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Scientific Sessions

Drug Chemistry Abstracts

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Update

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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. In light of the formation of the Organization of Scientific Area Committees (OSAC), 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 2016 and currently in 2017. Recent activities include publication of supplemental document 6 (SD-6) addressing measurement uncertainty for cases of weight or count extrapolations as well as an updated format to the SWGDRUG monographs. Current issues being addressed are validation of qualitative methods by way of revising the SWGDRUG Recommendations Ver. 7.1 Parts IIIB/IVB and a stats calculator to complement SD-6. 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

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The Forensic Science Standards Board (FSSB) has provided the opportunity for the OSAC Subcommittees to identify baseline documents and reference materials that best reflect the current state of the practice within their respective disciplines. The Seized Drugs Subcommittee focuses on standards and guidelines related to the examination of evidence to identify drugs and related substances. An update will be given on the activities of the OSAC Seized Drugs Subcommittee including the status of standards and guidelines under consideration for inclusion into the NIST Registry of Forensic Science Standards. Attendees will have an opportunity to ask questions.

A Risk Based Approach to Measurement Uncertainty and Data Integrity in Forensic Drug Analysis

Dave Cirullo, Brian Kear, Tucker Rubino, Mettler Toledo

Every forensic drug analysis begins with one crucial step that plays a major role in determining the severity of a criminal charge – recording the weight of a suspected illicit substance. To ensure this weight is accurate, labs often rely on the quality management system to define a weighing process, which includes proper recording criteria and the determination of measurement uncertainty. A concept that has been overlooked by many forensic institutions is the calculation of the minimum sample weight. This concept provides an additional level of accuracy and ensures proper weighing results.

Weighing is a key step in qualitative and quantitative drug analysis and strongly influences the integrity of the final result. The standout prerequisite for traceable and accurate weighing is the effective calibration of weighing instruments, which also comprises the estimation of measurement uncertainty. Historically, many laboratories set their own calibration procedures due to the lack of nationally or globally recognized calibration guidelines. Based on international cooperation from subject matter experts in the field of metrology, efforts were made to globally harmonize the methodology to calibrate weighing instruments. The major benefit of this harmonization is the ability to estimate measurement uncertainty at the time the instrument is calibrated and also provide guidance to estimate uncertainty during day-to-day usage. This resulted in the calculation of the minimum weight, the smallest amount of net substance that needs to be weighed in order to achieve a specified degree of accuracy. Minimum weight is a key parameter for quantitative drug analysis. It ensures the accuracy of the overall analysis is not negatively impacted by an insufficient sample weight.

To help meet requirements set forth by regulatory organizations it is also important to understand the benefits of incorporating components of the weighing process with an integrated data management system. In recent years an increasing number of assessments and internal audits have revealed incomplete data, the lack of audit trails, and falsification of results. While most labs have turned toward LIMS systems with the idea of replacing the manual workflow, these systems are designed primarily to aggregate result data from an array of analytical tests - not automate and document bench top workflows or bind instrument metadata to the measurement.

Regulatory organizations have recognized both the advantages and limits of electronic data systems, and have increasingly established further controls for the use of such systems all the way down to bench top instruments. The goal of reducing errors, simplifying processes, and reinforcing compliance can become further challenging when trying to directly integrate and automate bench top instruments. As regulators continue to tighten their auditing approaches, it is critical for forensic scientists to understand the key issues surrounding data integrity. This presentation will provide an overview on the harmonization of calibration procedures and the resulting concept of incorporating a minimum weight with measurement uncertainty. It will discuss the criteria for data integrity based on recent guidance issued by various regulatory agencies. It will provide practical solutions to improve data management processes and address many data integrity weaknesses typically found in a forensic laboratory.

Ambiguous Field Test Results Lead to Wrongful Arrest

Heather L. Harris, MFS, JD, D-ABC, Arcadia University

Across the United States daily, police officers conduct searches of automobiles and find suspicious materials. Often these materials are subjected to drug field test kits. A positive result on one of these test kits will lead to arrest for a controlled substance violation. In a number of jurisdictions, a positive result from a field test kit will lead to a guilty plea and a criminal sentence without any laboratory verification of the material's identity ever occurring. The danger of using these drug field tests in this manner is that they are not sufficiently selective to exclude non-controlled substances. In other words, false positives due to the improper use of drug field tests can lead to wrongful arrest and conviction.

This presentation will discuss one such case and the experiments conducted to determine the likelihood of false positives on the particular field test kit at issue.

"A Comparison of Portable Infrared Spectrometers and the Narcotic Identification Kit (NIK) Field Test for the On-Scene Analysis of Cocaine HCl

Dory Leiblein, University of New Haven, Meghann E. McMahon, M.S. Wisconsin State Crime Lab; Pauline Leary, Ph.D Smith's Detection; Peter Massey, M.S. University of New Haven; Brooke W. Kammrath, Ph.D University of New Haven

The majority of inmates across the country are incarcerated for drug related offenses. It is important that the technology used to test for controlled substances is accurate and reliable. For on-scene presumptive testing, the NIK test is most often utilized to test for the presence of a controlled substance. Recently, it has been discovered that there have been numerous cases where false-positive NIK tests at the scene were later disproven via confirmatory lab testing. As a result, there have been hundreds of wrongful convictions (1). This has resulted in press coverage condemning the NIK tests followed by significant public outrage. However, portable infrared (IR) spectrometers can be used to provide accurate and reliable identifications at the scene, with minimal risk of exposure to law-enforcement personnel when appropriate levels of personal protective equipment are employed. Infrared spectroscopy measures the absorption of IR radiation, specifically the vibrations of the bonds between atoms, to determine the structure of a molecule. This research compares the use of portable IR technology with NIK tests to determine which method is better suited for the on-scene analysis of illicit drugs, specifically cocaine HCl.

This research assessed important performance characteristics for each method, including a short- and long-term cost analysis, whether the method is destructive or nondestructive, the ease of use, knowledge and skill required of the operator, speed of analysis, limit of detection, susceptibility to false positives and false negatives, and the effect of common diluents on the ability to identify cocaine. The experimental determination of the limit of detection and the effect of common diluents on the recognition of cocaine HCl used common chemical diluents (e.g., lidocaine, mannitol, and caffeine) as well as common household diluents (e.g., artificial sweetener, and baby formula). A positive result for cocaine HCl with the NIK test was indicated by the appropriate color changes and was documented with photographs. A positive result with the portable IR spectrometer was a "hit" for cocaine using the library search function for the instrument. Manual spectral analysis was conducted as well to identify any instrument false-positive and false-negative results that could be due to the search algorithm used for library matching. Replicates of each analysis were conducted to ensure reproducible results.

This research concluded that although portable infrared spectrometers require a large initial financial investment, their high performance characteristics (e.g., ease of use, rapid analysis, non-destructive, acceptable limit of detection, minimal false positives and negatives) makes them a superior tool than the NIK tests for the on-scene presumptive analysis of cocaine HCl.

References:

(1) Gabrielson, R. (2017, July 14). Houston Police End Use of Drug Tests That Helped Produce Wrongful Convictions. Retrieved from https://www.propublica.org/article/houston-police-end-drug-tests-thathelped-produce-wrongfulconvictions?utm_campaign=bt_twitter&utm_source=twitter&utm _medium=social

Comparison of FTIR and Raman Spectroscopy for Safety and Security Applications

Suzanne Schreyer, PhD, Rigaku Analytical Devices

Raman and Infrared (IR) spectroscopy are mutually complementary techniques used for characterization and identification of materials. Raman spectroscopy utilizes inelastic scattering phenomena in order to determine the molecular fingerprint of a material; while IR spectroscopy is a vibrational spectroscopic method that relies on absorbance or transmittance of energy to generate the molecular fingerprint. Our study focused on the applicability of these two techniques to determine if they can be used interchangeably for hazardous material identification or if there are inherent advantages and disadvantages to either method. Results from instruments that use Raman spectroscopy with various excitation lasers were compared to an instrument using IR spectroscopy. These instruments were run against a common set of chemicals that included both precursors and active chemicals that are of interest in the production and detection of explosives and narcotics. Further, this study was focused primarily on chemicals of interest to hazardous material identification. Substances that are representative of what first responders would encounter were analyzed - including chemicals that are colored or in mixtures, as well as chemicals in a variety of containers.

