and reproducibility. Conversion from volumetric to gravimetric concentrations will be discussed. Steps for preparing standard and control solutions will be shown. Preparation of case samples (unknown powder samples) will also be done using the dosing system and will be highlighted how that was assessed in the validation.

"Phun with Phenylalanine" Jamie Foss, B.S., Perkin Elmer

The manufacture of methamphetamine has been on the rise in Maine since 2011, doubling every year, with greater than 100 labs and dump sites expected to be investigated this year. Most laboratories encountered are pseudoephedrine reduction laboratories, predominately One-Pot or "shake & bake" methods with a few scattered Red-P labs in between. Back in 2011 law enforcement encountered an interesting and unique methamphetamine synthetic route starting with a phenylalanine precursor. Phenylalanine is one of the essential amino acids. The subject had a prior history involving mushroom grows and LSD. He was homeless during the time of the investigation. The subject had a working knowledge of synthetic organic chemistry, and was receiving shipments of reagents at a Post Office box. This cooking process was unique and modeled after an Uncle Fester method. The investigation started just as the subject was starting the cooking process, so no final product was discovered. The trial spent a full day focused only on the chemistry, consisting of back-and-forth testimony from the State's chemist and a defense expert. The subject was found guilty, but later appealed the case on the basis that no final product was produced. The appeal was upheld and the

conviction overturned. This presentation will cover relevant case information including: the subject's history, the scene of the laboratory, the chemistry of this specific synthetic route, the trial, verdict, and the appeal.

"*LC-MS Method Development for the Identification of Route Specific MDMA Impurities" Rebecca Dunn, BS, Arcadia University, Heather L. Harris, MFS, JD, The Center for Forensic Science Research and Education, Warren Korn, Thomas Jefferson University Hospital, Karen Scott, PhD, Arcadia University

This presentation will demonstrate the simple dry extraction techniques possible with LC-MS instrumentation for impurity profiling of a popular club drug, MDMA. By analyzing the organic by-products, or impurities, in MDMA tablets, it is possible to identify the synthetic route used to prepare the sample. Two of the most common methods of synthesis are the reductive amination of MDP-2-P and the Leuckart reaction. The differentiation between these two synthetic routes can aid investigators in the identification of the manufacturer of the sample and to compare tablets from multiple drug seizures. The aim of this research was to develop a simplified method for the extraction and identification of 11 previously identified route specific MDMA impurities in order to differentiate between these two popular synthesis routes in the presence of MDMA and caffeine.

The LC-MS method developed for the identification of the analytes of interest was able to qualitatively identify all compounds at concentrations above 2µg/mL and identify all amphetamine analytes at concentrations above 1 ng/mL. All analytes were positively ionized and baseline separated.

Three dry extraction methods using methanol, 0.05 N hydrochloric acid in methanol, and 0.1% trifluoroacetic acid in methanol, respectively, were utilized for the extraction of the compounds of interest from simulated tablets. The compounds of interest were first extracted from cornstarch to determine the optimal extraction method. Of the three dry extraction methods, the methanolic HCl had the highest average percent recovery from cornstarch at 57% while the liquid-liquid extraction had 54%. When the average percent recoveries for each impurity extracted from cornstarch using each extraction method were compared, the single liquid extraction method using methanolic HCl was able to extract all of the impurities and be the optimal extraction method for a majority of the impurities. This method was then chosen as the optimal extraction method and was used to extract the impurities from three other tablet matrices: d-lactose, d-sorbitol, and microcrystalline cellulose.

"*Evaluation of a Handheld Raman Spectroscopy Instrument for the Preliminary Identification of Controlled Substances" Kristie McLaughlin, M.S., Bay Path University, Jamie Foss, B.S., Maine Health and Environmental Testing Laboratory, Maria Pease, B.S., Maine Health and Environmental Testing Laboratory

New England continues to be at the forefront of current drug trends from the abuse of prescription drugs to the emerging synthetic designer drugs, including bath salts, phenethylamines, and other synthetic hallucinogenic drugs. There has been a major increase in heroin and fentanyl seizures leading to a large number of fatal overdoses. Most drug cases are sent directly to the laboratory for testing, causing large backlogs for many forensic laboratories. Using the handheld Raman spectrometer, law enforcement officers will be able to scan and discern illicit substances on the street; possibly reducing the amount of drug evidence being sent to laboratories. The focus of this study is to evaluate the suitability and limitations of this analytical technique for the preliminary identification of controlled substances. Suspected illicit substances seized around the State of Maine were tested at the Maine Health and Environmental Testing Laboratory in Augusta, ME. Samples were analyzed neat, with no pretreatment or preparation and the results compared to traditional analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography/Mass Spectrometry (GC/MS).

"*Identification of Fentanyl and Other Synthetic Opiates using Ambient Ionization High Resolution Time-of-Flight Mass Spectrometry" Amanda Moore, B.S., Boston University School of Medicine, Biomedical Forensic Sciences; Jamie Foss, B.S., PerkinElmer; Sabra Botch-Jones, M.S., M.A., D-ABFT-FT, Boston University School of Medicine, Biomedical Forensic Sciences; Frank Kero, PhD, PerkinElmer

Fentanyl analogs and designer opioid drugs have received a large amount of media coverage due to numerous fatal overdoses. These drugs elicit analgesic effects similar to heroin making them desirable drugs to abuse. Fentanyl analogs and designer opioid drugs are expected to be more prominent in forensic casework in the near future. The compounds have been seen in forensic casework either alone or in combination with other drugs of abuse including heroin. It is therefore necessary to have an efficient methodology to identify these compounds. Currently, Gas Chromatography-Mass Spectrometry (GC-MS) is used to identify drugs of abuse and is considered the "gold standard" in seized drug analysis. However, analysis times can often range from 15-60 minutes in length. Another drawback is the need for spectra library matching, obtaining analytical reference materials for identification and the inability to identify new designer drugs before this material is available.

In this study, Direct Sample Analysis Time-of-Flight Mass Spectrometry (DSA-TOFMS) was utilized to provide rapid identification of fentanyl and related synthetic opiates. DSA is a direct ambient ionization source, requiring no chromatography and minimal sample preparation. High resolution time of flight mass spectrometry generates empirical formula information allowing for substance identification without a reference material as well as in-source collision induced dissociation (CID) resulting in additional structural information for confirmation. An overview of the instrumentation and use of DSA-TOFMS to rapidly generate exact mass data as well as fragmentation data from in-source CID for the identification of synthetic opiates will be presented. The analytes explored in this study include: heroin, 6-MAM, morphine, fentanyl, norfentanyl, acetylfentanyl, butyrylfentanyl, beta-hydroxythiofentanyl, furanylfentanyl, valerylfentanyl, AH-7921, U-47700, buprenorphine, norbuprenorphine, desomorphine, MT-45, W-15, and W-18.

"*The Stability of Mephedrone in Non-Alcoholic Beverages" Stephanie Lee Oddi, Karen Scott, PhD, Arcadia University, Glenside, PA

Mephedrone, also known by its street names "meow meow" or "bubbles," is a Schedule I synthetic cathinone that has increased in recreational use in the past ten years, especially in the club scene. When ingested, mephedrone triggers an amphetamine-like stimulation, which can cause euphoria, tachycardia, paranoia, and, in some cases, impaired memory or death. If mephedrone is combined with a depressant like alcohol, the result could be the ideal "date-rape" situation. Various synthetic cathinones are being reported in conjunction with sexual assault and are consequently showing up more frequently in forensic casework. It is valuable to understand the stability of the drugs in beverages to estimate if the drug can still be present in the beverage after it has been seized, and to evaluate the best storage conditions for the samples before and between testing. It is for this purpose that this project focuses on the stability of mephedrone in water and Coca Cola®.

Water and Coca Cola® were spiked with mephedrone at 1 mg/L and stored at room temperature, in the refrigerator, and in the freezer for 30 days. Mephedrone was extracted from the samples on days 0, 3, 7, 14, and 30 to record the amount of degradation over the month at each storage temperature. These extracts were derivatized and then analyzed by gas chromatography/mass spectrometry (GC/MS) in full scan mode.

Results of this research have shown that mephedrone is more stable in water than in Coca Cola®, as mephedrone in Coca-Cola® started to degrade after day 3 at all temperatures. It was also discovered that mephedrone was more stable when stored in the refrigerator and freezer, despite freeze-thaw cycles.

*****Denotes Peter R. De Forest Collegiate Competition Participant

FORENSIC BIOLOGY SESSION ABSTRACTS
Thursday, October 13, 2016

MODERATOR: AMY BRODEUR

"ATR-FTIR Spectroscopy as a Possible Screening Method for DNA Extraction from Burned Skeletal Remains" Allison Murray and Lawrence Quarino, Cedar Crest College

During mass disasters, fires, and airplane crashes forensic scientists may perform DNA analysis on numerous burned bone samples, as bone and teeth are often the only surviving tissue in such cases. This analysis is time consuming and may ultimately produce no workable DNA profile for analysis. Currently there is no universally accepted screening method for burned bone samples which would allow a scientist to select the bone with the best probability of producing a DNA profile. A screening method would be especially helpful in mass disasters when scientists have numerous samples to process and must do so quickly and efficiently.

In this study, ATR-FTIR was used to analyze animal and human bone samples which were burned at various temperatures ranging from 0-1000 °C for one hour and then subsequently pulverized into a bone powder. Peak height ratios and peak area were calculated in order to demonstrate the degradation within the bone. A phenol: chloroform extraction was then performed on these samples to extract DNA. The quantity of DNA recovered from the extraction was assessed by two different methods: (1) animal samples were quantitated using the NanoDrop 1000 UV/VIS Spectrophotometer and (2) human samples were analyzed with real time PCR. STR typing was performed on the human bone samples using the PowerPlex 16 HS System (Promega Coporation, Madison, WI).

The results indicate that ATR-FTIR can be used to determine the degradation of bones prior to a DNA extraction procedure. Spectral changes demonstrate that as bones are burned at higher temperatures, peaks correlating with the organic component of bone disappear. At these higher temperatures the quantity of DNA recovered is significantly lower. A screening method, such as ATR-FTIR, has the potential to be an invaluable tool for DNA analysts to assess the extent of DNA degradation in bones.

"Employing the Promega CTT Kit for Screening Triplex STR Profiles using PCR High Resolution Melt Analysis" Marie Kouspou and Kelly Elkins, Towson University

In this talk we present the use of the Promega CTT kit that probes three short tandem repeat (STR) loci, CSF1P0, TPOX and TH01, for DNA screening using a Polymerase Chain Reaction (PCR) High Resolution Melt (HRM) assay using LCGreen Plus. Employing HRM reduces the evidence processing time and sample cost incurred by performing additional multiplex STR DNA typing when differences between HRM profiles are observed using the three loci. Additionally, HRM can discriminate single nucleotide differences within the short tandem repeats that are not observed using capillary electrophoresis for DNA profile interpretation. A recent study evaluated the HRM method in single and multiplex reactions to differentiate individuals. PCR HRM is used in the six loci IrisPlex eye color single nucleotide polymorphism (SNP) screening assay and has been demonstrated for screening tissues using DNA methylation. The results of donor samples collected in accordance with the TU Institutional Review Board will be compared to the allele ladder supplied with the CTT kit and melt curves observed with purchased DNA standards. The DNA melt temperature varies with the length, sequence and the GC content of the amplicon. The purchased standards exhibit differences in melt peaks for the STR loci and alleles and difference plots.

"An Improved Capillary Electrophoresis System for Human Identification" Danielle Brownell, Promega Corporation

Rapid DNA and NGS hold great promise for the forensics community to extend the reach and depth of DNA typing. While powerful complements to traditional capillary electrophoresis (CE) STR typing, neither approach is likely to replace CE analysis for the majority of forensic samples. As such, improving CE technology will be critical for advancement of forensic DNA typing. The Spectrum CE System will offer increased spectral capacity, which will allow analysis of existing 4-, 5- and 6-color chemistry as well as a new family of 8-color multiplex STR systems. With the additional colors, improved multiplex configurations will provide more complete and consistent results with degraded and inhibited case samples as well as variable "direct amp" samples. The system will also offer increased flexibility and four continuously accessible plate positions. This design improves laboratory efficiency by reducing schedule conflicts, increasing overnight/weekend throughput and reducing the number of instruments

needed in the laboratory. The presentation will include an overview of the Spectrum CE System's features as well as a review of initial data generated with existing STR chemistries.

"Looking Back to Move Forward: Evaluating Historical Data to Guide Future Analysis Policies" Lynn Schneeweis, Massachusetts State Police Crime Laboratory

As the field of forensic biology has evolved over the past 20 years, the demand for DNA analysis in criminal cases has increased substantially. Technology has improved the sensitivity of testing and DNA profiles are routinely developed from items of evidence with minimal biological material present. The advancements in forensic biology have provided the criminal justice field with powerful investigative tools and have proven valuable in thousands of investigations and trials. However, even with the advanced technology at our fingertips today, limits still exist as to what types of evidence can be analyzed with the expectation that a meaningful DNA profile is likely to be developed. Additionally, labs must work within the limits of their resources when considering the number of items submitted in a case and each item's potential for DNA testing. Balance must exist between responsibly managing available resources while still providing high quality forensic services to the criminal justice community.

In 2010, the Massachusetts State Police Crime Lab developed a Forensic Biology Item Analysis Policy in an effort to utilize resources most appropriately as well as provide meaningful forensic analyses. This policy addresses among other things, types of evidence that will be accepted for analysis using current technology and what may be better suited for agencies to retain for potential future technology. It is a living document that is periodically updated based on data generated by the Forensic Biology Section, and distributed to our submitting agencies and District Attorneys' offices to assist them with requesting testing. These data are generated by analysts entering statistics into our LIMS for each case that is processed to address such variables as collection techniques, serology results, extraction methods, and amplification strategies. Additionally, DNA results statistics are collected that monitor the quality and forensic value of any profile that is developed from an item of evidence. The Biology Section reviews these data to ensure the currency of the acceptance policies within the Item Analysis Policy. This presentation will discuss data collected by our Biology Section from several different item types, collection methods, and analysis techniques over the past year and evaluate potential policy changes that could be considered from these data.

"Development and Validation of a Targeted Next Generation Sequencing Solution for Forensic Genomics" Ann Allison, Illumina

Sequencing (NGS) by Synthesis (SBS) enables the entire human genome to be sequenced in one day. As a simpler yet highly effective alternative, forensic scientists can choose to perform targeted sequencing of PCR products. By sequencing a dense set of forensic loci, casework and database efforts are directed toward the genomic regions that best answer forensic questions, relieving privacy concerns and simplifying analysis. Because it does not depend on allele separation by size, the number of targets interrogated is not limited, allowing a more comprehensive result to be generated.

We describe the development and validation of a targeted amplicon panel for forensic genomics that combines a core of global short tandem repeat markers used routinely today, along with additional forensic loci that can provide information when standard markers would fail to sufficiently resolve a case. Maximizing the number and types of markers that are analyzed for each sample provides more comprehensive and discriminating information for standard samples, as well as challenging samples that contain low quantities of DNA, degraded and/or inhibited DNA, and complex mixtures. The targeted amplicon panel will enable more complex kinship analysis to be performed, and can also reveal phenotypic and biogeographical ancestry information about a perpetrator to assist with criminal investigations. This capability is expected to dramatically improve the ability to investigate dead end cases, where a suspect reference sample or database hit are not available. We will describe the complete workflow, system, and data analysis tools, and present data from validation and collaborator studies including reproducibility, sensitivity, actual forensic samples, and concordance with standard capillary electrophoresis methods.