Screening for Drugs of Abuse at Music Festivals Using a Portable Gas Chromatograph Ion Trap Mass Spectrometer

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The variety of chemical evidence present at crime scenes is often quite extensive. While several analytical techniques have proven to be advantageous in combating the wide-ranging complexity of criminal evidence, limitations in sample throughput and on-site identification have caused forensic laboratories to become plagued by significant backlogs. The implementation of portable Gas Chromatography – Mass Spectrometry (GC-MS) instrumentation would permit on-site evidentiary analysis, expediting criminal investigations and reduce the burden of off-site laboratories. In this study, over 30 samples suspected of containing illicit substances were collected from Shambhala Music Festival (West Kootenay, British Columbia, Canada) and analyzed on-site using Torion T-9 Portable GCMS (PerkinElmer Inc., Shelton, Connecticut, USA) to successfully identify illicit substances. Samples were diluted in methanol and probed with a retractable coiled wire filament (CWF) (1). The CWF extraction device was removed from solution, dried for 10 to 30 seconds and injected directly into the portable GC-MS. The portable GC-MS equipped with a low thermal mass (LTM) gas chromatograph column and ion trap mass spectrometer provided rapid identification of compounds through NIST library matching in less than 5 minutes per sample.

An overview of the instrumentation, collected samples and the suitability of portable LTM GC-MS instrumentation to rapidly screen samples suspected of containing illicit substances will be presented.

References:

(1) Truong, T.V.; Nackos, A.N.; Murray, J.A.; Kimball, J.A.; Hawkes, J.E.; Harvey, D.J.; Tolley, H.D.; Robison, R.A.; Bartholomew, C.H.; Lee, M.L. Sample Introduction in Gas Chromatography using a coiled wire filament. J. Chromatogr A., 1216, 40 , 2009.

Validation and Characterization of an Ambient Sampling, Portable Mass Spectrometer for Field Screening of Forensic Evidence

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The backlog of evidence that currently affects publicly funded laboratories continues to grow, showing the need for higher throughput cost-effective methodologies. A significant gain in throughput can be realized by processing evidence of interest at the point of interest (e.g. traffic stop, crime scene), making portable mass spectrometers (MS) capable of direct sample screening via ambient ionization an interesting solution. Such a technology could not only allow one to rapidly screen for probative value from evidence, but also allow agencies to prioritize which evidence actually required off-site processing. However, any such technology that produces data with inherent legal ramifications must be shown to produce reproducibly valid results. To this end, a SWGDRUG-style validation study has been performed on the FLIR Systems AI-MS 1.2 portable cylindrical ion trap mass spectrometer featuring a custom-built paper spray ionization (PSI) source. Validation categories examined in this work include spectral accuracy, inter/intraday and inter/intra-user variability, reliability (in the form of false positive/false negative error rates), method robustness, and environmental ruggedness. Of emphasis in the presentation will be recent efforts to determine error rates for users of diverse education and experience, showing applicability to non-technical user classes. In an effort to demonstrate reliable and reproducible operation in the field, environmental ruggedness was examined by observing analytical performance at variable wind speed, ambient temperature, and relative humidity.

Psychedelics Leave Their Mark: Establishing Exposure To Psychoactive Plant-Based Legal Highs by MALDI Mass Spectrometric Imaging of Fingermarks

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In the U.S., a major cause of death is fatalities that occur as a consequence of driving under the influence of mindaltering substances. In 2015, ~1.1 million individuals were arrested for driving while impaired, and ~16% of motor vehicle crashes involved drugs other than alcohol such as cannabinoids, opioids, cathinones and other substances. The ability to identify these drugs and their metabolites hinges on the availability of protocols for their definitive identification. However, while there are established methods to test for exposure to most scheduled drugs, few are available for unscheduled mind-altering legal high substances. Cases involving the use of unscheduled psychoactive plants are particularly challenging, as methods for their detection are few or nonexistent. In such cases, although a toxicological screen may show the presence of an unknown substance, the cause of death remains inconclusive because it is not known if the compound is psychoactive and if it contributed to death. For these reasons, it remains virtually impossible to legislate the use of these substances, or to prosecute users and distributers. This situation is exacerbated by the fact that these drugs exist as complex matrices rather than as purified compounds, making the development of identification protocols by conventional approaches (which may involve complex and timeconsuming multi-step protocols) challenging. Therefore, it is essential that methods be developed that will enable efficient establishment of whether or not an individual is under the influence of such substances.

We propose that as a first step, it would be useful to demonstrate that an individual's exposure to a particular psychoactive plant can be established with relative certainty. We demonstrate here that this can be accomplished through the detection of diagnostic small-molecule biomarkers in fingerprints by SpiralTOF matrix-assisted laser desorption/ionization high-resolution mass spectrometry imaging (MALDI-HRMSI). Plant products of several

popularly abused species that have been identified by the United Nations Office on Drugs and Crime as plants of concern, were handled by rubbing the material between the fingers and then depositing a fingerprint, which was subsequently analyzed by MALDI-HRMSI. After analysis, ion images of selected m/z values showed the spatial distribution of molecular species that were indicative of exposure to psychoactive plant biomarkers, including mindaltering components. Among the species handled were Mimosa hostilis, Salvia divinorum, and the San Pedro cactus (Echinopsis pachanoi). Prints analyzed after exposure to these substances were found to contain dimethyltryptamine (DMT), salvinorin B, and mescaline respectively. Furthermore, the ion images of these psychoactive components were identical to those of prints generated by endogenous lipids such as oleic acid. This method not only establishes the presence of psychoactive components, but also provides definitive evidence, through their fingerprint, of an individual's exposure to the psychoactive drug. This approach provides a facile means by which to identify legal highs to which an individual under the influence of drugs has been exposed.

Developing Direct Analysis in Real Time (DART) Mass Spectral Libraries and Reverse Library Search Algorithm for Rapid Screening of Controlled Substances

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Routine screening techniques are employed by crime labs for identifying controlled substances in seized drug samples and determining the samples that need to be further analyzed using confirmatory techniques. These screening techniques commonly include quick colorimetric tests, melting point analysis, and UV or fluorescence spectrometry, all of which falls under Category C in SWGDRUG's categorization scheme. Although these techniques are rapid, they have the lowest potential discriminating power according to SWGDRUG recommendations. In order to increase laboratory efficiency and turnaround time, screening techniques with higher potential discriminating power is desirable. Direct Analysis in Real Time - Mass Spectrometry (DART-MS) is a technique that has been shown to be capable of accurately screening for controlled substances with speeds comparable to techniques such as color tests and FTIR. Since this is a mass spectrometry technique, it can be considered a Category A technique with high discriminating power. Thus far, the Alabama Department of Forensic Sciences and Virginia Department of Forensic Science are two forensic agencies that have adopted DART-MS as a screening tool for controlled substances in their casework. Although effective, an area that has been lacking for DART-MS analyses is the availability of DART-MS libraries.

Currently, the only available DART library for controlled substances and drugs of abuse is the library created by Steiner et al., in 2013; however, this library was created using a high-end mass spectrometry platform and is not up-to-date with respect to many new designer drugs and other substances seen in recent years. It is therefore important to have DART-MS databases that are up-to-date with the most current drugs and based on a cost-effective platform, particularly due to limited resources in forensic laboratories. We therefore demonstrate here a program that enables the creation and search of DART-MS databases using a cost-effective platform. A continual effort is being made to develop the database to include a wide array of controlled substances and drugs of abuse using certified reference materials. Currently, the library consists of approximately 150 substances, which includes primarily opioids, cannabinoids, stimulants, benzodiazepines, and novel psychoactive compounds. Preliminary tests thus far have shown no false negatives and all false positives were attributed to isomeric compounds. The library development process and data processing using the library program will be discussed. Future work includes plans to improve the algorithm to better differentiate isomeric compounds.