"Haplotype Data for 27 Y-STR Loci from Four Subpopulation Groups of the Garifuna Population" Nicole Bracci and Reena Roy, The Pennsylvania State University

The Garifuna are a unique population group who are descendants of West African, Central African, Island Carib, and Arawak people. Most of the populations in this cultural group currently live in Central America, primarily along the Caribbean Coast. Short tandem repeat (STR) Y chromosome DNA profiles are routinely used in forensic cases. The Y chromosome is passed through the paternal lineage, thus it is present only in males. While Y-STRs cannot be used for individualizing a single human, Y-STR profiles are useful in familial searches, in paternity cases, in the investigation of missing persons, in sexual assault cases, particularly when there are multiple donors; in mass disasters, and in other types of forensic cases including homicides.

The purpose of this study was to type 27 Y-STR alleles from the samples collected from four Garifuna subpopulations. One of the objectives was to determine if a small population, like the Garifuna, can be evaluated and perhaps differentiated using standard forensic genetic techniques. Another goal was to determine if the bloodstains collected on various types of paper substrates, some of which contained no preservatives, would yield complete Y-STR profiles when the samples were amplified directly with the Yfiler® Plus Amplification Kit from Applied Biosystems.

Blood samples were collected from four distinct Garifuna villages. The subpopulations in this study were from the following groups; Cristales, Rio Negro, and Santa Fe along the Honduran coast, and Punta Gorda on Roatan. Seventy-one donors were enrolled in this study and blood was collected from them on various types of papers. As per the Office of Research Protection (ORP) guidelines, all donors and samples were anonymized. Each bloodstain was labeled with its own unique identifier code.

The Yfiler® Plus Amplification Kit uses a six dye fluorescent system that allows detection of 27 Y-STR loci in one single polymerase chain reaction (PCR) amplification. Six of the 27 loci are known to mutate rapidly. Of these six loci, one is a multiple loci target. In addition, there are three highly diverse single allele loci. These rapidly mutating loci are a powerful tool for discrimination between individuals who are paternally related. The five dyes used in this system are 6-FAM™, VIC®, NED™, TAZ, and SID. The sixth dye is the internal lane standard dye, LIZ®.

The recommended protocol was used for amplification except where noted. A 1.2 mm punch was taken from each bloodstain and placed in a sterile tube. Prep-n-Go™ buffer (2 µL) and low TE buffer (8 µL) were added to each punch. A half reaction volume of 12.5 µL, which consisted of Yfiler® Plus Master Mix and Yfiler® Plus Primer Set, was then added to the punched sample. The amplification was performed using a Veriti® 96-Well Thermal Cycler from Applied Biosystems. DNA fragment analysis was performed on a 3130xl Genetic Analyzer from Applied Biosystems. Profiles generated were analyzed using the GeneMarker® HID Software, version 2.7.1 from SoftGenetics®.

Statistical analysis on the data indicated that certain alleles were represented more often (at a higher rate) when compared to the standard allele and haplotype frequencies obtained through the Y-STR **Haplotype Reference Database.**

The results also indicated that it was possible to amplify the bloodstains deposited on various types of papers, some of which contained no preservatives. Only one bloodstain out of the 71 samples failed to yield a complete profile. Complete profiles were generated when half of the recommended reagents from Yfiler® Plus Amplification Kit were used for direct amplification. Pretreatment with Prep-n-Go™ buffer was necessary to generate complete profiles.

"Making the Most of your Forensic Mixture in CODIS" Cristina Somolinos, NJSP OFS DNA Laboratory

Careful analysis of peak heights in two-person DNA mixtures can help interpret non-victim profiles, and improve the investigative leads generated by a database search. This presentation will introduce a simple tool to facilitate the entry of a robust profile into CODIS.

Three programs will interface in the analytical process.

In **GeneMapper ID-X,** the analyst processes data from electrophoresis, removing artifacts and running the profile comparison tool. A text table of genotype results can be prepared for export.

The NJSP OFS DNA Laboratory uses a **Microsoft Excel** workbook to harvest data from the export table to prepare reports and statistics. One worksheet of this workbook, developed for assessing two-person mixtures, can subtract a known component by comparison of allele calls and peak heights in the mixture. This tool determines obligate allele(s) for the foreign contributor, flagging the rarer of required alleles for forensic mixture entry in CODIS.

A DNA analyst may then refer to the Excel tool when entering a Forensic Mixture profile using STR/Y-STR profile entry in **CODIS Analyst Workbench**. The judicious application of a plus sign ("+") to flag a required allele from a perpetrator will markedly improve the calculated moderate match estimate (MME) of a mixed profile, in some cases qualifying it for NDIS upload. Another significant benefit of using required alleles is the filtering/reduction of "false matches" during moderate stringency searches at the SDIS level.

A review of allele patterns in mixtures, modeled with EPGMaker, will be used to illustrate underlying assumptions about peak height balance, mixture ratios, number of contributors, degradation/dropout, masking, analytical vs. stochastic thresholds, and

stutter. Flags are built into the Excel tool to help the analyst make an informed call based on these factors, and see the overall behavior of a mixture, which will be more necessary when CODIS requires expanded core loci.

"Furensics: Animal DNA in Criminal Investigations" Christina Lindquist, UC Davis Veterinary Genetics Laboratory Forensic Unit

While committing a sexual assault in a residential backyard, Rusfus Sito Nanez III rolled in some canine feces which later helped link him back to the victim's home resulting in his conviction. The Veterinary Genetics Laboratory Forensic Unit at the UC Davis School of Veterinary Medicine played a key role in the trial and conviction of this serial rapist. As the only crime laboratory in the country accredited for analysis of DNA from domestic animals, VGL-Forensics has been serving federal, state, and local law enforcement agencies as well as the general public for over a decade. The laboratory receives a wide variety of cases from all over the world, with sample types and species unlike those encountered by its human counterparts. Cases range from human-on-human crimes where dog or cat biological evidence links a suspect to the crime, to large-scale dog fighting, species identification, and animal cruelty cases. Recently, the laboratory worked with investigators in the United Kingdom on the first use of cat DNA in murder case in that country. Case examples from the north-eastern region of the United States, as well as other high-profile cases and cold cases will be presented.

"Temporal Effects in the Interpretation of Two-Component Low Copy Number DNA Mixture", Konrad Wojtas and Lawrence Quarino, Cedar Crest College

The purpose of this study is to determine if the time frame associated with the time of deposition of components of a two-component DNA mixture at a low copy number affects mixture interpretation.

Male and female leukocytes were used as the source of DNA in the study. The leukocytes were aliquoted into 1 mL vials and frozen at -80 °C until needed. Since this study was focusing specifically on the changes brought about by time, other variables such as light, humidity, and temperature were kept constant. Male and female mixtures were deposited on a glass substrate at a 50:50 ratio at a mixture concentration of 50 pg/μL. Samples were then stored at different intervals of 1, 2, 3 days and 1, 2, 3 weeks. Two sets of studies were designed: "lag between" and "lag after". The "lag after" study included the male and female components immediately placed on top of one another and tested at specified intervals. In the "lag between" study, one component was placed on the other component at longer and varied durations prior to analysis. DNA was extracted using a silica-based solid phase extraction and then quantitated using a SYBR® Green based assay. Gene amplification and genotyping were conducted using the Promega PowerPlex 16 HS kit.

Results showed no profile anomaly on the 1, 2, 3 day and 1 week delay for both the "lag between" and "lag after" experiments. Samples with a 2 week delay had allelic dropout and mixture ratios which deviated from the 50:50 ratio. The 3 week delay interval showed similar effects to the 2 week interval delays but deviations were exacerbated. Further studies are being done with 70:30 and 30:70 ratios for the "lag between" and "lag after" experiments. These studies will also have extended lag periods of up to 3 months.

"Validation Study Using Philisa® Thermal Cycler and Chemically Fixed Tissues" Jessica Bouchet, Megan Gillespie and Reena Roy, The Pennsylvania State University

Forensic DNA analysis often involves body fluids which are minute in quantity and must be amplified with polymerase chain reaction (PCR) DNA technology. This technology provides an analyst with short tandem repeat (STR) DNA profiles from the biological evidence left at crime scenes. The thermal cycling time can take up to three or more hours depending upon the amplification kits and the thermal cycler used.

The goal of this research project was to amplify DNA from chemically fixed tissues using the Philisa® Streck Thermal Cycler, which reduces the cycling time of the PCR process to only a few minutes.

Tissue samples from kidney, colon, liver, muscle, and small bowel were obtained from three deceased individuals (one male and two females). A piece of gall bladder was also collected from one of the three donors. Blood samples were obtained from these individuals also. Each donor and their samples were anonymized following The Pennsylvania State University's research guidelines. A piece of each tissue was designated as pristine and kept frozen until extraction. A minute amount of each tissue was

then fixed in 100% alcohol for varying lengths of time. All of the fixed tissue pieces were extracted at the end of each fixation period.

DNA from the tissue samples, fixed or pristine as well as the DNA from the blood from all three donors was extracted, and quantified. After quantification of the extracted DNA, the recommended amount of DNA was used for the amplification process. The PowerPlex® Fusion and the PowerPlex® 16 HS Systems were used for amplification of DNA from blood, and from pristine and fixed tissues. The PowerPlex® Fusion System targets 24 loci whereas the PowerPlex® 16 HS System amplifies 16 loci. The thermal cycling process took place in a Philisa® Streck Thermal Cycler. Thermal cycling using both amplification kits was completed in less than 20 minutes.

The generated DNA fragments were analyzed using the 3130xL Genetic Analyzer. GeneMarker HID 2.7.1 software from SoftGenetics was used to analyze the data. The profiles generated from the fixed tissues were compared to the known profiles of the corresponding donors' blood and also with the profiles obtained from the tissues not subjected to chemical fixative.

The amplification of the DNA extracted from the alcohol fixed tissues using PowerPlex® Fusion and the PowerPlex® 16HS Systems in the Philisa® Streck Thermal Cycler was successful. The previously published data using DNA from the same chemically fixed tissues and amplified in GeneAmp® PCR 9700 thermal cycler was concordant with the data generated using the Philisa® Streck Thermal Cycler.

In conclusion, the Philisa® Streck Thermal Cycler can be used to generate STR DNA profiles while reducing the amplification time to only 17 minutes. With industry leading ramp rates, thermal control and thin-walled plastic tubes this instrument can ultimately improve efficiency in the lab without compromising the accuracy of the amplification process.

MODERATOR: ANDREA BELEC LAJOY

"Simultaneous analysis of GHB and GHB-Glucuronide in urine using hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS)" Jozlyn Gibbs, Marianne Staretz, Ph.D., Thomas Brettell, Ph.D., D-ABC, Cedar Crest College

A new separation method has been developed to simultaneously detect GHB and its glucuronide metabolite in urine using hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS).

GHB is a Schedule I controlled substance that is highly addictive with low medicinal properties and has been abused in health clubs, raves, and in DFSA cases. GHB is an endogenous compound and rapidly eliminated from the human body after its absorption making it difficult to detect. GHB-glucuronide (GHB-Gluc) is a recently discovered metabolite of GHB whose role in the metabolism of GHB still requires investigation and is not well understood. There is currently no method to detect GHB and its metabolite, GHB-Gluc, simultaneously in biological fluids. Difficulty in the analysis of GHB and its glucuronide metabolite can arise due to the polarity of the compounds. Because they are small and polar molecules, hydrophilic interaction liquid chromatography (HILIC) can be utilized to achieve optimum separation.

A NUCLEODUR HILIC column (100 x 2 mm, 3 um) connected to a tandem mass spectrometer with an electrospray ionization (ESI) source operated in the negative-ion mode was used for all analyses. Mass spectrometric analysis was performed in the multiple reactions monitoring (MRM) mode. MRM transitions monitored for GHB included m/z of 103 to 85, 103 to 101 and 103 to 59 for quantitation. The MRM transitions monitored for quantitation of GHB-Gluc were m/z of 279 to 103, 279 to 113, and 279 to 59. Chromatography was performed at 50°C using a binary flow method with mobile phases of 0.1% (v/v) formic acid in water (pH=7) as the strong phase and 0.1% (v/v) formic acid in acetonitrile for the weak phase. The weak phase was held at 90% for 2 minutes, then decreased to 60% for 5 minutes and held for 3 minutes. The weak phase was increased back to 90% for 5 minutes to allow the column to re-equilibrate for the next sample. The total acquisition time was 18 minutes. GHB and GHB-Gluc eluted at approximately 2 and 9 minutes, respectively. The spiked urine samples were diluted 1:4 with deionized water, filtered and then 5 uL was injected into the HILIC column. The method displayed good linearity in the concentration range of 1 μg/mL to 100 μg/mL for GHB and GHB-Gluc.

The method was applied to urine samples. This method has the potential to be used in forensic toxicology laboratories for victims of Drug Facilitated Sexual Assaults (DFSA), driving under the influence (DUI) suspects, and postmortem investigations.

"Rapid Analysis of Ketamine and Xylazine in rat tissue by 2D LC/MS/MS technology" Sabra Botch-Jones MS, MA. D-ABFT (Boston University), Malorie Mella, BA (Boston University, Boston MA; Waters Corporation, Milford MA); Claude Mallet, PhD (Waters Corporation, Milford MA)

Introduction: In the field of veterinary medicine, xylazine is an approved compound by the Food and Drug Administration as an animal tranquillizer and often used in combination with ketamine. Since both drugs exhibit anesthetic properties, their recreational usage has also been reported in drug-facilitated sexual assaults cases. The increase in illicit usage prompted a re-classification of ketamine as a Schedule III drug in the United States Controlled Substance Act. Further, in post-mortem forensic toxicology casework, complex matrices can be difficult to analyze due to time-consuming extraction processes. Thus, in most tissue applications, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are commonly used as de-fatting and cleanup/concentration steps, respectively. With complex matrices, a more robust extraction and clean up methodology is required to reach target limit of detection (LOD) and to maintain instrument performance. The analysis of xylazine and ketamine in biological tissue specimens (brain, heart, lung, liver, kidney, spleen, stomach contents) entails several analytical challenges, predominately during the extraction phase. As with all solids matrices, the sample must undergo a complete disruption of the cell membrane to expose the inner portion of tissue cells. Homogenization is the first step to extract a target analyte into a liquid solution prior to further sample clean up and concentration. Forensic laboratories often employ extensive and time consuming sample preparation protocols to reach sub parts per billion (ppb or ng/mL) levels.

Advances in analytical capabilities with hyphenated instrumentation platforms have increased sensitivity and efficiency to detect trace levels of analytes. Therefore, today's analytical challenge resides with the sample preparation techniques, some of which have not been updated for decades. Traditional solid phase extraction techniques often require a lengthy evaporation step, which will inevitably delay analysis. A micro extraction protocol combined with a multi-dimension chromatography can decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques.