LADI Liberty: Development of a Mass Spectrometry Imaging Approach Free of Sample Preparation Steps for Analysis of Psychoactive Substances in Complex Matrices

Kristen L. Fowble, Rabi Ann Musah, PhD, State University of New York at Albany

Although hyphenated mass spectrometric (MS) techniques such as GC- and LC-MS continue to be the mainstays for drug analysis in crime labs, other more advanced MS methods such as mass spectrometry imaging (MSI) are increasingly finding a place in forensic science. For example, this approach has found application in the detection of forgeries in artwork, detection of compounds in fingerprints, and analysis of drugs in hair. However, more widespread use of this method by forensic labs is hampered by the fairly narrow range of samples that can be analyzed by this method, as well as various sample treatment requirements that are defined by the instrument. These numerous sample pretreatment steps can lead to bottlenecks in the analysis of evidence from crime scenes and thus are not practical for routine analysis of forensic samples. Nevertheless, multiple types of information of relevance to solving crimes could in principle be more routinely extracted if this challenge could be resolved.

Here, we describe a new approach to MSI that circumvents some of the challenges inherent in other MSI techniques for the analysis of forensic samples. The technique, termed "laser ablation direct analysis in real time imaging-mass spectrometry (LADI-MS)" combines a direct analysis in real time (DART) ion source with a UV laser, and interfaces this with a TOF-MS. The resulting platform allows for the analysis of a number of different forensic sample types without any sample preparation. It can be performed at room temperature in open air, thereby avoiding the need for high vacuum that is often required for other MSI techniques. Furthermore, there is no need for a matrix or solvent.

Here, LADI-MS was applied to the detection of drugs in fingerprints, and the identification of plant-based psychoactive drugs. With regard to latent prints, the preservation of the fingerprint image often precludes the acquisition of other information contained within the fingermarks, such as materials to which the perpetrator had exposure, prior to committing a crime. These may include "legal" mind-altering substances. LADI-MS enabled facile determination of psychoactive plant-derived drugs in the image of the print. In addition to mapping the spatial distribution of components of "legal" highs in fingerprints, unknown psychoactive plant materials themselves, such as seeds discovered at a crime scene, can be analyzed and identified using LADI-MS. Here, we show the ability to map the spatial distribution of abused compounds such as scopolamine and atropine in Jimson weed (i.e. Datura stramonium) and Datura leichhardtii seeds, and the technique allows the species to be distinguished from one another. The ability to map the spatial distribution of these psychoactive compounds within plant tissue could lead to the regulation of particular parts of plant products. The experiments require no sample preparation as the material can be analyzed directly in its native form.

Forensic Biology Abstracts

Rapid Processing, Normalization and Direct Amplification of Casework Samples

Danielle Brownell, Jeanne Bourdeau-Heller, Robert S. McLaren, Lotte Downey and Douglas R. Storts, Promega Corporation

DNA purification processes typically results in loss of precious sample, particularly in those casework samples with low-levels of cellular material. The Casework Direct Kit provides a method for the rapid generation of extracts from casework samples such as swabs or fabric cuttings, The lysate may be evaluated with the PowerQuant® System to quantify the abundance of human DNA, determine the male/female DNA ratio, predict PCR inhibition, and assess degradation of the DNA. Unlike direct amplification from a punch, extract quantification results can be used to normalize human DNA for STR amplification and aids in selection of the appropriate PowerPlex® Systems (autosomal versus Y-STR analysis) to use. Based on information gained by the PowerQuant® System, samples prepared with the Casework Direct Kit facilitate the generation of high quality laboratory results by directing workflow decisions and minimizing repeat assays and/or sampling. While DNA purification is not required for subsequent STR analysis, if IPC data in the PowerQuant® System indicates inhibition, the lysate may be purified by extraction with the DNA IQ™ Casework Pro Kit for Maxwell® 16. The analyst may choose to stop processing samples flagged as highly degraded.

An Evaluation of QIAGEN Investigator® 24plex GO! Direct Amplification of Body Fluids on Crime Scene Substrates

Marcel Burton and Reena Roy, Ph.D., The Pennsylvania State University, Forensic Science Program, Eberly College of Science

Short Tandem Repeat (STR) Analysis is a technique performed routinely in forensic laboratories. In violent personal crime cases, blood and other body fluids are regularly encountered on various substrates. Direct amplification of these body fluid stains without time consuming, labor intensive extraction and quantitation steps have proven to be useful in forensic science laboratories.

Direct amplification entails amplifying the DNA present in a body fluid stain without extraction or quantitation steps and injecting the amplified product in a capillary electrophoresis system. The QIAGEN 6-dye amplification system, Investigator® 24plex GO!, allows the identification of 22 polymorphic STR loci and includes two innovative internal PCR controls (Quality Sensors). The quality sensors, QS1 and QS2, provide information about whether there is degradation and/or inhibition. These also indicate the absence of DNA or a failed PCR reaction. The current research utilizes this amplification kit to directly amplify minute amounts of human body fluids deposited on various simulated crime scene substrates while the substrates remain in the reaction during amplification. Substrates chosen for placing of these body fluids included various types of fabric such as white cotton, blue denim jeans, and leather, as well as cigarette butts, chewing gum, woodchip, straw, grass and other objects.

Blood and other body fluids were collected from deceased and living individuals following the guidelines of the Office of Research Protection (ORP) at the institution. Each body fluid was diluted as necessary. Only 0.2 µL of each diluted sample was deposited on 1.2 mm punch or cutting of each substrate. In the next step, 5 µL of Investigator GO! Lysis Buffer was added to each punched substrate containing only one type of secretion, and left at room temperature for 20 minutes with occasional mixing.

Each substrate containing only one type of body fluid was then subjected to amplification. For this step, 20 μL of the reaction mixture was added to all tubes and amplification was performed following the recommended protocol. During the amplification step, each substrate containing only one of the body fluids remained in the reaction mixture. Amplified products were injected into the 3130xl capillary electrophoresis (CE) system. GeneMarker® HID analysis software v 2.9 from SoftGenetics® was used for fragment analysis.

All stains created from the four body fluids using simulated crime scene substrates were amplified successfully even when the substrates remained in the reaction mixture during amplification steps. All stains were created as single source samples in order to generate DNA profile from one single donor. Thus no mixture analysis was necessary in this project.

Consistent and concordant profiles were obtained from all of the body fluid-stained substrates. The S peak on QS2 locus occasionally dropped out, indicating inhibition in the sample, even when a complete profile was obtained. Known inhibitors such as soil or dye were present in some of the substrates. Therefore, this observation was not unexpected. In spite of the substrates being present during the thermal cycling steps, the reagents were able to overcome inhibition and amplify DNA from challenging samples.

This study suggests that the Investigator® 24plex GO! is a valuable tool which can be easily incorporated in the analysis of body fluids commonly encountered at crime scenes and analyzed in forensic laboratories. Since there is no extraction and quantitation involved in the procedure described above, the results can be obtained within a very short period of time. This in turn can help find a perpetrator or exonerate the innocent quickly.

Optimization and Validation of a Novel Direct-Lysis Differential Extraction Procedure

Anooja Rai, B.S., Mike Yakoo, M.S., Robin W. Cotton, Ph.D., Biomedical Forensic Sciences Program, Boston University School of Medicine

Forensic analysis of DNA from sexual assault kits is a laborious process. These samples may be a mixture of sperm and male or female epithelial cells (E-cells). Generally, it is the sperm cells that are of greatest forensic value. Since its introduction in 1985 by Peter Gill, differential extraction has remained an essential pre-PCR extraction procedure adopted by most forensic laboratories for the preferential lysis of E-cells and isolation of sperm cells/male fraction prior to DNA profiling. The differential extraction procedure operates based on the packaging of DNA in these two types of cells. The E cells are first lysed by SDS and Proteinase K which leaves the sperm cells intact. The mixture is centrifuged leaving E cell DNA in the supernatant and sperm cells in the pellet. After several wash steps to remove residual E cell DNA, the sperm fraction is then subjected to lysis using SDS, proteinase K, and dithiothreitol (DTT). DTT reduces the disulfide bonds present in the sperm nucleus, thereby releasing sperm cell DNA. This procedure, while proven to be highly effective in providing two separate fractions for a simplified interpretation of profiles, is a labor intensive and time-consuming process. Furthermore, the resulting fractions must be subjected to additional pre-PCR DNA purification procedures to remove PCR inhibitors such as SDS and Proteinase K which inevitably result in varying degrees on DNA loss. Progress has been made over the years to introduce methods that allow for PCR-ready lysates without additional purification steps, often referred to as direct lysis methods. However, none have been proven to be viable options for use in sexual assault samples. Our laboratory has developed a novel differential extraction procedure that is not only timeefficient and less laborious but also utilizes a direct-lysis procedure requiring no further pre-PCR purification for most samples. The novel procedure uses ZyGEM, which contains the thermophilic EA1 protease proven to effectively digest biological samples and produce PCR-ready lysates suitable for downstream nucleic acid amplification, thereby minimizing DNA loss. The procedure uses a multi-enzymatic approach and utilizes the different optimum activity temperatures of the enzymes to perform most of the process in a DNA extraction lab

thermocycler, requiring only a single centrifugation for the usual separation of the epithelial cell fraction and no subsequent washing steps for the sperm cell fraction. The procedure is currently being optimized and validated. It has the potential to be a rapid, robust procedure that should be easily implemented in any forensic laboratory. The talk will describe the procedure and report progress in the procedure optimization.