Method: Biological tissue specimens, including brain, heart, lung, liver, kidney, spleen, and stomach contents, were taken from 10 rat specimens, which were dose with xylazine and ketamine before being euthanized. After homogenization, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent. The mixed mode approach yields two eluting fractions, one fraction will comprise of neutral and acidic entities and the other fraction will concentrate the analytes with basic functionalities. When coupled to a 2D LC/MS/MS technology, several extraction steps can be eliminated from the protocol. Large volumes of organic extracts can be injected and pre-concentrated which allowed the elimination of the reconstitution and evaporation to dryness steps from the protocol. In this study, ketamine and xylazine were extracted from rat tissues using three extraction protocols for performance evaluation. The chosen 2D LC/MS/MS method used in this application was identified using a 6x6 automated methods development protocol, which target several key chromatography parameters for a total of 144 LC/MS/MS methods. The method evaluation was completed within 72 hours. The manual extraction of tissue samples were completed in less than 30 minutes. The analysis was performed using100 uL of the final organic solvent (MeOH or ACN) extracts. The limit of detection (LOD) for all drugs was measured at100 ng/mL from a 1 mL sample volume.

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"LC-MS/MS Analysis of Stimulants and Their Metabolites Extracted from Dried Blood Spots" Emily A. Williamson, Thomas Brettell, Ph.D., D-ABC, Cedar Crest College

In this project, a liquid chromatographic electrospray-ionization tandem mass spectrometry (LC-ESI-MS/MS) method was developed in order to analyze seven different stimulant type drugs that have been extracted from dried blood spots (DBS). FTA DMPK-C blood cards were used as the medium to collect and store the spotted blood samples. The extracts from 30 µL of blood deposited on blood cards were analyzed using a Shimadzu LC system connected to an ABI Sciex 3200 QTRAP triple quadrupole mass spectrometer operating in positive-ion mode. Using a Restek Ultra® C_{18} column (5.0 cm x 2.1 mm, 3.0 □m) the liquid chromatographic separation of the compounds was completed and optimized. The HPLC method used a gradient mobile phase system consisting of a 0.1% formic acid weak phase and a 0.1% formic acid in acetonitrile strong phase with a total run time of 6.5 minutes. A retention time versus column temperature optimization study provided the most favorable separation conditions for the compounds at 25°C. Optimum MS conditions (Q1 and Q3 ions, collision energy, declustering potential) were determined for each of the compounds as well as their internal standards. The extraction procedure for the DBS was optimized through testing various extraction solvents, mixing techniques, blood spot sizes, and drying down techniques. The most favorable extraction conditions involved the use of a 1:1 ratio of methanol and acetonitrile as the extraction solvent and allowing the extracts to mix in a shaking well plate. The finalized method will be used to analyze authentic blood samples from toxicology laboratories to test its fit-for-purpose. The developed method has the potential of being used by forensic toxicology laboratories as a road-side method for driving under the influence of drugs (DUID) cases due to its ability to decrease the amount of time between the time of the stop and the time of sample collection.

"What Keeps You up at Night: Targeted vs Untargeted Drug Screening" Mike Timmons, Ph.D., AB Sciex

The modern forensic lab in being asked to search for more than ever. Everyone is being asked to cover more drugs at lower levels in multiple matrices. The ability to screen for as many drug, many that change constantly, is more important to the modern toxicologist than ever. Today we will discuss screening for drug with LCMSMS focusing on two different workflows. We will explore the advantages and differences between targeted screening and untargeted screening by LCMSMS.

"Analysis of Narcotic Analgesics in Postmortem Blood using Biocompatible Solid-Phase Microextraction (BioSPME), GC/MS, and LC-MS/MS" Chandler M. Grant, B.S., Thomas Brettell, Ph.D., D-ABC, Cedar Crest College

Forensic toxicology laboratories are responsible for analyzing postmortem samples, such as bile, vitreous humor, urine, blood, liver, gastric contents, brain, and kidney, for common drugs or poisons. For criminal investigations, analysis of these postmortem samples may be time consuming and could cause backlog. The application of in-vivo solid-phase microextraction (SPME) has grown due to its ability to be directly injected into a biological matrix without the physical removal of sample. Biocompatible SPME (BioSPME) fibers have been developed to absorb any drugs that may be present within the sample, leaving behind any substances that may cause interference, such as macromolecules.[1,2] The aim of this study is to provide a new

procedure for postmortem toxicology testing that is faster than current toxicology testing methods. The use of BioSPME coupled with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) allows for minimal sample collection, preparation, and analysis for illicit substances in a shorter time frame.

In this study, an extraction, screening, and quantitation method has been developed to analyze 6-monoacetylmorphine (6-MAM), codeine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, and oxymorphone in a blood matrix. Two types of BioSPME fibers have been explored when developing this method, C-18 and mixed-mode coated fibers. These fibers are conditioned, directly injected into blood for extraction of possible drugs, desorbed into solution, screened by GC/MS, and finally analyzed and quantified by LC-MS/MS. This method was optimized using bovine blood looking at multiple variables, which included extraction time, desorption time and volume, drying time, and reconstitution volume and solvents. This procedure utilized a screening method, which comprised of an HP 6890 Series GC system using a Rxi-5sil MS (29 m x 250 m, 0.25 mm) column, coupled with an HP 5973 Mass Selective Detector. The quantitation method of this procedure comprised of a Shimadzu® LC system using an Ascentis® Express Biphenyl Column (50 mm x 2.1 mm, 2.7 mm) with the weak mobile phase consisting of 0.1% (v/v) formic acid in water and the strong mobile phase consisting of 0.1% (v/v) formic acid in acetonitrile along with a AB SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer with an electrospray ionization (ESI) source operated in the positive ion mode. After optimization, this method is then being applied to postmortem blood samples provided by the Lehigh County Coroner's Office of Allentown, Pennsylvania.

"Results of the National Safety Council ADID Survey of Drug Testing in DUID and Traffic Fatality Investigations"

Amanda L. D'Orazio, Amanda L.A. Mohr, MS[1]; Karen S. Scott, PhD[2]; Jennifer F. Limoges, MS[3]; Amy Miles, BS[4]; Colleen E. Scarneo, MS[5]; Marilyn A. Huestis, PhD[6]; Laura J. Liddicoat, BS[1]; Sarah Kerrigan, PhD[7]; Barry K. Logan, PhD, F-ABFT[1,8]
[1]Center for Forensic Science Research & Education; [2]Arcadia University; [3]New York State Police, Forensic Investigation Center; [4]Wisconsin State Laboratory of Hygiene; [5]New Hampshire Department of Safety; [6]University of Maryland School of Medicine; [7]Department of Forensic Science - Sam Houston State University; [8]NMS Labs

After attending this presentation, attendees can compare their laboratory practices for toxicological testing in drug-impaired driving and traffic fatality cases and evaluate their cutoff limits for screening and confirmation of commonly encountered drugs to other U.S. and Canadian laboratories.

This presentation will impact the forensic science community by providing results from a survey conducted under the National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID) regarding laboratory practices to update the current guidelines and recommendations for laboratory testing in driving under the influence of drugs (DUID) and traffic fatality investigations to improve standardization.

The purpose of this project is to provide toxicology laboratories with a list of commonly encountered analytes and appropriate screening and confirmation thresholds in DUID cases and motor vehicle fatalities. Standardization of analytical testing addresses concerns highlighted in the 2009 National Academy of Sciences (NAS) Report.[1] Additionally, having a standardized approach will improve the quality of statistics reported for DUID and motor vehicle fatality cases.

Toxicology laboratories were surveyed about their drug testing practices, specifically with respect to the matrices tested, scope of testing, cutoff concentrations for screening and confirmation, and whether they are in compliance with the 2013 guidelines and recommendations.[2] Changes in drug trends and improvement in testing technologies and capabilities of forensic toxicology laboratories were also addressed. The survey was sent via SurveyMonkey™ to individuals who confirmed their participation, and ultimately 70 completed surveys were included in the data analysis.

Of the responding laboratories, 90% test blood samples, 68% urine samples, and 1% oral fluid samples in DUID casework. Screening methods for blood include Enzyme-Linked Immuno-Sorbent Assay (ELISA) (71%), Gas Chromatograph/Mass Spectrometry (GC/MS) (50%), Liquid Chromatography/Mass Spectrometry (LC/MS) (34%), Enzyme Multiplied Immunoassay Technique (EMIT) (11%), and Liquid Chromatography Time-Of-Flight (LC TOF) (9%). Urine screening included ELISA (46%), GC/MS (37%), EMIT (27%), LC/MS (26%), and LC TOF (6%). Confirmatory methods were 87% GC/MS and 73% LC/MS for blood samples, and 77% GC/MS and 46% LC/MS for urine samples. A total of 34% of respondents report unconfirmed screen results, with many commenting that the report states the result is not confirmed. Reasons for reporting unconfirmed results included legal time constraints, confirmatory testing not available, relevance of the drug, or poly-drug case policy.

Compliance with the scope of testing and cutoff limits from the 2013 recommendations showed that 15% of the laboratories met or exceeded the recommendations, while 47% are currently changing the method to meet them. For blood, screening for opiates and confirmation of cannabinoids and opiates were the most frequent categories for which the recommendations were not met.

Updates to the 2013 cut-offs and recommended test menu will be determined at a consensus meeting of the participating laboratories in November, with distribution by NSC ADID in early 2017.

"Detection of Cocaine and Its Major Metabolites in Rodent Bone Following Outdoor Decomposition after Chronic Cocaine Administration using 2D-LC/MS/MS" Malorie Mella B.A., Sabra Botch-Jones, M.S., M.A. (Boston University Biomedical Forensic Science Program; Claude Mallet, Ph.D (Waters Corporation; Tara Moore Ph.D., (Boston University Forensic Anthropology Program)

In the field of forensic toxicology, several challenges exist with quantification analysis of cocaine and metabolites in post mortem samples. Cocaine can prove difficult to detect and quantify in blood, urine, and soft tissues following extensive decomposition. Alternative matrices, such as hair, nails, and bone could prove useful in detecting chronic drug use in post-mortem toxicology cases. Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes, and inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated detection of drugs following decomposition of remains, specifically outdoor decomposition. The objective of this study was to develop a robust extraction and clean up methodology, in which a homogenization step precedes, to efficiently extract drugs from complex matrices, reach a target limit of detection (LOD) and to maintain instrument performance.

Method:
All rat specimens used for this study underwent 10-12 weeks chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before being euthanized. Average daily dosages for each rat fell within a range of 13-19 mg/kg. 14 cocaine positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable pelt and skeletal samples were collected for testing. A second group consisting of 16 cocaine-positive rats was placed outside and above ground in a gated facility for 1 week. A group of 4 cocaine positive rats were removed for testing on the second week, and every week following. All recoverable skeletal samples were collected for testing. Drug free control rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred. In this study, a method analyzing cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester was developed.
After homogenization of whole bones, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent, which yields two eluting fractions—one with neutral and acidic entities, one with basic analytes. The use of a 2D LC/MS/MS technology eliminates the need for a lengthy evaporation step in the extraction method. The chosen 2D LC/MS/MS used in this application was identified using a 6x6 automated method development protocol. The manual extraction of the bone samples was completed in less than an hour. The analysis was performed using 100µL of the final organic solvent (MeOH) extracts.

The limit of quantitation (LOQ) for cocaine and its metabolites was measured at 100ng/g sample material. The response factor of analytes was high enough that the limit of detection (LOD) was estimated at 10ng/g (10ppt).

The micro extraction protocol combined with a multi-dimension chromatography used in this study decreased sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques. The procedure developed in this study can be utilized on bone and completed in less than an hour before injection of 100ul final extract into the 2D-LC/MS/MS system.

"Amitriptyline Bacterial Degradation on Post-Mortem Pericardial Fluid" Bruno A. Rodriguez-Rodriguez, B.S., Reena Roy, Ph.D. The Pennsylvania State University – Forensic Science Program

Under climatological conditions where both decay and putrefaction occur, soft tissue degradation proceeds from within due to the action of enteric microorganisms. Microorganism-body fluid interactions take place under these conditions, stimulating potential chemical modifications that impact toxicological analyses. Several studies support the use of pericardial fluid as an alternate source for the determination of drug concentration in postmortem body fluids. It is expected that non-characterized metabolites will be produced when *Clostridium perfringens* start interacting with available carbon sources such as amitriptyline in postmortem pericardial fluid. Previous studies have shown the degradation of psychoactive compounds by bacteria in blood, but only limited research has been performed with pericardial fluid.

In this research, bacteria were inoculated into amitriptyline spiked media at literature reported overdose concentrations of 500 ng/mL, 100 ng/mL, and 15 ng/mL. The results indicated that the growth curve of *Clostridium perfringens* was slightly affected at 100 ng/mL and 15 ng/mL, while no growth was observed at 500 ng/mL.

The samples were extracted using the solid phase micro extraction (SPME) and solid phase extraction (SPE) techniques. With extraction using SPME, the recovery of amitriptyline was above 80%. The recovery with SPE was consistent with that using the SPME technique.

A GC-MS method for detecting amitriptyline metabolites was developed using a Shimadzu GC-MS QP-2010 with a rxi5-ms column. The method was linear for amitriptyline and doxepin for concentrations of 25 - 500 ng/mL with a coefficient of determination higher than 0.99. For desipramine and nortriptyline, the coefficient of determination was higher than 0.96.

Research, including the qualitative and quantitative degradation of amitriptyline on bacterial media as a way to compare the metabolites produced by CYP450 cytochrome, is continuing and the data will be published in peer-reviewed journal and presented in forensic conferences.

"The Effects of Filtration Sterilization on the Stability of Ketamine, Selected Benzodiazepines and Metabolites in Female Urine" Lin Zhen, Boston University

Benzodiazepines (BZPs) and ketamine (K) are compounds which have been encountered in Drug-Facilitated Sexual Assault (DFSA) cases. Due to the intimate nature of these crimes, evidence collection is often postponed due to delays and/or reluctance in reporting these crimes. Further delays in analysis may be encountered in laboratories with large caseloads and/or backlogs. Drug identification is important to determine whether the victims knowingly or unknowingly took an impairing substance, however the results could be negative due to chemical degradation over a long storage period.

The purpose of this project was to study if degradation could be prevented with a new preservation method at the time of collection. The samples were prepared by the addition of K, selected BZPs and metabolites, subjected to different sample pre-treatment techniques, and were analyzed after storage at room temperature (25°C), in refrigerator (4°C) and freezer (-20°). The samples were pre-treated with preservative (0.5% toluene) and filtration sterilization (sterile filter kit) within two hours after the samples were collected, and a control group with no pre-treatment was also incorporated into the study for comparison.

The changes in concentrations over 50 days (BZP group) and 210 days (K group) were evaluated between different pre-treated methods and different temperature conditions. Sample that were treated with 0.5% toluene showed the most degradation, 44% of oxazepam (least) and 96% of diazepam (most) lost after stored for 10 days, and 80% of dehydronorketamine lost after stored for 150 days regardless the storage conditions. Clonazepam and flunitrazepam concentrations were reduced by 80% of the original concentration when stored at room temperature for 10 days. The majority of benzodiazepines evaluated in this study were stable when stored in freezer. In K group, ketamine and norketamine that were stored at room temperature and refrigerator over 210 days were stable, however degradation was observed after 150 days when the samples were stored in freezer.

There was no statistically different change observed among the samples pre-treated with or without filtration sterilization. Each samples pH was measured and it was determined that those stored at room temperature had an average pH of 8.5, samples stored in refrigerator and freezer had an average pH of 6.7 and 6.5 respectively. This finding revealed that pH could be the major factor affecting compound degradation rather than the bacterial contamination with high pH contributing to degradation, and low pH potentially preventing sample loss.

"Practical Considerations for Optimal Collection of Trace Explosive Residue"

Marcela Najarro, Jessica Staymates, Jeff Lawrence, Ed Sisco, Jennifer Verkouteren, Matt Staymates, and Greg Gillen

Effective field sampling is one of the most critical steps in the trace explosives detection analysis chain. Without the proper collection of the residue, subsequent evidence analysis may result in the absence of an analyte present at the site of a blast. Field training typically lacks in-depth discussion of factors that lead to optimized particle collection during swipe sampling. Research data shows that proper sampling techniques can lead to an increased likelihood of residue collection and detection. Specifically, data shows that there is a minimum threshold in pressure that must be applied on a surface for the optimal transfer of trace explosive residue off of a surface and onto a collection media. In some cases, an increase in pressure well above that threshold correlates with more residue collected, while in others, the collection efficiency saturates. Explosive type, characteristics of the surface being sampled, as well as type of collection media affect the pressure required to remove the trace residue. In addition, some cases benefit from the focused collection of the residue onto a particular area of the wipe, therefore following techniques that facilitate collection onto a target area of the collection media is best. Finally, the direction in which a surface is swiped can improve the likelihood of interacting with trace particles and avoid the re-deposition of trace particulate onto the surface. NIST researchers developed a prototype training module based on laboratory measurements that includes both training material and training aids. The training module focuses on three key concepts that contribute to best sampling protocols – PAD: Pressure, Area of the wipe/coverage Area, and Direction. The goal of the study was to determine whether this new training material and interactive hands-on training could modify and/or standardize sampling techniques. Results quantitatively demonstrate the feasibility of standardizing and/or improving sampling techniques.

"Surface Detection of Explosives using Thermal Desorption DART-MS and Reverse Library Search"

Frederick Li, Joseph Tice, Steve Shrader, Paul Liang, Brian Musselman

Detecting and identifying explosives on individuals, on clothing, on surfaces in houses, cars, or at crime scenes and other related items is of interest for security and forensic applications for determining if contact with explosive materials has occurred. Establishing contact may indicate illegal possession or handling of explosives and therefore it is crucial that the identification of explosives is highly reliable. Currently the most common analytical methods for detecting explosives from surfaces involve swabbing of the surface followed by analysis of the swabs using GC-MS, LC-MS, or IMS. Although GC-MS and LC-MS are extremely sensitive and reliable, they are relatively time and labor intensive, and they are typically not field-usable. Conversely, IMS has proven to be field-usable, however, they are limited to drift time and therefore are not as reliable as mass spectrometry-based methods.

In our laboratory, we are developing an innovative technology that is capable of detecting explosives on surfaces in real time by employing DART and a swab and detect approach. Our detection system employs a Direct Analysis in Real Time (DART) ambient ionization source that is interfaced to a compact single quadrupole mass detector in order to create a rapid and sensitive as well as mobile platform for explosives detection. In addition, a thermal desorber that is able to accommodate a variety of different swab types, including Nomex and Teflon swabs that are typically used for security applications, is employed to permit rapid thermal desorption of explosives from swabs for DART ionization. This platform was used to analyze 10 different explosives and explosive-related materials that were collected from different types of surfaces. Furthermore, we have used in-source fragmentation to generate a nominal mass library of the most characteristic precursor and product ions for 10 explosives using a reverse library search program. An investigation of the utility of this platform and reverse library search program as a means to improve the level of confidence in explosives identification for security and forensic applications will be discussed.

"Examination of hypergolic mixtures involving potassium permanganate and select fuels"

Stephanie R. Harrold and Wayne Moorehead

Bombings due to criminal and terrorist activities are in the news on a weekly or daily basis. The heightened alertness of law enforcement and security personnel does not seem adequate to prevent the destructive actions of terrorists while explosive materials are so easily obtained. Hypergolic mixtures are chemicals useful for retail or commercial purposes, but when mixed create a type of oxidation-reduction reaction that results in spontaneous combustion, without the need for external ignition. Hypergolic mixtures can be created with a number of different oxidant and fuel combinations. Based on a literature search, little research has been published exploring the time to reaction, temperature, and the variety of commercial products involved in initiating these reactions. These mixtures are simple to construct and are made with materials that are readily available for purchase such as pool chlorine, pool shock, water softening chemicals, and fuels such as brake fluid or antifreeze.

Typically a delay in the combustion reaction occurs after the reactants are mixed. This delay can allow a person to leave the area before the reaction occurs. After combustion, the post burn products may include the remnants of the containment vessel and the post burn hypergolic products. Original ingredients may also be present.

This study involves the creation and examination of the hypergolic mixture of potassium permanganate (a strong oxidizer) and selected fuels including polyethylene glycol, ethylene glycol, and glycerin. Potassium permanganate can be found in select water softening chemicals. Polyethylene glycol has several uses in cosmetics, pharmaceuticals, commercial products, and industry. Ethylene glycol can be used as anti-freeze and in the fiber industry in the manufacture of polyester fibers. Glycerin has uses in the food, pharmaceutical, and chemical industries.

Hypergolic mixtures were created and recovered on a small scale (approximately 0.5 g of powdered potassium permanganate and approximately 0.25 mL of a chosen fuel) in open Pyrex® glass petri dishes (100 mm x 15 mm) in a fume hood (with a flow of at least 100 fpm) to explore the reactions and analyze the residues. After brief period, typically less than one minute, a small flame with white smoke occurred that moved across the mixture. After the reaction, the resulting products were examined visually, with the aid of a stereo light microscope, and with a diamond attenuated total reflectance Fourier transform infrared spectrometer (FTIR).

The small scale reactions were varied to determine optimal ratios. The resultant ratio of 2:1 weight/volume (g/ml) of potassium permanganate to liquid fuel consistently produced combustion. Still images and video recordings captured the reactions. The video recordings showed differences in both the speed and duration of combustion.

Preliminarily, the results of hypergolic mixture residues analyzed by FTIR, which has been used as a discriminatory technique for explosive residues on other types of explosives, may not have the discriminating power necessary to identify post combustion products to determine original reactants.

*** "An Expansion of the Griess Test for the Detection of Nitrites in Gun Shot Residue"**

Erin Noval and Jeanne Berl

The current Modified Griess Test detects the nitrites present in GSR by reacting them with sulfanilic acid to form a diazonium ion. This reactive ion then reacts with alpha-naphthol to form a colored azo dye. The current method involves taking the item suspected of having GSR on it and processing it by laying it over a paper substrate which has been pretreated with the reagents and dried. Cheesecloth soaked in dilute acetic acid is then laid over the top of the item and the stack is ironed without steam. If nitrites are present an orange color dye is visible on the paper substrate.

An update to the work presented in 2015 at this conference will be presented. Several studies were conducted to find a new method for performing the Modified Griess Test, including a paper substrate comparison to find the best paper to preserve the results, a sensitivity study to see if the new reagents have a limit of detection comparable to the original. Once new reagents were selected a stability study was carried out to see how long pre-treated paper could be used; as well as a distance study to determine the distance at which nitrite particles could be detected. Bloodied substrates were also tested to see if there was any interference with blood. Finally testing was done to determine the compatibility of the new reagents with the currently used Sodium Rhodizonate Test for lead.

The new method that will be presented tested m-nitroaniline and 2-aminoanthracene in place of alpha-naphthol and sulfanilic acid, respectively. These changes resulted in a pink dye when reacted with nitrites. Our results show that these reagents detected

nitrites with comparable detection limits to the previous version, but the new bright pink color provides greater visual contrast. The studies mentioned above yielded the following results: The paper study found that high gloss copy paper made an appropriate substitute for the traditional photographic paper. The sensitivity study found that the new reagents were as sensitive as the original and the reagent stability study found that the substrate could be pretreated up to three months ahead of its use. We learned from the distance study that both methods are no longer able to detect nitrites at a firing distance of four feet. We saw better contrast with our new reagent in blood, although this has not been tested at a distance. Finally, the Sodium Rhodizonate study found that the new reagents do not interfere with lead detection.

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*** "Inexpensive Microscope and an iPhone Fun and Simple Ways to Determine Relevance of Hair Examination in Forensic Cases"**

Jessica Bouchet, Nicole Bracci and Reena Roy

Evidence such as hairs and fibers are commonly found at crime scenes, however, not every item found is of significant value or relevant to the crime. There are many tests, which can be performed on hairs and fibers, but the most advantageous and least expensive is microscopic examination, particularly for the purpose of exclusion. This undergraduate research project examined hairs from several sources ranging from humans to animals, human hairs from different ethnic groups, and compared them to various hair-like objects. Since hairs are uniquely mammalian, the study included other non-mammalian items, as well as human hairs which had been subjected to chemical treatments. The result of this research indicated that it is possible to distinguish human hairs from animal hairs and from miscellaneous items encountered at crime scenes using inexpensive microscope and an iPhone.

A human hair, however, can never be individualized using physical and microscopic examinations only. Two head hairs from the same individual can appear to be quite different. Hairs are biological evidence and thus variation is natural and common. Alternately, two humans can have very similar hairs and their hairs cannot be distinguished from each other by microscopic examination only.

Thus, when physically and microscopically comparing two hairs from the same or different individuals, reporting of hair analysis should always include a disclaimer statement. This statement ensures that conclusions are not overstated and cannot be used to incriminate an individual without analysis using nucleic acid assay. In most cases DNA analysis using STR profiles (nuclear DNA), and mitochondrial DNA analysis (from mitochondria) should be employed for identification and discrimination purposes. When animal hairs cannot be positively identified as coming from a certain species of animal, RNA analysis should be employed for identification of the species of animal. DNA analysis can also be performed on animal hairs.

Microscopic and macroscopic analysis employed in this research indicated that hair examination with inexpensive tools such as a simple instructional microscope and an iPhone can be useful in forensic crime laboratories. In this modest study it was possible to distinguish human hairs from animals, and determine the racial characteristics of the human hair. When animal hairs were identified it was possible to determine the species of animal. Microscopic examination distinguished relevant evidence from all other extraneous material. This study demonstrates that simple assays using easily available tools ensures the minimization of the number of evidence processed for nucleic acid analysis, thus preventing unnecessary backlog in DNA analysis laboratories and minimizing expensive tests.

"SirchVAC Evidence Vacuum Sweeper Validation"

Julianna Moge and Beth Saucier Goodspeed

The SirchVAC Evidence Sweeper is a piece of equipment used at a scene as a form of collecting evidence as well as in the laboratory. This vacuum is used for surface vacuuming with a hermetically sealed, single-use filter assembly. This eliminates cross contamination among different crime scenes. The entire sealed filter is then packaged and sent to the laboratory for examination. In order to test this piece of equipment, a certain number of fibers were added to different types of clothing to be collected by the

SirchVAC. The other vacuum that was being used by the Criminalistics unit was looking for a vacuum that was more applicable to the items being examined that would not cross contaminate. The experiment that took place was to ensure that fibers could be collected and the vacuum was sufficient in not breaking or becoming contaminated. The SirchVAC could be used both within the laboratory as well as at a crime scene. Nine articles of clothing were used during the validation. A certain number and variety of fibers were deposited on the different articles of clothing. The types of clothing used were non-shedding, shedding, and possible shedding material. The inventory of fibers collected from each sample was documented and the percent recovery was calculated from the inventory. The average percent recovery was 71.2%. Also, one of the shedding samples was used to test the endurance of the filter as well as the power of the vacuum. The shirt was vacuumed for over an hour. Every 20 minutes, more fibers would be planted on the garment and every five minutes the bag within the vacuum was checked to see if there was any contamination. After an hour and ten minutes, the filter did not collapse nor did the vacuum become contaminated. Four carpet mats from a car were also vacuumed and the trace materials were observed. There was also no issue when vacuuming the car mats and the vacuum was proven to be reliable. It was also determined that it is more beneficial to vacuum the samples using the non-venturi-controlled method, having the end of the vacuum completely touch the sample. The SirchVAC has proven itself as reliable and successful in collecting evidence and could be used both in the laboratory and at a crime scene.

"Investigation of Factors Affecting Formation of Three-Dimensional Fabric Imprint Patterns in Automotive Finishes"

Rachel Downey, Zoltan Rado, Ted Schwartz, Wayne Moorehead and Ralph R. Ristenbatt

In vehicle-pedestrian impacts with sufficient force, imprint patterns from clothing fabric may be formed on vehicle bumpers and in automotive finishes. Even at low speeds, hit and run vehicle-pedestrian impacts are far too common.[1-4] In the event of a hit and run, a crime lab's focus is often on the individualization of paint left behind by the vehicle, either on the pedestrian or in the surrounding area.[5-6] Without paint, hair, or tissue evidence transfer, it is often difficult to prove an evidentiary link between the vehicle and the victim. The aim of this study is the elucidation of pattern production mechanics—type of fabric, surface coating, angle of impact, and impact force of the vehicle—and the eventual individualization of imprint patterns to specific fabrics.

This study is currently in its preliminary stages, and thus utilizes a 6-ft. pendulum rather than full-sized vehicles for simulated impacts. Two custom mounts have been designed and produced: one for the pendulum arm to support a dome-shaped weld cap covered in a thin layer of foam and fabric, simulating a human knee; and one in which a section of either a door or fender—cut to a rough 9-in. x 9-in. square—is mounted using c-clamps. To vary the impact force, the pendulum arm is raised or lowered by 5-in. increments.

Preliminary results do show the consistent, repeatable formation of fabric imprint patterns at or exceeding a narrow range of pendulum heights. Surface coating, fabric type, and angle of impact have not been altered to allow for focus solely on impact force. Precise impact force can be calculated through the analysis of high-speed video, which is currently being assembled and optimized and will be utilized in further tests repeating the above procedure. This will allow for the determination of impact force and the study of pattern variability with changing fabric type, surface coating, or angle of impact.

Initial examinations of imprint patterns have been performed with DSLR photography and optical microscopy. In the future, micro-level terrain mapping may be able to provide individualizing characteristics. As little research in this area has been published, continuing aspects of this study will be supported by a survey of industry professionals. This survey is undergoing IRB evaluation prior to distribution.

Once these variables—impact force, fabric type, surface coating, and angle of impact—are fully examined on a small scale, a continuation of this study seeks to analyze a more true to life impact, using manikins, an 80-ft. pendulum, and full-sized vehicles.

For the forensic community, analysis of the variables involved in fabric imprint formation may provide the means to establish an alternate evidentiary link between a vehicle and pedestrian, as well as additional information for the purpose of reconstruction.

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"The use of a fiber optic Micro-spectrometer to measure color in blue glass chips and dyed fibers"

Tiffany J Millett, Thomas A. Kubic and Mircea Comenescu

Micro-spectrophotometry has long been employed as a technique for adding evidential value to the microscopical and forensic examination of micro transfer evidence, such as glass chips and fibers, as discussed here. A number of commercial units have been available over the years to make transmission, reflectance, and fluorescence measurements on these types of evidence, although many of these current and past units are quite costly. In this paper, we describe an Ocean Optics USB-2000+ fiber optic array detector connected to existing laboratory microscopes and a lab top computer. This spectrometer performed more than adequately for the determination of reflectance and transmission visible spectra of glass chips and dyed fibers. Sample preparation of the glass samples to complete these measurements were minimal and nondestructive, keeping with the integrity of the evidence if further testing is necessary.