Characterizing Double-Back Stutter In Low To Multi-Copy Number Regimes In Forensically Relevant STR Loci Jennifer L. Sheehan, Kelsey C. Peters, Biomedical Forensic Sciences Program, Boston University School of Medicine; Catherine M. Grgicak Ph.D., Biomedical Forensic Sciences Program, Boston University School of Medicine and Department of Chemistry, Rutgers University

Mixtures are commonly encountered in forensic casework, and the presence of artifacts can lead to uncertainty and require the implementation of probabilistic interpretation strategies to manage the uncertainty. One commonly encountered artifact, stutter, is the result of strand slippage during PCR extension. Reverse stutter, also known as back stutter, is prevalent and is one repeat unit shorter (n - 1) than the template strand. Given the strange slippage mechanism, if a reverse stutter amplicon is produced then the generation of stutter product from stutter is also likely. This artifact, usually referred to as double-back stutter (DBS) or n - 2 stutter, is important to characterize given that its presence may obfuscate signal acquired from minor contributors to a mixture.

Recently there has been interest in examining signal approaching baseline levels. As the sensitivity of the process improves, so does the probability of detecting DBS. Therefore, studies that examine the peak height distributions, rarity, stutter signal-to-noise distances and the general impact of DBS on the signal are warranted. The work presented herein builds upon a preexisting dynamic model and generates synthetic DNA profiles comprised of 21 autosomal STRs consistent with the GlobalFilerTM multiplex. The expansion of the model included the incorporation of a three-type Galton-Watson branching process allowing DBS to be added to the simulated electropherogram (EPG).

The in silico model was used to simulate the amplification of a 1:43 and 1:73 mixture at a total DNA concentration of 0.3 and 0.5 ng, respectively, and the effects of DBS on the signal from the minor contributor were evaluated. At 0.3 and 0.5 ng both the noise and stutter signal histograms are rightskewed and a Kolmogorov-Smirnov (KS) test indicates that the noise and DBS were significantly different (p-value < 4x10-6). The average peak height of DBS for all loci in both scenarios were less than 50 RFU (Relative Fluorescence Units) and the DBS median ratios were less than 0.5%. In addition, a per locus analytical threshold (AT) was calculated for both the 0.3 and 0.5 ng targets. The percentage of DBS peaks greater than AT ranged from 0 to 7.08% and 0 to 10.50%, respectively. When the AT was increased the rate at which DBS was detected reduced to 0 to 1.08% and 0 to 0.17%, respectively. This demonstrates that DBS from an extreme major using these laboratory conditions is not in the same signal regime as the signal from alleles; however, signal from DBS was significantly different from noise and surpassed the AT in a number of cases. Therefore, laboratories should carefully consider the laboratory conditions, DBS detection rates, DBS resolution from baseline and allele signal and the software expert pair when determining if DBS ought to be considered during interpretation.

CleanIt: An automated procedure for filtering electropherogram artifacts

Lauren E. Alfonse, Biomedical Forensic Sciences, Boston University School of Medicine; Catherine M. Grgicak Ph.D., Biomedical Forensic Sciences, Boston University School of Medicine, Department of Chemistry, Rutgers University, Center for Computational and Integrative Biology, Rutgers University

The analysis of forensic STR data frequently entails manual removal of well-characterized artifacts arising from PCR or spectral overlap. Several factors indicate that the forensic DNA processing pipeline is trending towards big data and

that an automated approach to artifact filtering would therefore be advantageous. First, the implementation of robotic and high throughput technologies has led to an increase in the amount of data being generated. Second, with the advent of probabilistic genotyping systems capable of utilizing baseline signal, high analytical thresholds (ATs), designed to mitigate the effects of false noise detection, are rapidly being replaced by lower ATs; this has resulted in an increase in the number of peak detections (though a decrease in overall detection error rates). Third, validation of increasingly sensitive technologies and comprehensive mixture interpretation schemes has begun to require complex, large-scale datasets inclusive of low template samples containing many peaks. Given the above, it is likely that data management — whether for purposes of casework, research, or validation, will require automated solutions. To fill the gap between the ability to generate large datasets and the capacity to efficiently process signal artifacts in such datasets, we describe CleanIt, a visual basic script that filters pull-up, complex pull-up, and minus A. CleanIt, which runs in the Microsoft® Excel environment and works on comma-separated values files exported from any genotyping software, presents a straight-forward, automated approach to artifact filtering. The user inputs the data of interest, selects which artifacts to remove, and provides values for the relevant parameters. Automated filtering then proceeds by comparing the base pair size and RFU intensity of the suspected artifact peak to the peak-ofinterest, or parent peak, for all loci in a given profile; artifact peaks which fulfill the size and intensity criteria specified by the user are removed from the data. CleanIt has been tested on data generated with commercially available 16- and 24-locus STR amplification kits which target tri-, tetra-, and pentanucleotide repeat loci, and thousands of profiles have been analyzed in one run. Total run time depends on both the size of the dataset (i.e., number of loci per profile and total number of profiles) and the computing power of the system. For example, analysis of 96 megaplex STR electropherograms proceeds in approximately forty minutes on a standard 2.6 GHz dual- core laptop. By translating a series of steps present in a laboratory SOP to a computer script, we have developed an automated procedure for filtering electropherogram artifacts which reduces handson time for the analyst and confers sample-to-sample consistency throughout a dataset.

Production of High-Fidelity Electropherograms Results in Improved and Consistent Match-Statistics: Standardizing Forensic Validation

Catherine M. Grgicak, Ph.D., Rutgers University and Boston University School of Medicine; Kelsey C. Peters, Harish Swaminathan, Lauren E. Alfonse, Boston University School of Medicine; Ken R. Duffy (NUIM, Ireland); Desmond S. Lun, Rutgers University

Samples containing low-copy or complex DNA mixtures are routinely encountered in operations. The signal acquired from these sample types are difficult to interpret as they do not always contain all of the genotypic information from each contributor, where the loss of genetic information is associated with sampling and detection effects. The present work focuses on developing a validation scheme to aid in mitigating the effects of the latter to produce high-fidelity electopherograms (EPGs) that can be effectively interpreted by all probabilistic genotyping systems.

To this end, we have devised a computational system, named RESOLVIt (Resolving Evidentiary Signal for Objective Laboratory Validation), that generates synthetic EPGs in a laboratory-specific manner. As an input to the system, a large number of single source profiles of known genotype are provided by the laboratory. From these data, the distribution of the peak heights at noise positions is modeled as a function of the starting template amount using a log-normal distribution. The electrophoresis sensitivity, which is used to generate the DNA height distribution, is also acquired from the single source experimental data procured from the laboratory. Other pertinent laboratory conditions, such as the number of PCR cycles, injection time, starting template mass, etc. are input parameters and are easily modified by the user.

Since RESOLVIt utilizes a simulation approach, which is based upon experimental data acquired from the laboratory, multifarious scenarios may be explored by each laboratory in a cost-effective manner. Metrics such as signal1copy-to-noise resolution, false positive and false negative signal detection rates are used to select tenable laboratory conditions that result in high-fidelity signal in the single-copy regime. We demonstrate that the metrics acquired from simulation are consistent with experimental data obtained from two capillary electrophoresis platforms and various injection parameters. Once good resolution is obtained, analytical thresholds can be determined using detection error tradeoff analysis, if necessary.