Traditional forensic visual examination of glass fragments and fibers includes color comparison. Although color is the logical first step towards inclusion or exclusion of a possible contributing source, color in a single fragment can vary with size and thickness, making direct comparison of two similarly colored glass chips largely subjective. Of the forty-six blue glass samples analyzed, differences between spectra were used as a discrimination factor amongst various manufacturers and similarities were observed within the same manufacturer. Throughout the work on this project, surprising facts were found about blue glass that will be discussed. We aim to improve the technical capabilities of the field of forensic science by making the sorting of glass samples within a single color class simpler and instrumentally supported. Future hopes for this project include branching to other classes of evidence and other instrumental techniques to better support our conclusions.

*** "Studies into the Preservation and Storage Conditions of Ignitable Liquid Residue Extracts"**

Clare Fried and Thomas Brettell

Forensic Scientists commonly use passive headspace to sample and concentrate ignitable liquid residues onto activated charcoal strips. Little work has been reported on the preservation and reanalysis of activated charcoal strips that have been previously extracted with carbon disulfide. Presently, there is no accepted standard practice for storing and preserving these extracts. ASTM E2451-13 states "…changes to a preserved sample extract and the length of time it remains viable under storage conditions are unknown". Clear standards and validation of the conditions for preservation of the extracts should be documented and universally accepted within the field.

In this study, GC/MS was used to analyze passive headspace extracts to study the changes of a standard accelerant mixture (SAM) adsorbed onto activated charcoal strips and preserved under different storage conditions. The SAM consisted of a mixture of 1:1:2 ratio of gasoline, kerosene, and diesel fuel. Two different types of chromatographic vials were compared, screw cap vials and snap cap vials. Four different storage conditions for the extracts were studied – room temperature (25°C), refrigeration (4°C), freezer (-20°C), and freeze and thaw cycles. The study examined three different conditions in regard to the preservation of the sample in a chromatographic vial during analysis: (1) no change to the septum after each injection of multiple injections; (2) septum replaced after each injection, and (3) cap removed and carbon disulfide evaporated in order to reconstitute with carbon disulfide once the strip is dried. Area normalization of peak abundances was used to calculate recovery and reproducibility of gas chromatographic patterns. Chromatographic peaks used for the quantitative comparison were verified through mass spectral analysis and comparison to library standard reference spectra.

The results indicate activated carbon strip samples extracted with carbon disulfide may be dried and reconstituted at least two times with no loss of sample integrity or diagnostic chromatographic peaks used for identification. After three evaporation/reconstitution cycles lighter constituents began to evaporate and affect the chromatographic validity of the data. Sample vial, storage temperature and conditions are crucial aspects of fire debris sample preservation. Further studies should be done to investigate long-term storage conditions.

*** "Using Solid Phase Extraction to Reduce Interference in GC/MS Analysis of Fire Debris Samples"**

Julia Maier and Thomas Pritchett

The objective of this research is using solid phase extraction or SPE along with gas chromatography/mass spectrometry (GC/MS) increasing the chances of correctly determining the presence or absence of petroleum based accelerants even in the presence of interferences.

Interferences can be broken down into three types: substrate products, pyrolysis products and combustion products[1]. Their presence can hinder the interpretation of arson samples and make it more challenging to either determine whether an accelerant is preset or correctly identify which accelerant was used. To reduce the interference and make it easier for identification, a simple method of extraction with SPE cartridges can be used to purify the samples. Silica and amino propyl cartridges were looked for the following reason: they remove any interfering compounds that hydrogen bond while not having any significant bond interactions. Accelerants containing hydrocarbons were used in the study.

There are two phases to the research. First phase is to observe how solid phase extraction effects the relative compound distribution in neat accelerants. Passive headspace was used with activated charcoal strips to adsorb the compounds from the samples injected onto KimWipes placed in arson analysis cans [2]. About 1 mL of carbon disulfide (CS_2) is used to extract the compounds from the strips in amber vials. Three activated charcoal strips are used per one accelerant. The extract from one strip goes through a SiO_2 cartridge, another through a NH_2 cartridge and the third extract is untreated. All three extracts are then analyzed by GC/MS.

The chromatograms from the neat, silica cartridge and amino propyl cartridge are compared for each accelerant by isolating six most prominent peaks which were identified using the reference library from the GC/MS and verifying from the National Center for Forensic Science (NCFS)[3]. Each accelerant ran in triplicates and the areas were normalized. The mean and standard deviation were then determined and the difference in the means for each peak in both the SiO_2 and NH_2 extracts were computed against the means for the same peaks in the untreated sample. These differences were then tested to see if they were statistically different at the 95% confidence level. None of the mean normalized areas were found to be statistically different from the mean normalized areas in the corresponding peaks in the untreated extracts.

Phase two of research is to focus on testing the effectiveness against interferences. Interfering substances will be heated until smoldering in the arson cans. At this point, the cans will be spiked with the reference accelerant mix and sealed with the carbon strips. After the cans have been heated overnight in the oven, the three strips will be removed and extracted. Again, one extract will be purified with a SiO2 cartridge, another with the NH_2 cartridge and the last will be untreated. All three will also be analyzed by GC/MS. In this case all the areas of interfering peaks will be normalized against the largest peak from the reference accelerant and then the percent removal of each will be computed relative to the untreated extract.

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"An Update on the Activities of the OSAC Chemistry and Instrumental Analysis Specialty Area Committee"

Vincent Desiderio and Adam Hall

The various subcommittees under the OSAC Chemistry and Instrumental Analysis Specialty Area Committee have had a busy year of evaluating standards in an effort to move them to the OSAC Registry, guiding standards through the standard development process, and developing new standards to address areas of need. In addition to the specific work with respect to standard development, there has been great effort put forth to identify research needs that are required to support the OSAC mission as well as the needs of the forensic science community at large.

This presentation will provide a brief introduction to the OSAC mission and structure, discuss the general accomplishments of the Chemistry and Instrumental Analysis Specialty Area Committee, and provide an in-depth view of the activities of the Fire Debris and Explosives Subcommittee. To this latter point, an emphasis will be placed on the activities of the Fire Debris and Explosives Research and Training Task Group which has been working diligently to evaluate gaps and identify specific needs with

respect to fire debris and explosives research. The audience will be guided through the thought process and steps taken to address new research needs to include the topic, description, background literature search and finally to address the current body (or lack there of) knowledge within this topic area. Research needs defined by each of the subcommittees within OSAC will likely contribute to the defined research needs of the Forensic Science community in an effort to strengthen Forensic Science within the United States. Time will be allowed for attendee participation through a guided discussion and Q/A session at the end of the presentation.

EDUCATOR'S FORUM ABSTRACTS
Wednesday, October 12, 2016

MODERATOR: JOHN DRAWEC

"Challenges in creating laboratory Forensic Science Textbooks for the high school and Undergraduate level"

Dr. John Allison

While laboratory manuals for General Chemistry or Organic Chemistry courses abound, there are much fewer for Forensic Chemistry. Often, those available are very general, in part because the chemical and instrumental pool varies so much between schools. While you may have an excellent LC/MS experiment in a lab manual, only a few percent of the targeted schools will actually do that experiment.

In an introductory Forensic Science course, one must learn the vocabulary and begin to understand some of the major topics (fingerprints, blood analysis, etc.), but what talents do we really want students at this level to develop? I propose a few here:

1. Allowing oneself to imagine scenarios, guided by facts, information and stories of past crimes.
2. Understanding that information is power, and (almost) all information is available thanks to the Internet.
3. Understanding that one can do many experiments without spending a lot of money.
4. Developing an ability to define how to prepare to consider a cold case ("going back in time").
5. Developing an ability to attempt back-of-the-envelope calculations that could answer quantitative questions.
6. Understanding when to be complete, overlooking nothing, and when one need only consider a single targeted question.

We have been doing research on parts of the Lindbergh baby kidnapping (cold) case, and are considering writing a laboratory book around some parts of it that every school could use, while learning some important American/International history. We would like to share some teaching/learning ideas with the Educator's forum group, as well as ask what capabilities one would most like to develop in students who are interested in pursuing a career in the Forensic Sciences.

"The Council of Forensic Science Educators: Strengthening the Forensic Sciences Through Education, Research and Collaboration"

Amy Brodeur

This presentation will discuss the role that the Council of Forensic Science Educators (COFSE) is playing in strengthening the forensic science disciplines through education. COFSE is a national professional organization dedicated to promoting high academic standards and excellence in programming to educate students seeking careers in the forensic sciences. Over the past few years, COFSE has taken on a much more active role in recruiting new members, soliciting forensic science education presentations and workshops, advocating for more federal funding dedicated to forensic science research, and encouraging collaboration between forensic educators at all academic levels. In addition to highlighting COFSE's recent achievements, the presenter will ask the audience to share new ideas and propose future goals for the organization.

EVENING SESSION
Thursday, October 13, 2016

MODERATOR: BETH SAUCIER GOODSPEED
"The Eastbound Strangler"

AUTHOR BIO – M. William Phelps

Crime and serial killer expert, creator/producer/writer and former host of DARK MINDS, acclaimed, award-winning investigative journalist M. William Phelps is the *New York Times* best-selling author of 32 books and winner of the 2013 Excellence in (Investigative) Journalism Award and the 2008 New England Book Festival Award. A highly sought-after pundit, Phelps has made over 100 TV media-related television appearances: *Early Show, The Today Show, The View, Fox & Friends,* truTV, Discovery Channel, Fox News Channel, *Good Morning America,* TLC, BIO, History, Oxygen, OWN, and many others. He's appeared on USA Radio Network, Catholic Radio, Mancow, Wall Street Journal Radio, Zac Daniel, Ave Maria Radio, Catholic Channel, EWTN Radio, ABC News Radio, and many more. He is one of the recurring experts frequently appearing on two long-running series *Deadly Women* and *Snapped. Radio America* calls Phelps "the nation's leading authority on the mind of the … murderer," and *TV Rage* says, "M. William Phelps dares to tread where few others will: into the mind of a killer." A respected journalist, Phelps has written for numerous publications—including the *Providence Journal, Connecticut Magazine* and *Hartford Courant*—and consulted on the first season of the hit Showtime cable television series *Dexter.* Phelps grew up in East Hartford, Connecticut, and now lives in a reclusive Connecticut farming community just north of Hartford.

Beyond crime, Phelps has also written several history books, including the acclaimed, *New York Times* bestseller NATHAN HALE, THE DEVIL'S ROOMING HOUSE, THE DEVIL'S RIGHT HAND, MURDER, NEW ENGLAND, and more.

Phelps has close to 2 million copies of his books in print, has sold well over 1.2 millions copies of his print books (as well as 350,000+ eBooks), and has been a constant presence on cable TV with his own series and providing guest commentary as a leading expert in his field on several additional series. Phelps dominates the nonfiction crime space. Beyond being a *New York Times* best-selling author he created, produced, wrote, and starred in "Dark Minds" on Investigation Discovery (22 episodes). He has sold numerous nonfiction TV ideas, a few of which are currently in development with different production companies, and two of his books (NATHAN HALE and THE DEVIL'S ROOMING HOUSE are under contract for film with A7SLE FILMS, Peter Facinelli). Phelps has built his true crime dominance with over 300 appearances in print and radio, nationwide book tours, magazine and television profiles, his immense backlist of books (all of which are *still* in print!), a "Dark Minds" Nielsen average of .810/.480 viewers/episode/demo, along with his legions of dedicated readers. He has a combined fellowship of social media followers close to 30K.

Website: www.mwilliamphelps.com
Twitter: @MWilliamPhelps
Facebook: https://www.facebook.com/mwilliamphelps
"Dark Minds" Facebook: https://www.facebook.com/pages/Dark-Minds/192493800815468

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GENERAL SESSION
Friday, October 14, 2016

MODERATOR: BETH SAUCIER GOODSPEED

"PTSD in Forensic Science"

RAYMOND J. DAVIS

Raymond is a forensic scientist with 32 years experience in the field of criminalistics, toxicology and crime scene investigation. He has testified in excess of 1600 criminal, civil and court martial cases from Fairbanks, AK to Dallas, TX. He has a bachelor's degree in chemistry and began his career with the California State Department of Justice Crime Laboratory system in 1972.

Raymond spent 13 years in private practice in Seattle, WA before returning to California to work for the San Mateo County Sheriff's Forensic Laboratory.

Later, he accepted a position as supervising criminalist with the Santa Clara County District Attorney's Office Crime Laboratory. He retired in 2004 from the Department of Justice DNA laboratory in Richmond, CA. He was responsible for training the technical staff in general criminalistics and courtroom testimony and participating in the hiring of new criminalists.

He has published and presented over sixty technical articles in forensic journals and at scientific symposia. He has coauthored two books including the just published, "Crime reconstruction", by Chisum & Turvey.

Raymond has also published two novels based on his experience, 'Dark side of justice' and 'Parabellum.'

Raymond has drawn upon his extensive courtroom experience to train forensic scientists, CSI personnel, nurses and police officers the skills to survive and thrive in the courtroom. His company, *Courtskills* has trained over 6000 law enforcement professionals across the United States.

Raymond is the past president and former editorial secretary of the California Association of Criminalists. He is also a life member of the CAC.

PLENARY SESSION
Friday, October 14, 2016

MODERATOR: BETH SAUCIER GOODSPEED

"The Sexual Assault Kit Backlog – Real Life Examples"

Debbie and Rob Smith

Debbie Smith is a mother of two grown children, with seven grandchildren and has been married to Rob for over 44 years. She was a stay at home mom in 1989 when a masked intruder entered her home, abducted, robbed and repeatedly raped her in the woods behind her home in Williamsburg, Va. For six and a half years this crime went unsolved until DNA make a cold hit on a repeat offender who was serving time for other crimes. Since the end of a lengthy and drawn out trial, Debbie has become a crusader intent on helping other victims and trying to prevent others from becoming victims. She has appeared on several TV shows including 60 minutes, Oprah, John Walsh, Jane Pauley, etc. She has been interviewed in numerous national magazines and newspapers. Federal law with Debbie's name attached(the Justice For All Act of 2004) passed and was signed by President G. W. Bush, which will provide funding to eliminate the backlog of untested rape kits, to train first responders such as police, EMTs and nurses and it will standardize rape kits nationwide. This five year law was passed again for an additional five years as the Debbie Smith Act in 2008. Debbie speaks at conferences all over the U.S. and the world in an effort to help others. She is the founder and CEO of H-E-A-R-T, Inc., a non-profit foundation established to aid victims of sexual assault.

Rob Smith retired from the Williamsburg Police Department as a Lt. Detective with more than 25 years of service. He graduated Summa Cum Laude from St. Leo's University with a BA in Criminal Justice. He retired in order to help Debbie with H-E-A-R-T and serves as president/treasurer for the foundation. Together with Debbie he speaks at conferences bringing his police experience together with his experience as a secondary victim and the husband of a rape survivor. His primary focus is in training first responders how to better understand the mindset of a victim of sexual assault. He has served on the board of directors for the local Williamsburg area battered women's shelter, AVALON, currently for the local SANE program, and on the Colonial Area Taskforce on Domestic and Sexual Assault.

Both Debbie and Rob are past members of the IACP(International Association of Chiefs of Police) and OVC (Office of Victims of Crime) national advisory committee for establishing a new victim's service model for law enforcement. They were both members of the board for NREP (National Rape Evidence Program) which raised funds to help with the cost of testing rape kits until the federal grant program started. They were part of the advisory board that established the Virginia Victim/Witness Academy. Debbie was appointed by one governor of Virginia(Kane) to the Criminal Sentencing Commission, by the next governor (McDonnell) to the Virginia Criminal Justice Services Board and most recently by current Governor McCaulliffe to the Juvenile Justice Advisory Board. To date they have presented in 34 different states, Canada, Croatia, South Africa and Australia.

Lifetime TV aired the movie, "A Life Interrupted: The Debbie Smith Story" on April 23, 2007.

Michelle Bowdler

Michelle Bowdler is the Senior Director of Health & Wellness at Tufts University, where she has been actively involved in sexual assault prevention treatment and response for well over a decade. She was also the Principal Investigator for a Dept. of Justice the Campus Violence Prevention Grant at the University. Michelle is on the Speakers Bureau of the Boston Area Rape Crisis Center as well as RAINN and has consulted on the rape kit backlog issue as a survivor since 2011 for a number of different initiatives. She is an Advocacy Partner for RKAP – the Rape Kit Action Project. Ms. Bowdler has a master's degree from the Harvard University School of Public Health, with a concentration in health policy and management.

Deb Calhoun

Ms. Deborah Calhoun is the Scientific Services Division Director for the Pennsylvania State Police Bureau of Forensic Services. Ms. Calhoun has spent over 27 years working in various capacities throughout the State Police Crime Laboratories. Though trained in drug identification and trace analysis, she spent the majority of her career in serology, first as a forensic scientist and later as a supervisor and technical coordinator. She gained experience and extensive training in both traditional serology and bloodstain pattern analysis. She is a past member of the International Association of Bloodstain Pattern Analysts and the American Academy of Forensic Sciences and is currently a member of and the American Society of Crime Laboratory Directors. Ms. Calhoun completed her training as an ASCLD/LAB-*International* Assessor. She has participated on several committees including those involving the Pennsylvania Commission on Crime and Delinquency, the Pennsylvania Coalition Against Rape and the Department of Health. She has qualified as an expert witness in nineteen (19) counties in Pennsylvania and in the United States District Court offering expert testimony in serology, bloodstain pattern analysis, hair and fiber analysis and drug analysis. Ms. Calhoun earned a B.S. in Biochemistry with a minor in Criminal Justice from the University of Scranton and later earned a Master's degree from Duquesne University in Pittsburgh. She is a graduate of the West Virginia University Forensic Management Academy and the Commonwealth of Pennsylvania Leadership Development Institute.

Michelle Levasseur

MICHELLE LEVASSEUR is the technical leader of the Criminalistics Unit of the Massachusetts State Police Crime Laboratory, where she supervises laboratory staff responsible for the identification, testing and collection of biological fluids, trace materials and gunshot residues. Currently, there are four laboratories within the crime laboratory system that perform these functions. She is a graduate of National University and UMASS Boston.

MODERATOR: MELISSA SMITH

"**The Determination of GHB from Urine using Hollow Fiber Microextraction and Quantification by LC/MS/MS,**" Marianne Staretz, PhD, Mackenzie Beyer, BS; Cedar Crest College

GHB is becoming a popular topic of conversation in forensic science toxicology laboratories. At high concentrations, GHB can cause sedation, short-term memory loss, and increased libido, which all contribute to its use in drug facilitated sexual assaults. GHB is quickly excreted from the body, with a peak concentration between 3 and 6 hours and almost total excretion occurring between 12 and 24 hours. This, along with the fact that GHB is highly polar, makes analysis and detection troublesome.

The goal of this study was to develop a GHB hollow fiber microextraction method with good efficiency and requiring minimal time and cost compared to previously reported methods. In HFME the pores of a porous fiber are filled with an organic solvent forming a supported liquid membrane (SLM). The internal cavity, or lumen, of the fiber is filled with an aqueous acceptor phase. The analyte passes through the SLM into the acceptor phase. The acceptor phase can then be removed with a syringe and the analyte can be detected. Detection and quantification of the GHB extracts was performed using liquid chromatography coupled with tandem mass spectrometry.

The liquid chromatographic conditions for the analysis of GHB were optimized using a Macherey-Nagel Nucleodur HILIC column (125mm X 2mm ID X 3 □ m) with an isocratic solvent system consisting of 20% 5 mM ammonium acetate (pH 7) and 80% acetonitrile. The instrument was monitored in negative ionization mode with multiple reaction monitoring (MRM). The transitions monitored were m/z 103-57, 103-58, and 103-101. The retention time was approximately 2.5-3 minutes.

The initial hollow fiber microextraction conditions were based on a study by Hyder[1]. The donor phase was prepared by adding 0.1 mL of concentrated sulfuric acid to 25 mL of synthetic urine yielding a concentration of 0.05 M. The donor phase was then spiked with GHB to a concentration of 20 mg/L. The organic phase was HPLC grade acetone. The acceptor phase was 0.1 M ammonium carbonate (pH 9.5). The optimum length of the fiber was determined to be 30 cm resulting in a 50 □ L extraction volume on average. GHB was extracted and detected using initial conditions. Various donor, acceptor and organic phases were compared using extraction efficiency. The extraction efficiencies were determined by calculating a ratio between the amount of GHB extracted in the hollow fiber to the amount of GHB spiked into the sample, which can then be converted into a percentage.

The current study indicates that HFME can be used to extract GHB from urine. Optimal HFME conditions will be presented along with the application of the method to the analysis of GHB in urine samples.

Reference:

Hyder M, Genberg J, Jönsson JÅ. Application of hollow fiber liquid phase microextraction for pinic acid and pinonic acid analysis from organic aerosols. Anal Chim Acta 2012;713:79–85.

"Detection of Handwriting Indentation," Lauren Blackmore; Hofstra University

The purpose of this research was to determine which technique, the Electrostatic Detection Apparatus, or the Reflective Transform Imaging, detected indentations from handwriting more consistently. The Foster and Freeman Electrostatic Detection Apparatus (ESDA) was already accepted as a reliable instrument to detect indentations. The Reflective Transform Imaging (RTI) technique, however, is new to the field of Question Documents. Instead of using an instrument that creates an electrostatic charge, specific photographs were taken to capture fixed light, using reflective spheres. The photographs then interface with software where light was detected using the reflective spheres. Finally, once the light was captured, it was determined whether indentation was detected. Four different types of gel pens and ball-point pens were used to compare both techniques. Three people submitted five samples for each pen. When the data was collected a visible examination was conducted to determine if any indentations were visible. If there was indentations found in either of the techniques, it was then determined if the indentation was decipherable. There were some indentations detected on the RTI, mostly from the same writer. Not all of the indentation that was detected was decipherable. When using the ESDA, indentations was detected on all of the samples submitted, and almost all of the samples were clear. As a result, so far in my experiment, it is concluded that ESDA gives more accurate results. While conducting my experiment, however, there have been recommendations with my procedure with the RTI technique that could have given more accurate results. This proves that with further experiments RTI can develop into a reliable technique.

"The importance of resource quality, arrival order, and bacterial cues on the colonization behavior of the black blow fly, Phormia regina (Meigen)," Melissa Branker, Jennifer Rosati; John Jay College

During decomposition, there are many different insects groups that utilize carrion as a resource. In particular, blow flies (Order: Diptera; Family: Calliphoridae) are considered to be a forensically important family due to their ability to rapidly locate and colonize a carrion resource. As a result, blow flies are commonly used as indicator species in PMI estimations. However, recent research indicates that the colonization behavior of these species can be influenced by a variety of abiotic and biotic factors. In

this study, the effects of arrival order, resource quality and bacterial or species cues on the oviposition behavior of Phormia regina were investigated. Colony cages containing gravid P. regina females (100) and males (50) were exposed to resources of different quality (fresh vs. aged pork liver), species presence/absence (with or without Lucilia sericata eggs) and bacterial cues (sterilized vs. unsterilized L. sericata eggs). To test the effect of resource quality, fresh and aged pork liver was used. To test the effect of arrival order, colonization by L. sericata was simulated using eggs collected from L. sericata colony cages that were immediately placed on both the fresh and aged liver. To test the effect of bacterial cues, freshly collected L. sericata eggs were immediately placed on liver or were subjected to a sterilization treatment to remove bacteria prior to placement on the resource and subsequent exposure to P. regina for colonization. These factors affected not only the amount of eggs laid on the resource and the locations of oviposition, but also influenced the time of colonization in each treatment. The colonization behavior of P. regina was quantified by measuring the time to colonization, location and amount of eggs laid in each treatment condition. Fresh liver without L. sericata eggs was used as a control. The results from this study can improve our knowledge and understanding of the mechanisms driving the colonization behavior for forensically important blow flies and validate their use as indicator species for the estimation of the minimum time of colonization.

"Chemical Assays for Recognition of Originator Attributes from Fingerprints," Erica Brunelle, Crystal Huynh, Anh (Ami) Minh Le, Lenka Halámková, Juliana Agudelo, Leif McGoldrick and Jan Halámek; University at Albany, State University of New York

The analysis of fingerprint samples via pictorial comparisons has been largely accepted by the scientific community as a dependable method of identification. While this method is fairly well established, it is not applicable for all situations. For example, when only partial or smudged fingerprints are collected, a match is unlikely to be found. In cases such as these, the chemical composition of the samples would be of more use than the image of the fingerprint. It has recently been demonstrated using bioaffinity-based enzyme cascades and chemical assays that the amino acid content in fingerprints can be used to differentiate between male and female fingerprints. The research displayed here further investigates the use of straightforward chemical assays instead of the more complex biochemical assays. Chemical tests are fairly well-known, especially in the field of forensic science, where there are field kits that are used for the on-site analysis of drug samples. The most common tests for illicit substances are Marquis, Simon's and Chen's test.

As with any multi-analyte system – enzymatic or chemical – it is possible for multiple amino acids to correspond to the same attribute and can, therefore, compromise the overall results. To eliminate this possibility, it is pertinent that there be systems developed that are restricted to one analyte (amino acid) or a specific combination of analytes that are correlated to the desired originator characteristics. To insure that the methods presented here are practical and can be used on samples left on more than one particular surface, research showing the performance of the system on samples collected from various surfaces is also provided.

The developed chemical assays also have the potential to be coupled with a portable apparatus for use directly onsite where the assay can subsequently be performed and the results interpreted by non-scientific personnel, unlike most currently available techniques. This can be done in a manner that is similar to water test kits and the VOCkit system which is a small strip that has a grid of several dozen indicator chemicals imprinted on it which is used by the Army for the detection of threat agents such as anthrax, sarin and mustard gas.

"Automated Desorption, SPE Extraction, and LC/MS/MS Analysis of Dried Blood Spots," Oscar G. Cabrices; GERSTEL, Inc.

The extraction of dried blood spots (DBS) typically involves manual intervention. First, a small disc is punched out of the center of a dried blood spot placed on a DBS card. Following solvent extraction of the sample, it is also common to include further cleanup steps, using solid phase extraction (SPE) to improve detection limits or exchanging solvents for compatibility with subsequent chromatographic separations. Modern analytical labs are looking to automate the process to help reduce solvent usage and to increase sample throughput while ensuring the high quality of the resulting data.

In this report, the complete automation of dried blood spot analysis is demonstrated and the results evaluated. A novel autosampler automatically inserts DBS cards into a flow through cell in which individual blood spots are rapidly and effectively desorbed. The DBS elution is integrated into a complete cleanup and analysis system using online SPE with replaceable cartridges combined with automated injection to an LC/MS/MS system. Automated DBS extraction methods were optimized for a variety of analytes from rat and bovine blood.

Drug compounds of forensic interest (e.g., ketamine, amitriptyline and ketoprophen) were successfully extracted from dried blood spot samples using an automated DBS-SPExos procedure coupled to an LC/MS/MS system. The automated workflow proved to be accurate and precise. For dried rat blood spots, accuracy data averaged 91.7% (range: 82.6% - 104%) and precision data averaged 5.75% CV (range: 2.47% -11.5%) for all compounds analyzed. For dried bovine blood spots, accuracy data averaged 108% (range: 98.6% - 119%) and precision data averaged 5.31% CV (range: 0.288% -10.2%) for all compounds analyzed.

"Prep-and-Shoot": A High Throughput Automated Sample Preparation and Analysis Workflow for Comprehensive Toxicology Urine Screenings using LC/MS/MS," Oscar G. Cabrices; GERSTEL, Inc.
A major mechanism of the metabolism of many drugs of abuse involves conjugation of the analyte with glucuronic acid. To ensure the accuracy of results, analyses of drug analytes from urine matrices first requires the deconjugation of the analyte. This deconjugation is typically performed by enzymatic hydrolysis using enzymes such as beta-glucuronidase, which selectively cleave the conjugated compounds leaving the free analyte. Typical hydrolysis procedures involve long incubation periods at specified temperatures and have traditionally been performed manually.

Automating the entire hydrolysis, extraction, and subsequent analysis by LC/MS/MS provides high throughput analysis for drugs in urine. Using a GERSTEL Multi Purpose Sampler (MPS), syringe transfer of all liquids involved in the enzymatic hydrolysis procedure, controlled incubation of the samples for a defined prior of time, as well as extractions of the subsequent hydrolyzed urine samples using Disposable Pipette Extraction (DPX) were performed.
As a result of this study, we were able to show that an automated enzymatic hydrolysis and subsequent DPX cleanup method was shown to be successful using a dual head GERSTEL MPS for glucuronide conjugated analytes in urine. Using this method, analytes can be rapidly and reproducibly isolated from hydrolyzed urine samples using an automated DPX cleanup procedure coupled to LC/MS/MS analysis. Linear calibration curves resulting in R^2 values 0.99 or greater were acheived upon the complete automated hydrolysis of glucuronide conjugated analytes with LOQs of 1ng/mL for both Morphine and Oxazepam.
The automated hydrolysis, DPX extraction and LC/MS/MS method provided good accuracy and precision. The accuracy data averaged 102% for Morphine and 96.3% for Oxazepam and precision data (%CV) averaged 3.52% CV for Morphine and 4.70% for Oxazepam.

"Development and validation of an automated liquid-liquid extraction GC/MS method for the determination of THC, 11-OH-THC and free THC-carboxylic acid (THC-COOH) from blood serum," Oscar G. Cabrices; GERSTEL, Inc.
The analysis of □ 9-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-□ 9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-□ 9-tetrahydrocannabinol (THC-COOH) from blood serum is a routine task in forensic toxicology laboratories. For examination of consumption habits the concentration of the phase I metabolite THC-COOH is used. Recommendations for interpretation of analysis values in medical-psychological assessments (regranting of driver´s licenses, Germany) include threshold values for the free, unconjugated THC-COOH.
Using a fully automated two-step liquid-liquid extraction THC, 11-OH-THC and free, unconjugated THC-COOH were extracted from blood serum, silylated with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and analyzed by GC/MS. The automation was carried out by an x-y-z sample robot equipped with modules for shaking, centrifugation and solvent evaporation. This method based on a previously developed manual sample preparation method.
Validation guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh) were fulfilled for both methods, at which the focus of this article is the automated one. Limits of detection and quantification for THC were 0.3 and 0.6 µg/L, for 11-OH-THC were 0.1 and 0.8 µg/L and for THC-COOH were 0.3 and 1.1 µg/L, when extracting only 0.5 mL of blood serum. Therefore the required limit of quantification for THC of 1 µg/L in driving under the influence of cannabis cases in Germany (and other countries) can be reached and the method can be employed in that context.
Real and external control samples were analyzed and a round robin test was passed successfully. To date the method is employed in the Institute of Legal Medicine in Giessen, Germany, in daily routine. Automation helps avoiding errors during sample preparation and reduces the workload of the laboratory personnel. Due to its flexibility the analysis system can be employed for other liquid-liquid extractions as well. To the best of our knowledge this is the first publication on a comprehensively automated classical liquid-liquid extraction workflow in the field of forensic toxicological analysis.