Decreasing the limit of detection of the forensic process to one copy of DNA is a powerful mechanism by which to increase the information content on alleles from minor components of a mixture, which is particularly important for probabilistic system inference. By utilizing another fully continuous probabilistic system, CEESIt (Computational Evaluation of Evidentiary Signal), we demonstrate that if the forensic pipeline is engineered to produce high-fidelity EPG signal then the likelihood ratio (LR) of a true contributor increases and the probability that the LR of a randomly chosen person is greater than one decreases. CEESIt has been developed to not only compute the LR but also the probability that the LR is greater than one for millions of randomly chosen contributors, making it a powerful validation tool. This systematic, in-silico, laboratory-specific, computational-based approach to improve allele information content is, potentially, the first step towards standardization of the bio-analytical pipeline and DNA validation process across operational laboratories.

Storage of Amplified DNA on FTA Cards

Jillian Fesolovich and Michael Chermak, Keystone College

A quote by Joe Nickell states, "All objects in the universe are unique. No two things that happen by chance ever happen in exactly the same way. No two things are ever constructed or manufactured in exactly the same way. No two things wear in exactly the same way. No two things ever break in exactly the same way." As forensic scientists, we know his statements to be true. In forensic biology, no two swabs, even of the same evidence, will produce the same genetic profile with the same peak heights.

Commercially available DNA storage cards, such as FTA® and FTA® Elute, have been used by crime laboratories for convenient sample collection, purification, and storage. Direct sample processing and their ability for sample storage at room temperature make this product attractive to high throughput laboratories. This project aims to determine if amplified DNA products can be stored on FTA® and FTA® Elute cards and how this impacts the sample upon eluting the amplicons. Although forensic biologists always aim to retain as much sample material as possible for future testing or testing and analysis by another lab, this can often be difficult. With new approaches to testing low copy number DNA samples; including decreasing the DNA extraction elution volume, the entire extracted DNA sample may be added to the PCR reaction. If this is the case, no more of that sample remains for further testing. It has been observed that amplified products begin to evaporate with refrigeration within 1 week, thus you will not get reproducible results through capillary electrophoresis separation. If amplified products can be stored on FTA® paper after receiving passing CE results, this amplified sample can be retained and stored at room temperature for future analysis.

In this research project, multiple amplified samples of control DNA were pooled and deposited on FTA® and FTA® Elute cards. Stored amplicons were eluted off at pre-determined time points. This presentation will include up to 3 months of storage time. Newly developed elution protocols will be described for each DNA preservative card. Other forensic and non-forensic applications of this storage approach will be described.

STRMix™: The Journey from Validation to Casework

Cristina L. Rentas, DNA Labs International

Over the past several years, the introduction of probabilistic genotyping software has had a dramatic impact on the forensic science community. Since probabilistic genotyping software options have become available, DNA mixtures that were previously deemed inconclusive are now capable of being fully resolved. Previous statistical models, such as random match probability are still acceptable; however, probabilistic genotyping software can now consider the entire DNA profile including additional parameters such as peak heights, mixture ratios, stutter contribution and allele sharing.

In an accredited forensic laboratory, it is a requirement that internal validations are performed when new technologies are brought online prior to implementation into casework. In June of 2015, the Scientific Working Group on DNA Analysis Methods (SWGDAM) published guidelines for the validation of probabilistic genotyping systems in order to assist the forensic community in bringing this new technology online to a reliable standard. In December of 2015, STRmix™ v2.4 was validated at DNA Labs International (DLI) for use with the Applied Biosystems AmpFLSTR® Identifiler® Plus PCR amplification kit in accordance with the published SWGDAM guidelines. The first reports at DLI utilizing probabilistic genotyping software were issued in January of 2016. However, the initial internal validation and report releases were the tip of the iceberg for the changes that would be implemented over the next 18 months.

Following the completion of the initial internal validation and the training of the senior DNA analysts, it became necessary to decide how the program would be implemented into casework. Since DLI has a numerous clients throughout the United States and Caribbean with different technologies online, it was decided that the previously utilized statistical methods would still be retained as an option within our standard operating protocols. Once it is determined that a particular profile is inconclusive using previous statistical models, our analysts evaluate several aspects of the mixture in order to determine if a sample is eligible for additional analysis with probabilistic genotyping. These aspects include at a minimum; evaluating the potential number of contributors, how many loci have data and the overall quality of the profile.

With the expansion of the new core CODIS STR loci, effective January of 2017; STRmix™ v2.4 validations were also undertaken at DNA Labs International for use with the Applied Biosystems Globalfiler™ and the Promega PowerPlex® Fusion 6C PCR amplification kits. Additionally, the Applied Biosystems AmpFLSTR® Identifiler® Plus PCR amplification kit was validated with the STRmix™v 2.4 software package. Validations plans are in place for STRmix™ with the Qiagen Investigator® 24plex QS system. To further add to the massive validation undertaking, all systems are slated to be validated with the pending STRmix™ v2.5 release.

In addition to the challenges of validation and implementation, one of the greatest hurdles that analysts face is how to properly report the probabilistic genotyping results and present this technology in the courtroom. The entire journey our laboratory undertook from the beginning stages of validation to the final implementation into casework and court testimony will be discussed.

A Comparison of Quantitative Efficiencies of the ABI 7500 SDS (Real-Time PCR) versus NanoDrop Instrumentation

Raeghan Steelberg and David San Pietro Ph.D., University of New Haven

Accurate DNA quantitation is important in the analysis of forensic DNA evidence to properly amplify and develop a profile and is currently achieved using RT-PCR. The Thermo Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer is a more cost-effective method for quantifying DNA. The ABI 7500 SDS RT-PCR instrument has been found to be very sensitive and requires minimal sample, but there is a high cost associated with the running of each sample. The extraction kit alone costs approximately $3.20 for each reaction. The Nanodrop is established for its ability to measure the absorbance of small volumes, having a wide detection range, providing quick measurement, all with low cost. This study is a comparison of the precision of the NanoDrop One to the Applied Biosystems 7500 Sequence Detection System (SDS). Following IRB approval, dilution series ranging from 0.625 ng of total DNA to 10 ng of total DNA were prepared using extracted buccal samples. Replicates of the dilution samples were measured on both instruments for a total of 100 samples. The results showed that the RT-PCR method is more precise and thus better suited for low level forensic samples. The average deviation of the measured concentrations of each sample for each dilution concentration ranged from 0.13 to 0.23 ng of DNA for the NanoDrop results. The RT-PCR results showed an average standard deviation range for the same samples of 0.008 to 1.9 ng of DNA. The NanoDrop was determined to have a more consistent variation in its measurements for all the dilution concentrations, while the precision of the RT-PCR method increased as the amount of DNA decreased. As the nature of forensic evidence is minimal in quality, concentration, and quantity, confidence in quantification is critical. It is possible that the NanoDrop is sufficient for the quantification of DNA in exemplars since there is inherently more DNA usually present, but further research would need to be performed to assess the accuracy of the NanoDrop with lower level evidentiary DNA samples.

An Assessment of the Effect of PreCR™ DNA Repair Treatment on Mixture Ratios in Two Person Mixtures

David San Pietro, Ph.D., University of New Haven

The nature of forensic specimens often exposes them to environmentally harsh conditions and various degradative factors. This is most often the case with biological specimens. It has been observed that these conditions have direct consequences on the ability to obtain useful interpretative information.

Enzymatic "cocktails" have been developed that mirror the in vivo repair mechanisms associated with cellular DNA damage and its repair. The PreCR™ cocktail, made by New England BioLabs, was developed as a treatment kit to repair damaged regions allowing for STR analysis. Initial studies performed to assess the efficacy and fidelity of the PreCR™ system focused on single source samples.

In this study, DNA extracted from known buccal samples was combined into two component mixture samples. These were subjected to UV exposure prior to their amplification with the Promega PowerPlex® 16HS amplification kit, and subsequent capillary electrophoresis on the ABI 3130xl instrument. Damaged samples were subjected to enzymatic repair treatment and retested to assess the amount of repair. Data showed that there is fidelity associated with the application with profile concordance after its use, and a corresponding increase in the amount of recovered alleles post damage. Results also showed changes in the stochastic relationship between mixture components that appear to be induced by the repair process itself. The mixture ratios of DNA samples were altered from an approximate original 1:3 ratio, to a ratio of 1:2 or greater. This variation can have a significant effect regarding the ability to reliably de-convolute DNA mixtures that have been subjected to the repair process. The application of this process will also be discussed in light of the more recent advances in the area of probabilistic genotyping. With consideration, this process may find itself a valuable addition to the casework analyst's "toolbox".

High-throughput DNA sequencing of environmentally insulted latent and partial bloody fingerprints after visualization with the nanoscale columnar-thin-film technique

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The National Research Council has recommended the determination of the underlying scientific basis of forensic testing procedures. Identification failures, particularly of suspects, occur for a variety of reasons. Fingerprint matches are impeachable, with approximately 10% error in repeatability and substantial inter-examiner variability for challenged samples. Both fingerprints and DNA evidence degrade due to environmental insults such as temperature and humidity. This proposal is aimed at using nanotechnology for development of environmentally insulted latent (EIL) fingerprints and analysis of DNA from partial bloody (PB) fingerprints on forensically important substrates. The nanomaterial used in the columnar-thin-film (CTF) has been shown not to be detrimental for short tandem repeat (STR) DNA analysis. A pilot study with 144 fingerprints and post-developmental ageing of prints indicated that nanomaterials in CTF did not produce any noticeably different degradation pattern when compared to undeveloped fingerprints. The basic-science goals of this project are to determine the currently unknown effects of ageing, relative humidity, and temperature on (i) fingerprint residue emulsion topology, (ii) the CTF development of fingerprints, and (iii) the Human Identity (HID) SNP Genotyper Report from DNA analysis of cellular material in fingerprints. The effects of CTF development on the SNP genotypes will also be determined. The applied-research goals are to determine (i) the adequacy of the CTF development of latent fingerprints subjected to real-world conditions, (ii) to determine concordance and identity from sequence data from EIL and PB fingerprints that have been either CTF-developed or not, and (iii) the efficacy of identity determination from a combination of fingerprint visualization and SNP profile. Finally, the design of a field-deployable CTF system is a subsidiary goal.

Tracking the Sexual Assault Kit Backlog

Kallie Crawford, B.A., Lyndsie Ferrara, M.S., Forensic Science & Law Program, Duquesne University

The backlog of untested sexual assault kits is a national problem. Numerous federal funding opportunities offer the forensic science and law enforcement communities valuable resources needed to test the kits, but issues still remain. The majority of resources are focused on the collection and testing of sexual assault kits, but the tracking of the kits has not been a primary focus. This research highlights improvements that can be made to better understand the current backlog and improve the future processing and tracking of kits. Given the lack of a universal evidence tracking database among agencies, tracking sexual assault kits seems impossible. In Allegheny County, over 100 different law enforcement agencies exist, each with their own policies and procedures. As a result, the number of untested kits is unknown. Through a comprehensive review of improved practices in proactive jurisdictions, including Ohio, Houston, and Detroit, valuable data was gathered about improved tracking mechanisms. Additionally, interviews with key stakeholders identified issues in Pennsylvania. This information led to the development of a survey that will aid in data collection related to sexual assault tracking practices across the country. A multidisciplinary, collaborative approach is needed to better understand the true sexual assault kit backlog in order for agencies to more effectively use grant funding aimed at testing the kits. The results of this research will provide valuable information to enhance sexual assault kit tracking methods.

Autosomal STR Variation in Four Garifuna Subpopulations on the Honduran Coast

Megan Gillespie, Kristine G. Beaty, Norberto Baldi, Edwin Herrera-Paz, Michael H. Crawford, and Reena Roy, Penn State University Forensic Science Program

The Garifuna are an Afro-Caribbean population group who are descendants of escaped slaves that intermarried with the West African, Central African, Island Carib and Arawak people. Four villages of the Garifuna can be found in Punta Gorda, on Roatan, and Cristales, Rio Negro, and Santa Fe on the Honduran coast.

Short Tandem Repeat (STR) DNA profiles are routinely used in forensic and paternity cases. The purpose of this study was to generate autosomal STR profiles from blood samples collected from the four villages using the PowerPlex® Fusion 6C System. This kit is a six-dye fluorescent amplification system allowing for the simultaneous detection of 27 loci. These include 23 autosomal STRs, three Y-STRs and the amelogenin locus.

This research was a collaborative effort between The Pennsylvania State University Forensic Science Program, University Park, Pennsylvania and Laboratories of Biological Anthropology, University of Kansas, Lawrence Kansas. Other institutions involved in this project include the Facultad de Medicina, Universidad Católica de Honduras, Campus San Pedro y San Pablo, San Pedro Sula, Honduras, the Escuela de Anthropología, Universidad de Costa Rica, San Pedro, San José, Costa Rica and the Dirección de Medicina Forense, Ministerio Público de Honduras, Tegucigalpa M.D.C., Honduras.

Blood samples from the four villages were collected by the institutions mentioned above, each organization following their Office of Research Protection (ORP) guidelines. Each blood sample was deposited on one of three types of paper substrates. These substrates included large and small FTA cards (containing lysing agents) and non-FTA filter papers (containing no lysing agents). All donors and samples were anonymized and each bloodstain was labeled with a unique identifier. An approximate 1.2 mm cutting of each bloodstain was extracted using the EZ1 Advanced BioRobot and EZ1® DNA investigator kits from QIAGEN. After quantification of DNA in the extract, an appropriate amount of DNA was amplified with the PowerPlex® Fusion 6C System following the recommended protocol and a Veriti® 96-Well Thermal Cycler from Applied Biosystems. DNA fragments were separated on a 3130xl Genetic Analyzer and analysis of the generated profiles was accomplished with GeneMarker® HID analysis software version 2.9 from SoftGenetics®. Profiles were successfully generated from the bloodstains collected from inhabitants of all four villages on all three types of paper substrates. Genetic diversity of the villagers living in Punta Gorda, Cristales, Rio Negro and Santa Fe will be discussed.

The RoarPlex - A Novel Tetranucleotide Microsatellite and Sex-ID Panel

Colton L. Ames, Nickolas P. Walker, B.S., Lisa Ludvico, Ph.D., Jan E. Janecka, Ph.D., Duquesne University

The snow leopard (Panthera uncia) is an elusive species native to the mountainous regions of Central and South Asia. Targeted for its fur and bones or to protect the livestock they prey upon; the snow leopard is an endangered species that requires immediate conservation action. Due to their cryptic nature, research based on direct observations required for effective conservation is problematic and can be supplemented by noninvasive surveys and genetic analysis. A multiplex containing 8 tetranucleotide microsatellite markers and a sex-determining region Y marker was developed to aid in the investigation of illegal killings of snow leopards and assist with population abundance and distribution studies

on a larger geographic scale. Previously, studies relied on dinucleotide repeats that were complicated to score and difficult to combine across data sets. Dinucleotide microsatellite markers are vulnerable to errors associated with high stutter percentages and misinterpretation of adenylylation stutter. The previous panel also included only 4 microsatellite markers in one reaction. Tetranucleotide repeats drastically reduce the errors caused by slippage and decrease the visual impact of adenylylation stutter, making for unambiguous allele interpretation. The addition of more microsatellite markers in one reaction exponentially increases the individual identification information available, a component comparable to CODIS increasing the number of core loci accepted. An allelic ladder was developed to ensure accurate allele designation across laboratories, with validation according to SWGDAM and ISFG recommendations. To accomplish the multiplex design, 16 tetranucleotide microsatellite markers originating in the domestic cat (Felis catus) were screened. Results indicated that 15 markers could be successfully amplified using the available primer sets due to common ancestry and similar genome structure. Using M13 labeling, the 15 amplified microsatellites were fluorescently tagged and genotyped. Resulting peaks of the successful microsatellites were more distinct and allele calling was simplified. An informative multiplex containing 8 of the most optimal microsatellite markers was constructed through selective data analysis, primer redesign, and compatibility determination. A sex-determining region Y marker was inserted into the reaction mix to simultaneously accomplish sex identification. The tetranucleotide panel provided more information, including sex, in one reaction, reducing the cost, error, and time required to perform the assay. The enhanced panel and allelic ladder simultaneously improved research methods, assisted with transboundary initiatives, and enabled data sharing thereby increasing the impact of population studies. Despite multiplex design being focused on the snow leopard, initial studies indicate that the RoarPlex could be successful in obtaining the individual identification of other large felids including the bobcat (Lynx rufus), lion (Panthera leo), and the cheetah (Acinonyx jubatus), expanding its utility. Due to the constantly evolving and largely emerging nature of wildlife forensics, the RoarPlex could have a tremendous influence on future studies involving large felid species.