"The Effect of Environmental Conditions and Substrate Material on the Weathering of Gasoline and Light Petroleum Distillates," Matthew Ciano**,** University of New Haven Forensic Science Department; Robert Powers, University of New Haven Forensic Science Department; Erika Chen, NYPD; Michael Valetutti, NYPD; Brooke Kammrath, University of New Haven Forensic Science Department
In fire investigations, the determination of the presence of an ignitable liquid at the scene is often important, as they are frequently used as accelerants. Many different ignitable liquids exist which can be used to help start or maintain a fire. The most commonly encountered is gasoline, in part because it is widely available and cost effective. Light petroleum distillates, such as paint thinners, lighter fuels, and cleaning fluids, are also a commonly used class of ignitable liquids because they too are easily obtainable and highly flammable. Both of these classes of ignitable liquids contain many highly volatile compounds. This makes them particularly susceptible to weathering, the effect of evaporation on their chemical makeup. Criminalists must be capable of recognizing ignitable liquids in their weathered forms as samples recovered from fire scenes rarely show the characteristics of unweathered materials.
It has long been stated that a wide variety of conditions can have effects on the weathering process of ignitable liquids. These conditions can include extent, temperature, air composition, airflow, exposure to sunlight (specifically ultraviolet light), and several others. Various studies have sought to use pattern recognition software to either classify the ignitable liquids and/or

determine the original conditions under which a sample was weathered. Few published studies have investigated how these conditions affect the presence of the various different compounds detected in ignitable liquids.
In this research, samples of three different ignitable liquids were studied: gasoline, lighter fuel, and a simulated ignitable liquid mixture made up of ten compounds from classes commonly found in ignitable liquids (alkanes, aromatics, and condensed ring aromatics). The simulated ignitable liquid mixture serves as a simpler system in which the effects of the different conditions on specific homologous chemicals can be studied before looking at the more complex mixtures that make up actual ignitable liquid samples. All samples were evaporated to 25%, 50%, 75%, and 90% extents by volume while varying the following conditions: container size, temperature (a greater range of temperatures than in prior studies), and exposure to UV. Samples of each were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), and the resulting chromatograms compared to determine any notable differences.

"Acetyl Artifact Formation in Seized Methamphetamine Samples," Tamar Daniels, M.S.F.S, Heather Harris, M.F.S, J.D., D-ABC, Francis Diamond, B.S., Christopher Merrill, M.S.F.S.; NMS Labs
The goal of this presentation is to present data to provide a better understanding of the possible reasons for the formation of the N-acetyl Methamphetamine artifact in seized methamphetamine samples.
In the most recent report issued by the National Forensic Laboratory Information System (NFLIS), a program of the Drug Enforcement Administration (DEA), methamphetamine reports have had a significant increase in recent years. Methamphetamine reports accounted for an estimated 17.37% (the second most prevalent) of all case submissions to state and local crime laboratories nationwide.[1] This study is a response to the detection of an unknown artifact peak, identified as N-acetyl Methamphetamine (CAS# 27765-80-6), in several methamphetamine case samples upon analysis by Gas Chromatography/Mass Spectrometry (GC-MS). The sample types that presented this artifact varied from crystalline to illicit tablets and other compounds detected included aspirin breakdown products and caffeine.
In case samples, methamphetamine is not produced from pure materials, which results in the presence of impurities and artifact formation through secondary reactions. This research shows that in the presence of aspirin, methamphetamine will be converted into N-acetyl Methamphetamine. This research also will explore the possible cause(s) of this formation and processes to prevent its formation. The standards used for this research were greater than or equal to 99% purity, which demonstrates that the acetyl artifact is being formed during analysis rather than being present in the case samples as an impurity.
To determine the factors contributing to this artifact formation, methamphetamine, aspirin and caffeine solid standards were mixed in varying concentrations to test under different analytical conditions. Solvent dilutions and acid/base extractions of the standard mixtures in different solvents were performed and analyzed by GC-MS. All preparations yielded the formation of the acetyl artifact. The pH in extractions will be altered to determine if the pH of the chemical environment can be controlled to prevent artifact formation. In addition, thin layer chromatography (TLC) was performed on all GC-MS preparations that yielded the acetyl artifact in order to determine if an analytical method that does not place the sample under high temperatures will result in any suspected artifact bands. All of those same preparations did not yield any suspected artifact bands on TLC.
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"Characterization of cartridge cases based on case deflector marks from Tavor rifles," Andrew J. Winter, Middlesex County Prosecutor's Office, N.J; Peter Diaczuk, Penn State University, PA
The Tavor rifle is one product in a long line of firearms produced by Israel Weapon Industries (IWI). The authors had the opportunity to test such a rifle for several reasons, one of which was to determine if any parts of the rifle mark fired ammunition components in a specific way. A case deflector is a simple device used by some firearm manufacturers to redirect the travel of ejected cartridge cases away from the firearm user and also sometimes away from an individual shooting right next to the firearm as well.
When a case is ejected from a Tavor rifle, it impacts the case deflector and is sent careening off to the side, away from the shooter, exactly as designed. The spent case incurs an indentation mark as a result of this impact, which is the focus of this presentation. Due to a novel design feature of the Tavor rifle, it can be made to suit either a left handed shooter or right handed shooter by simply rearranging a few key parts, one of which is the removable case deflector. The authors obtained a total of six case deflectors for this project. The same ammunition was fired from the same firearm but with the different case deflectors to keep all other parameters identical, such as recoil spring tension, thereby applying the same force to the cartridge case during the ejection process.
All fired cases were examined microscopically and compared to one another to determine if the impact with the different case deflectors could be individualized.

"Identification of Controlled Substances in Seized Street Drugs Using a Portable Gas Chromatograph Ion Trap Mass Spectrometer," Heather Dyer, Maine Health and Environmental Testing Laboratory; Jamie Foss, Bill Hahn, Charlie Schmidt, Frank Kero, PerkinElmer Health Sciences

Gas Chromatography-Mass Spectrometry (GC-MS) has long been considered the "gold standard" for the identification of controlled substances1-2 as it allows for the detection of a broad range of analytes in diverse sample mixtures.3 However; samples must be sent to forensic laboratory for analysis, which can take months due to current case backlogs and analysis times are relatively long versus other analytical techniques, often ranging from 15-60 minutes per sample. The use of portable instrumentation has been used for the preliminary identification of substances in the field triaging which samples need to be submitted to the laboratory. Portable GC instruments such as GC-FID and GC-PID lack specificity and the range of compounds they can identify. This portable low thermal mass (LTM) gas chromatograph equipped with an ion trap mass spectrometer allows for the rapid identification of substances using retention time indices and NIST library matching.

In this study, a variety of illicit substances that were seized within the State of Maine, and submitted to the Maine Health and Environmental Testing Laboratory, were analyzed using a PerkinElmer Torion T-9 Portable GC-MS. The illicit material was dissolved in water for analysis. Analyte extraction was performed using a solid phase microextraction (SPME) syringe consisting of a polymer coated SPME fiber. The fiber was directly immersed in the solution for approximately 60 seconds with gentle manual agitation. After sampling, the SPME fiber was injected into the injection port of the GC-MS, the analytes were allowed to desorb from the fiber and directed onto the LTM capillary GC column followed by a rapid GC temperature program. Instrument analysis time was under 3 min per sample.

An overview of the instrumentation and the suitability of portable LTM GC-MS instrumentation to rapidly generate NIST searchable GC-MS spectra for the identification of controlled substances will be presented.

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3. Cheng, J. Y. K., Chan, M. F., Chan, T. W. and Hung, M. Y., 2006, Impurity profiling of ecstasy tablets seized in Hong Kong by gas chromatography-mass spectrometry, Forensic Science International, Vol. 162, No. 1, pg. 87-94.

"Identification of Fentanyl and Other Synthetic Opiates using Ambient Ionization High Resolution Time-of-Flight Mass Spectrometry," Amanda Moore**,** [1]Boston University School of Medicine, Biomedical Forensic Sciences; Jamie Foss[2,3], Sabra-Botch Jones[1], and Frank Kero[3] - [2] Maine Health and Environmental Testing Laboratory [3]PerkinElmer-Human Health/Environmental Health

Fentanyl analogs and designer opioid drugs are a hot topic in the news right now contributing to numerous fatal overdoses. These drugs elicit analgesic effects similar to heroin making them desirable drugs to abuse. Fentanyl analogs and designer opioid drugs are expected to be more prominent in forensic casework in the near future. Fentanyl analogs and designer opioid drugs can be seen in forensic casework either alone or can be mixed with other drugs of abuse such as heroin. It is therefore necessary to have an efficient methodology to identify these compounds. Currently, Gas Chromatography-Mass Spectrometry (GC-MS) is used to identify drugs of abuse and is considered the "gold standard" in forensic casework. However, analysis times can often range from 15-60 minutes in length. Another drawback to GC-MS is need for spectra library matching, giving the need for analytical reference materials for identification leading to an inability to identify new designer drugs before a reference material is available. In this study, Direct Sample Analysis Time-of-Flight Mass Spectrometry (DSA-TOFMS) was utilized to provide rapid identification of fentanyl and related synthetic opiates. DSA is a direct ambient ionization source, requiring no chromatography and minimal sample preparation. High resolution time of flight mass spectrometry generates empirical formula information bypassing the need for a reference material, and in-source collisionally induced dissociation (CID) produces additional structural information for confirmation. An overview of the instrumentation and use of DSA-TOFMS to rapidly generate exact mass data and fragmentation data from in-source CID for the identification of synthetic opiates will be presented. Analytes explored include: heroin, 6-monoacetylmorphine, morphine, fentanyl, acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, U-47700, and W-18.

"**A Comparative Analysis of Commercially Available Protein and Peroxidase Reagents for Blood Detection and Enhancement on Laundered Clothing of Various Fabric Types,"** Gabrielle A. Hartley, Dr. Claire L Glynn; University of New Haven Forensic Science Department

Blood on a suspect or victim's clothing is not uncommon in criminal cases involving violent incidents, and often these stains will be washed away in hopes to clear away the evidence. A number of products are available for the detection and enhancement of dilute bloodstains, including both protein and peroxidase based reagents. This study aims to produce a comprehensive analysis of three protein based reagents and three peroxidase based reagents commonly used and commercially available for the detection of trace amounts of blood on laundered clothing. Staining reagents Hungarian Red, Coomassie Blue, Amido Black, luminol, Bluestar® Forensic Magnum, and aqueous Leuco Crystal Violet (LCV) were purchased from Sirchie and used to detect 100 µl human blood stains on varying fabric types and colors (white cotton, black cotton, blue denim, white polyester, and black polyester) at a range of blood dilutions (neat, 1:10, 1:100, 1:1000, 1:10000, 1:100000, 1:1 million) after laundering to determine the usability and sensitivity of the reagents.

Following informed consent and approval from the Institutional Review Board at the University of New Haven, venous blood was obtained from volunteers into sterile vacutainer EDTA vials and stored at 4 ºC. 100 □l of human blood was deposited onto each fabric type at each dilution. Each sample was performed in triplicate and photographed prior to laundering and enhancement. Following laundering with a standard detergent and washing machine, each sample was enhanced and photographed following the manufacturer's instructions provided with each of the six reagents. The results of each reagent, dilution, and fabric type were compared using a scale from 0-4 (0 = no reaction; 4 = strong positive reaction).

This study revealed that the peroxidase based reagents produced the greatest sensitivity on the natural fabrics, reacting positively down to a blood dilution of 1:1000. The protein reagents produced greater sensitivity on the synthetic fabrics, reacting positively down to a blood dilution of 1:10. Peroxidase stains relying on chemiluminescent properties rather than colorimetric results produced better results on the dark colored fabrics. As the protein based reagents are based on color and not chemiluminescence, their use on dark fabrics produced indiscriminate results. The results of this study suggest the importance of laundered clothing as evidence and provides an analysis of these six reagents for blood detection on fabrics after blood evidence has been washed. It suggests peroxidase based reagents to be the superior method for use on natural fabrics and chemiluminescent peroxidase reagents to be superior on all dark fabrics. Protein based reagents were best suited for use on synthetic fabrics.

"**Determination of Biological Sex from Fingerprints via Bioaffinity-based Cascades,"** Crystal Huynh, Erica Brunelle, Lenka Halámková, Juliana Agudelo, Leif McGoldrick, Jan Halámek; State University of New York, University at Albany

Fingerprint analysis traditionally refers to the process of comparing fingerprint patterns by an expert and/or an automated fingerprint identification system. Currently, the analysis ends with this matching methodology causing the field to be dependent on the presence of a stored matching print or a matching print from an individual that is physically present. Due to this limitation, a latent fingerprint may be judged to be too smudged or smeared to be of use. What is often overlooked is that those latent prints are created by sweat and sebum emulsions excreted by the fingertips. Those emulsions have their own unique chemical compositions for each individual making them possible biological samples for analysis. Our lab has developed a bioaffinity-based cascade for the determination of biological sexes from the chemical composition of the sweat/sebum left as the latent prints.

The research presented here addresses the current limitations in fingerprint analysis using a bioassay system that focuses on the components of fingerprints. Bioaffinity-based assays have been developed for the determination of biological sexes from those components. In one assay, L-amino acid oxidase was used to target the amino acids present in the sebum and sweat left on latent fingerprints. Further research has led to the testing of authentic fingerprint samples collected from various surfaces as well as the development of other bioaffinity-based assays capable of differentiating between biological sexes via less complex systems. Other bioaffinity-based assays will also be developed in the future for the determination of other physical attributes such as age group and ethnicity.

"**Identification using bioaffinity-based assays of body fluid samples,"** Leif McGoldrick, Sarah Farrell, Juliana Agudelo, Erica Brunelle, Crystal Huynh, Lenka Halámková, Jan Halámek; State University of New York, University at Albany

In the field of forensic science, biomarkers are widely used for purposes of identification. The main method using this technique currently is to use DNA from blood in order to identify a person. However, DNA analysis is a lengthy process which requires a specific match in a database to be present for results to be obtained. This is the main drawback to DNA analysis and is a factor that leads to backlogs in the lab analysis in criminal cases. By using single analyte bioaffinity-based assays (bioassays) one is able to use the content of certain biological markers (biomarkers) in blood and sweat in order to obtain identifying characteristics of a person. This bioassay approach to forensics would be able to provide some individual characteristics that would narrow down the subject pool and expedite the process of criminal investigations. Along with blood, the contents of fingerprints can also be used for this approach, enabling the use of multiple body fluids to be able to be analyzed. Currently, fingerprint analysis is mainly focused on pictorial comparison which draws the need for perfect, usable prints and would be inapplicable to partial or smudged prints. Since bioassays look at the content within the actual print, these prints would then be usable in forensic investigations.

Single analyte bioassays depend on the varying levels of biomarkers between people based on age, sex, race, and general lifestyle. By using similar bioassays, other information is able to be compiled such as the time since a bloodspot was deposited. The aim of the research would be for it to be compiled into a kit for direct crime scene analysis and be able to be used by people who have little to no science background.