Development of the Field Isolation and Amplification of DNA Assay (FIA-DNA) kit: A Revolutionary Method for Species Identification of Unknown Samples

Nickolas P. Walker, Jan E. Janecka, Ph.D., Lisa Ludvico, Ph.D., Duquesne University Dept. of Biological Sciences

A major challenge in biodiversity conservation is the identification of unknown samples collected in the field. Most endangered species are elusive; therefore, many monitoring programs rely on noninvasive sampling using scat or hair. One difficult hurdle is identifying unknown biological samples in the field due to the reliance on genetic analysis in a laboratory. The use of specialized software and equipment is generally tied to labs located in developed countries such as North America, Europe, India, and Japan. However, sample collection and management are often carried out in rural and developing countries in Africa, South America, Asia, and remote areas of North America where most large animal populations remain.

This can cause delays in obtaining important information and is often infeasible for many on-theground conservation efforts with limited resources. Because there is no method of on-site species identification for these samples, money and time are wasted sending non-target samples to labs for expensive and time-consuming analyses. Therefore, our goal was to develop a Field Isolation and Amplification of DNA Assay (FIA-DNA) Kit for genetic field identification of species for both international conservation efforts and management of Pennsylvania wildlife without the need for PCR and agarose gel electrophoreses.

Once successfully implemented, the FIA-DNA Kit will allow field researchers and wildlife managers to perform a simple genetic analysis for species identification without requiring samples to be sent to a laboratory. The FIA-DNA Kit makes use of Loop-Mediated Isothermal Amplification (LAMP) and visual product detection using calcein, a novel

technique which eliminates the need for a thermal cycler for DNA amplification and gel electrophoresis detection. This technique was coupled with a modified extraction protocol using Whatman® Non-Indicating FTA Elute Micro Cards, allowing for portability and ease of use outside of the laboratory setting. Preliminary results tested efficacy on snow leopard (Panthera uncia), bobcat (Lynx rufus), and coyote (Canis latrans) samples for species-specific identification. Our method using LAMP amplification of DNA has exhibited 100% specificity for over 30 previously extracted Snow Leopard scat samples. Coupling LAMP amplification with the modified FTA extraction protocol has yielded successful, repeatable detection in over 50% of tested scat samples to date. This method can be completed in under one hour with minimal equipment, using UV fluorescence within the reaction tube to confirm the species identification.

Due to LAMP amplification's ease of use, rapid reaction time, and isothermal conditions which do not require a thermal cycler, this kit can be used in the field to effectively identify samples at a species level. The ability to pre-screen samples will also increase the efficiency and turnaround time for the genetics laboratory. Because our FIA-DNA Kit is easily modified to test for other species by developing primers, it will prove invaluable for other time-sensitive applications including population surveys of game species, poaching cases, and outdoor crime scenes involving unidentified biological samples. Due to the expanding field of wildlife forensics, the FIA-DNA Kit holds endless potential for wide applicability across genetic and forensic disciplines.

Dissolution of Organic Remains in Nitric Acid

Brianna Hill, Maria Panepinto, R. Christopher O'Brien, Ph.D., University of New Haven Center for Wildlife Forensic Research

There are several documented cases in which people dissolve bodies in acid to avoid personal identification of the body. These cases run from historic to modern and span the globe. However, there has been little published research in this area. Published research has only been done on constituents of a body. The research that has been published ranges from examination of teeth dissolved in household chemicals to examination of microscopic residues using Scanning Electron Microscopy. These experiments can be classified as studies of forensic taphonomy. While taphonomy is the study of decay over time, forensic taphonomy focuses on the effects of the environment on the decay. This research aimed to put a process that scientists have conflicting theories about into a harsh environment, nitric acid, to observe the effects. This was accomplished by dissolving full squirrel, chipmunk, and rabbit carcasses in nitric acid over specific time intervals. At predetermined times, the remains were removed from the acid and weighed. In addition, individual constituents of remains were also dissolved in acid over specific time intervals. The change in weight over time for each animal and material were analyzed statistically. This study was a proof of concept study that determined that the type of material and weight group of organic remains affected the rate of dissolution of nitric acid. This study also determined that the type of animal does not affect the rate of dissolution of organic remains in nitric acid. The future implication of this research includes the proof of concept that nitric acid can dissolve organic remains, and the ability to quantify the process of dissolution in nitric acid using time and percent change in weight.

Investigation and Comparison of mtDNA heteroplasmy rates across various haplogroups using massively parallel sequencing

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Massively parallel sequencing (MPS), a high-throughput form of next generation sequencing, allows increased resolution of mitochondrial (mt) DNA heteroplasmy and is at the forefront of efforts to expand the utility of forensic mtDNA typing. Maternally inherited mtDNA is present in hundreds to thousands of copies within one cell, has a high mutation rate, and passes through multiple bottlenecks. These factors allow for a range of variant percentages in the mtDNA sequence. Maternally related individuals will share the same collection of major variant single nucleotide polymorphisms and insertions/deletions called the mtDNA haplotype. Certain haplotypes are common in population groups and may be shared among unrelated individuals1. In line with this, a haplogroup consists of similar haplotypes which have risen from related ancestral lineages. Heteroplasmy is a heterogeneous collection of sequence variants in the cytoplasm of the cell. Heteroplamic rates from a population of European haplogroups (for example, H, J, K, T and U) have provided evidence that heteroplasmy is relatively common in the control region (CR) of the mtgenome; greater than 40% of Europeans have SNP-based heteroplasmy. It is hypothesized that there is potential for differences in rates of heteroplasmy linked to population haplogroups, based on assumption and empirical observation that the position and rate of heteroplasmy may be linked to the haplotype sequence. This presentation will discuss a comparison of findings from the European study to preliminary findings of a current project which used a robust MPS approach to measure, analyze, and report rates of heteroplasmy on a per sample and per nucleotide basis; 550 samples reporting to be European (NIJ-2014-DN-BX-K022) and 750 samples in population groups reporting to be non-European (NIJ-2016-DN-BX-0171), respectively. Buccal cells were collected unrelated individuals of European and non-European descent, and MPS analysis was conducted on the CR using an Illumina Miseq with Nextera XT library preparation and 300X300 paired-end reads. Secondary data analysis was performed using GeneMarker® HTS software to evaluate haplotype and heteroplasmy, and Haplogrep to determine haplogroups. The forensic science community requires further validation of MPS analysis of mtDNA to encourage adoption of this technology into working laboratories and investigations. These projects utilized a reliable MPS workflow developed within our laboratory, along with effective software for evaluation of MPS mtDNA data. Implementation of this optimized procedure combined with establishment of rates of heteroplasmy across the CR in various haplogroups will significantly enhance the accessibility to, and discrimination potential of, mtDNA typing.

1. Carracedo A, Bär W, Lincoln P, Mayr W, Morling N, Olaisen B, Schneider P, Budowle B, Brinkmann B, Gill P, Holland MM, Tully G, Wilson M. DNA commission of the International Society of Forensic Genetics: guidelines for mitochondrial DNA typing. Forensic Sci. Int. 2000;110:79-85.

Impact of DNA degradation on MPS-based autosomal STR, iiSNP and mitochondrial DNA typing systems: simulations and application to skeletal remains from the 9th to 18th centuries

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Biological samples exposed to environmental insults for extended periods of time, including skeletal remains, exhibit increasing levels of DNA damage and fragmentation. Human identification methods typically use a combination of mitochondrial (mt) DNA sequencing and short tandem repeat (STR) analysis, which require target segments of DNA ranging from 80 to 500 base pairs (bps) in length. Templates in the upper range are often unavailable as samples age and the associated DNA degrades. Analysis of single nucleotide polymorphism (SNP) loci may serve as an answer to the problem, as they can be typed by targeting templates in the lower end of the range. Recently developed assays for

STR and SNP analysis using a massively parallel sequencing (MPS) approach, such as the ForenSeq kit from Illumina, offer a means for generating results from highly degraded samples as they target templates as small as 60 to 170 bps. We performed a modeling study that demonstrates that SNPs can increase the significance of identification when analyzing highly fragmented DNA down to an average size of 100 bps, and for input amounts between 375 pgs and 1 ng of nuclear (n) DNA. An improved extraction method for DNA from skeletal material was used to assess the performance of the Illumina ForenSeq (n=14) and Promega PowerSeqTM Mito (n=70) kits on remains from the 9th to the 18th centuries, resulting in successful coverage of 99.29% of the mtDNA control region at 50X coverage or more, and partial autosomal STR and identity informative (ii) SNP profiles. These findings aligned to expectations resulting from the modeling study.