"**Evaluation of a Handheld Raman Spectroscopy Instrument for the Preliminary Identification of Controlled Substances,"** Kristie McLaughlin, Bay Path University; Jamie Foss, Maria Pease; Maine Health and Environmental Testing Laboratory

New England continues to be at the forefront of current drug trends from the abuse of prescription drugs to the emerging synthetic designer drugs, including bath salts, phenethylamines, and other synthetic hallucinogenic drugs. There has been a major increase in heroin and fentanyl seizures leading to a large number of fatal overdoses. Most drug cases are sent directly to the laboratory for testing, causing large backlogs for many forensic laboratories. Using the handheld Raman spectrometer, law

enforcement officers will be able to scan and discern illicit substances on the street; possibly reducing the amount of drug evidence being sent to laboratories. The focus of this study is to evaluate the suitability and limitations of this analytical technique for the preliminary identification of controlled substances. Suspected illicit substances seized around the State of Maine were tested at the Maine Health and Environmental Testing Laboratory in Augusta, ME. Samples were analyzed neat, with no pretreatment or preparation and the results compared to traditional analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography/Mass Spectrometry (GC/MS).

"**The Screening and Confirmation of NBOMe Designer Drugs on Blotter Paper by Ambient Ionization Mass Spectrometry and FTIR Chemical Imaging Spectroscopy,"** Amanda Moore; [1]Boston University School of Medicine, Biomedical Forensic Sciences, David Barajas[1], Jamie Foss[2], Frank Kero[3], Tom Byron[3], Sabra Botch-Jones[1 2] Maine Health and Environmental Testing Laboratory [3]PerkinElmer-Human Health/ Environmental Health

Introduction: N-(methoxybenzyl)-phenethylamine compounds (also known as "NBOMe's") are an emerging threat to public health with fatality and severe injury related investigations increasing throughout the country. The NBOMe compounds are obtained as "legal" highs through unregulated internet purchases and are typically administered via blotter paper. The user experience has been described as a more potent, but a similar high when compared to lysergic acid diethylamide (LSD). Users often believe they are ingesting LSD when in fact it was one or a combination of the NBOMe compounds. Previous reports from this group have demonstrated the utility of non-targeted high resolution mass spectrometry platforms for this application paired with both ambient ionization and electrospray ionization sources since authentic case work samples are often obtained as mixtures of true unknowns (reported at NEAFS 2015). This previous work employed a minimal sample preparation via a 5 min liquid extraction of the target compound(s) from the blotter paper. This work was completed in collaboration with the State of Maine Department of Health and Human Services (Augusta, Maine), the University of Central Florida (Orlando, Florida) and Boston University School of Medicine Biomedical Forensic Sciences Program (Boston, Massachusetts). **Objective:** In an extension of this work, chemical imaging using Fourier Transform Infrared Spectroscopy (FTIR) was evaluated to provide an orthogonal workflow solution for confirmation and to leverage the advantage of Direct Sample Analysis-Time-of-Flight Mass Spectrometry (DSA-TOFMS, PerkinElmer, Waltham, MA, USA) for screening to reduce the speed of analysis, cost in consumables and waste. FTIR chemical imaging method development variables considered included variation in sampling depth and scan modes to generate a spatial chemical map of the sample. **Method:** Certified reference material of 25C-NBOME (Cerilliant, Round Rock, TX, USA) was analyzed as "cast" films by allowing a liquid solution in methanol (ThermoFisher Scientific, Waltham, MA, USA) to evaporate before analysis. In addition, 25C-NBOMe was spiked onto blotter paper. Further, certified reference material of LSD (Cerilliant) was also analyzed for comparison. A macro Geranium (Ge) Attenuated Total Reflectance (ATR) sampling accessory was used. The scan range was 4,000-748 cm-1, spectral resolution was 8 cm-1, scans per pixel was 4, field of view was 500 x 500 um, special resolution 1.56 um, measures spectra of 102,4000, and the total number of scans were 64. By utilizing the Ge ATR, the diffraction limits were 2.5 um at 1,000 cm-1. **Results:** Utilizing the spectral library and chemical imaging, LSD was identified and able to be separated from the blotter paper components which were identified as polyethylene/carbonate, cellulose, hydrocarbon/fluorocarbon/carbonate mixtures. The correlation for the NBOMe was determined to be ~0.78 and the same components were identified from the blotter paper. There are indications that this technique may be used to identify the presences of LSD and NBOMe on a paper matrix. High spatial resolution is needed to isolate the chemical entity from the paper constituents, however it results in a small field of view or area measured limiting the ability to examine the whole matrix. If drug is not well dispersed within paper matrix, technique may produce a negative result do to limitation of area measured. It is anticipated that this workflow would be of broad-based interest to the forensic community on the topic of emerging drugs of abuse, designer and synthetic drugs.

"Determining the Probative Value of Amylase-only Case Samples in Sexual Assaults," Courtney Mower, Arcadia University; Christine Schlenker, New Jersey State Police Office of Forensic Sciences

A review of 225 sexual assault cases submitted to the New Jersey State Police Office of Forensic Sciences (NJSP OFS) was conducted in order to determine the probative value of amylase-only samples in sexual assaults. It was determined that amylase testing is of value; however, 75% of the 476 positive amylase-only samples analyzed did not generate DNA profiles sufficient for national upload to CODIS (≥ 8 non-victim loci). Only 8.8% of the total samples also had a named suspect and suspect reference sample. If a suspect reference sample has not been obtained, the evidence is less likely to undergo processing for YSTRs. The 476 samples reviewed consisted of 126 swabs and 350 non-swabs (i.e. underwear and bras). Underwear accounts for more than one-third of the probative amylase-only samples. Current serological analyses incorporate the Phadebas® Forensic Press Test to determine the presence of saliva, but it has been known to cross-react with fecal matter, perspiration, semen, vaginal secretions, and breast milk, producing false positive results which are further carried through to DNA analysis. In Drug Facilitated Sexual Assaults (DFSA) the victim is unable to recall the event clearly. Thus the contents of the sexual assault kit and the victim's clothing are tested for the presence of seminal fluid. Subsequent amylase testing of the clothing and external genital samples is conducted if seminal fluid is not identified or if previous consensual activity is indicated. Results indicate that additional screening, such as RSID testing for saliva or Y-screening, would generate more conclusive results during serological analyses, minimize the production of non-probative DNA profiles, and decrease the DNA backlog.

"**An Assessment of Pipette Calibration Stability Using Statistical Process Control Charts,"** Rachel Pruckler, Sabra Botch-Jones, Catherine M. Grgicak; Boston University School of Medicine, Biomedical Forensic Sciences
Routine pipette calibration is an essential part of any quality assurance and quality control program in the forensic sciences and beyond. Pipette calibration standards in a forensic laboratory are typically set to the limits outlined by the document ISO8655, published by the International Organization for Standardization for the general scientific community. Alternative methods exist that may be capable of monitoring pipette stability across time in a forensic setting. Statistical process control charts, or Shewhart charts, are one such form of process control, which is being investigated for its potential application to pipette calibration monitoring for forensic laboratories.

To investigate the applicability of process control charts for monitoring pipette stability, a series of *X*-bar and *S* charts, a type of Shewhart chart, have been produced from eight years of collected calibration data. A total of 71 pipettes of the following sizes were examined: 1-10 µL, 1-10 µL multi-channel, 10-100 µL, 100-1000 µL, 1-3 µL, 30-300 µL, 5-50 µL, 5-50 µL multi-channel, and 500-5000 µL pipettes. The ISO8655 calibration recommended volume limits of these pipettes have been added to the charts for the purposes of comparison. With these charts, it is possible to assess pipette performance over time in comparison to the ISO8655 calibration standards and to the control limits imposed by the Shewhart charts. The completed charts suggest that the methodology proposed by Shewhart shows promise as a supplement to ISO8655 recommendations for monitoring pipette stability across time.
To corroborate the value of using Shewhart charts to monitor pipette performance, a simulated serial dilution with dynamic modeling software, Stella v10.0.2, was performed. The simulations investigated multiple hypothetical pipetting scenarios concerning various levels of systematic bias, including: no bias, in Shewhart and ISO control, out of Shewhart and in ISO control, out of both Shewhart and ISO control, and the selected pipette's most recent calibration. The simulations consistently corroborated the value of Shewhart charts to enforce better compliance between a pipette's nominal and actual volume delivery.

"**Implementing an In-House Service Team in your Laboratory,"** Sara M. Ritter; Burlington Labs
With laboratories increasingly looking for ways to run leaner and cut operating costs, very often service contracts for laboratory instrumentation are not a priority. Having specially trained in -house staff on hand is key. The need to balance production uptime and routine maintenance is vital to the health of the lab. Investing in these specially trained staff will reap rewards when they are able to then help other employees learn best lab practices when working day to day with their lab equipment. These best practices will reduce wear and tear on instrumentation, and decrease the need for major repair.

Members of your team should have some past experience with instrumentation, and a willingness to work with vendors to research and compare products. Communication between team members needs to be clear, and duties should be well defined. Opportunities for training should be made available. This can include webinars that are available through many vendor's websites. Onsite training can be a large cost savings for the lab. Networking with other lab managers with similar equipment can help give you a baseline for service.

Maintenance logs should be user friendly and easily accessible. The maintenance log serves a dual purpose as a resource for inventory management and a laboratory record for regulatory agencies. Routinely recording all consumables used for instrumentation upkeep will make the process for developing an in-house preventative maintenance plan much easier. You will be able to balance the real time needs of your laboratory with the maintenance plans set forth by the vendors. Having a clear, well-kept record of maintenance will also be helpful for regulatory audits.

This presentation will give an examples of training and evaluating staff. It will also provide examples of cost savings analysis, determining appropriate times for preventative maintenance, and creating in-house tools for inventory tracking and regulatory compliant maintenance logs. To conclude it will provide resources for staff training and continuing education.

"Analysis of Sand from New Jersey Beaches Using Fourier Transform Infrared Spectroscopy," Courtney Vander Pyl**, Danielle Coombe**, Jillian Fesolovich; University of the Sciences in Philadelphia
The United States of America has 12,383 miles of coastline, which is broken into roughly 350 beaches. Out of those 12,000 miles, 130 of them belong solely to New Jersey. Beaches located in South New Jersey are famous for attracting an abundance of tourists every year during the summer months. The purpose of this research project is to determine if sand from different beaches in South New Jersey are uniquely identifiable. The beaches selected for this project are Cape May, Wildwood, Stone Harbor, Ocean City, and Atlantic City. Within each beach, a sample was taken from three locations; the water line, the middle of the beach, and the dunes. The specific longitude and latitude coordinates were recorded for each sample collected. To characterize each sample, Fourier Transform Infrared Spectroscopy was used. The individuality of each sample was determined by the shift, intensity, and sharpness, of each peak present in the IR Spectrum. Each sample was first analyzed in the condition in which it was collected. Then each sample went through a wash phase to rid of any impurities, and analyzed again. Preliminary results from these tests

show that differences are apparent from beach to beach. Although there are minor shift changes in the spectra between locations on a specific beach, there are major spectral differences found between the five different South New Jersey beaches. The data from this research can be used by investigators to link evidence to crime scenes in New Jersey. Analysis in the future will include analyzing sand samples using Raman spectroscopy and inductively coupled plasma-mass spectrometry.

"**Changes in the probability distribution of the number of contributors when signal thresholds are applied to complex DNA mixtures,"** Xia Yearwood-Garcia, Harish Swaminathan, Catherine M. Grgicak; Boston University School of Medicine, Biomedical Forensic Sciences

During forensic DNA analysis, the analytical threshold (AT), which is an RFU value assigned to an electropherogram designed to discriminate between allelic and non-allelic data, is applied. Its application allows the laboratory to eliminate noise from further analysis with some degree of certainty. Since implementation of an AT that is either too large or too small can result in substantial levels of false negative or false positive peak labeling, it is arguably one of the most important thresholds applied during analysis. However, there has been development of a probabilistic software, NOCIt, which has the ability to model characteristics of noise and integrate that information into its interpretation. Therefore, it brings to question whether the AT remains a necessary part of the analysis pipeline. NOCIt is a computational method designed to provide an *a posteriori probability* (APP) distribution on the number of contributors (NOC) that give rise to a DNA sample. It takes into account signal peak heights – from alleles, noise and stutter - population allele frequencies and allele dropout. To determine how the application of an AT affects NOCIt's ability to accurately identify the NOC to a sample, a total of 335 known DNA samples were tested using the Identifiler® Plus Caucasian population frequency table [1]. The samples were amplified using the AmpFlSTR® Identifiler® Plus PCR Amplification Kit and analyzed using an Applied Biosystems® 3130 Genetic Analyzer with a 10 second injection time. The samples ranged in complexity and contained up to 5 contributors. Template masses ranged from 0.008 to 1 ng. The multi-sourced samples contained various mixture ratios. The APP distribution of these 335 samples were tested at an AT = 1 RFU and then again at an AT = 50 RFU. Preliminary results show that 168 of 299 samples resulted in a maximum APP consistent with the true NOC when the AT = 1 RFU. The accuracy increases to 237 samples when a minimum APP of 1% is used. This indicates that the application of an analytical threshold may not be needed when probabilistically evaluating forensic signal. Further, it highlights the complexities associated with inferring the NOC to complex stains. The results of the aforementioned study will be compared to the APP that is generated when an AT = 50 RFU is applied.

References

1. Life Technologies. AmpFlSTR® Identifiler® Plus PCR Amplification Kit User Guide. Carlsbad, CA: 2014

"**The Effects of Filtration Sterilization on the Stability of Ketamine, Selected Benzodiazepines and Metabolites in Female Urine,"** Lin Zhen, Boston University

Benzodiazepines (BZPs) and ketamine (K) are compounds which have been encountered in Drug-Facilitated Sexual Assault (DFSA) cases. Due to the intimate nature of these crimes, evidence collection is often postponed due to delays and/or reluctance in reporting these crimes. Further delays in analysis may be encountered in laboratories with large caseloads and/or backlogs. Drug identification is important to determine whether the victims knowingly or unknowingly took an impairing substance, however the results could be negative due to chemical degradation over a long storage period. The purpose of this project was to study if degradation could be prevented with a new preservation method at the time of collection. The samples were prepared by the addition of K, selected BZPs and metabolites, subjected to different sample pre-treatment techniques, and were analyzed after storage at room temperature (25°C), in refrigerator (4°C) and freezer (-20°). The samples were pre-treated with preservative (0.5% toluene) and filtration sterilization (sterile filter kit) within two hours after the samples were collected, and a control group with no pre-treatment was also incorporated into the study for comparison. The changes in concentrations over 50 days (BZP group) and 210 days (K group) were evaluated between different pre-treated methods and different temperature conditions. Sample that were treated with 0.5% toluene showed the most degradation, 44% of oxazepam (least) and 96% of diazepam (most) lost after stored for 10 days, and 80% of dehydronorketamine lost after stored for 150 days regardless the storage conditions. Clonazepam and flunitrazepam concentrations were reduced by 80% of the original concentration when stored at room temperature for 10 days. The majority of benzodiazepines evaluated in this study were stable when stored in freezer. In K group, ketamine and norketamine that were stored at room temperature and refrigerator over 210 days were stable, however degradation was observed after 150 days when the samples were stored in freezer.

There was no statistically different change observed among the samples pre-treated with or without filtration sterilization. Each samples pH was measured and it was determined that those stored at room temperature had an average pH of 8.5, samples stored in refrigerator and freezer had an average pH of 6.7 and 6.5 respectively. This finding revealed that pH could be the major factor affecting compound degradation rather than the bacterial contamination with high pH contributing to degradation, and low pH potentially preventing sample lost.

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