Toxicology Abstracts

The Investigation into the Analysis of Narcotic Analgesics in Postmortem Blood using Biocompatible Solid-Phase Microextraction (BioSPME)

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The United States has seen an increase in the misuse and abuse of narcotic analgesics and other drugs in recent years. The number of drug-related deaths has become larger due to this slow motion mass disaster. Majority of the drug-related deaths that occur are due to narcotic analgesics. Forensic pathologists have the responsibility of collecting various postmortem samples which are then sent to a toxicology laboratory to be analyzed for prescription drugs, illicit drugs and poisons. This process can be time consuming and may result in a backlog, hindering a potential criminal investigation. The use of BioSPME fibers can be a solution to this problem. A small coated fiber can be directly injected into a biological matrix and absorb any drug present without the interference of macromolecules. This process allows for a faster time frame, from autopsy to toxicological results. A BioSPME method has been developed to analyze nine narcotic analgesics, 6-monoacetylmorphine, codeine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone and oxymorphone in postmortem blood using BioSPME fibers followed by GC-MS screening analysis and LC-MS/MS confirmation analysis. BioSPME fibers were conditioned, directly injected into blood, washed, filtered, desorbed into solution, dried down and reconstituted. GC-MS screening analysis was performed using splitless injection on Rxi-5SilMS column (30.0 m x 0.25 mm, 0.25 μm) in the SIM mode. Samples were confirmed using an AB SCIEXTM 3200 QTRAP® triple quadrupole mass spectrometer with electrospray ionization (ESI) source in the positive ion mode. Liquid chromatography was performed on a Shimadzu® LC system using an Ascentis® Express Biphenyl column (50 mm x 2.1 mm, 2.7 μm) with the weak mobile phase of 0.1% (v/v) formic acid in water and the strong mobile phase of 0.1% (v/v) formic acid in acetonitrile. The flow rate was 0.30 mL/min, column temperature was 30o C, injection volume was 1 μL, and an analysis time of seven minutes per sample. This BioSPME method was developed using bovine blood and then applied to 43 postmortem blood samples from the Lehigh County Coroner's Office (Allentown, Pennsylvania).

2D LC/MS-MS Analysis of Synthetic Cannabinoids in Urine, Plasma and Edibles

Kayla Benvenuto, M.S., Boston University School of Medicine, Sabra R. Botch-Jones, MS, MA. D-ABFT., Boston University School of Medicine, Claude R. Mallet, Ph.D., Waters Corporation

Introduction: Synthetic cannabinoids (SCs) present a multitude of problems in terms of maintaining up-to- date methods of detection. They are novel psychoactive substances originally synthesized for medical use and research

purposes. Abuse has demonstrated a variety of effects ranging from euphoria to aggressive behavior and death. The marketing, similar naming, and described pharmacological interactions create the dangerous and false perception that SCs are similar to, or the same as, tetrahydrocannabinol in cannabis products. This research focused on the optimization and development of a sample preparation, chromatographic and mass spectrometry method to detect and quantify seven SCs in urine, plasma, and gummy bears. The method was successfully applied to 17 authentic urine case samples.

Method: Protein precipitation using 2mL of methanol (Fisher Scientific, Hampton, NH) and solid phase extraction utilizing an anion exchanger Oasis® MAX (Waters Corp.), were used to prepare samples for two dimensional liquid chromatography tandem mass spectrometry (2D LC/MS-MS) analysis (Waters Corp., Milford, MA), The final chromatography method utilized an ACQUITY UPLC BEH C8 2.1 x 30mm (Waters Corp.), 10µm trap column with an ACQUITY UPLC HSS T3 analytical column 2.1 x 150mm, 1.7µm (Waters Corp.).

Results: The urine calibration curve produced a linear dynamic range (LDR) of 0.05-2.5ng/mL for UR-144 5-COOH and AB-PINACA 5-COOH and 0.05-5ng/mL for the other five synthetic cannabinoids. R2 values included 0.992 and 0.993 for UR-144 5-COOH and AB-PINACA 5-COOH, respectively and 0.995 or above for XLR-11, 5F-PB- 22, AM-2201 4-hydroxypentyl metabolite, JWH018, and JWH-018 5-hydroxypentyl metabolite. SCs were detected at varying concentrations in all 17 case samples examined. Plasma and gummy bear calibration curves had a LDR of 0.05-10ng/mL or 0.05-2.5ng/mL with R2 values above 0.995. All recovery values were greater than 80% with the exception of 63% recovery for AB-PINACA 5-COOH in the gummy bear matrix. Suppression effects of 8%, 18.9%, and 6.6% were observed for urine, plasma, and gummy bears, respectively.

Conclusion: Overall, a sensitive, specific, and reliable method was developed for efficient and rapid analysis of SCs at trace levels. The overall sample preparation time required for each matrix assessed in this research was less than one hour including protein precipitation, heating, and SPE steps. Urine samples totaled 15 minutes of preparation while plasma and edible samples were completed in approximately 30 minutes. A calibration curve was developed for each analyte in each matrix assessed with high R2 and recovery values, remarkable linear dynamic ranges (LDR), and low suppression effects. A variety of SCs and concentrations were detected in all 17 urine case samples.

The Weeds Seem Taller This Year

David J. Nemeth, Ph.D., F-ABFT., Monroe County Office of the Medical Examiner

Analyses for delta-8- tetrahydrocannabinol (THC) and its metabolites 11-hydroxy- delta-9 tetraydrocannabinol (THCOH) and 11-nor-9-carboxy- tetrahydrocanabinol (THCCOOH) remain among the most common tests performed as part of our Driving Under the Influence of Drugs (DUID) investigations. We present a survey of our results from 2010 through 2016, which show an upwards trend in concentrations of THC in DUID blood samples as well as an increased prevalence of concentrations above 5 or 10 ng/mL. In addition, we present the distribution of cases in which cannabinoids are found alone or with other drugs of abuse.

Ethanol Determination in Human Blood and Oral Fluid Samples by Headspace Gas Chromatography

Emily R. Parchuke, B.S, Cedar Crest College, Thomas A. Brettell, Ph.D., D-ABC, Cedar Crest College, Marianne E. Staretz, Ph.D., Cedar Crest College, Matthew Wood, Ph.D., DABC, Ocean County Sheriff Department

The use of oral fluid as a forensic specimen for driving while impaired cases have the potential to provide a better estimation of blood alcohol concentration (BAC) at the time of the incident. The ability to collect oral fluid at the scene can potentially reduce the lag time between traffic stop and sample collection. In order for oral fluid to be utilized in casework for this purpose, a correlation between ethanol levels in both blood and oral fluid must be determined. A method for the analysis of ethanol in blood and oral fluid was validated for this purpose and a human dosage study was performed. In this study, a Perkin Elmer HS-Clarus 580 headspace gas chromatograph with two flame ionization detectors and a TurboMatrixTM 40 autosampler was utilized. A single headspace injection was split between two columns, Elite-BAC1 (30 m x 0.32 mm x 1.8 μm) and Elite-BAC2 columns (30 m x 0.32 mm x 1.2 μm). Helium carrier gas at a flow rate of 12.30 mL/min was utilized, and the column temperature was set to 70°C.

Calibration curves were created by analyzing eight standard ethanol calibrator solutions (10 mg/dL to 500 mg/dL) in duplicate over five days, and pooling the data; correlation coefficient values exceeded 0.999 for both BAC1 and BAC2, showing good linearity. Aqueous solutions, bovine blood, human blood, and human oral fluid samples were created at ethanol concentrations of 0.01, 0.08, 0.15 and 0.30%. Samples of each type were run in triplicate over five days to assess interday variation. Solutions were also run in triplicate five times in one day to assess intraday variation. Error thresholds (<10%) were met for all solutions. Samples from a controlled dosing study were analyzed using the validated method. The study was performed utilizing 12 female subjects who consumed a pre-determined amount of wine (11.5%) in order to reach a target BAC of 0.05 g/dL for each individual. Blood, breath, and oral fluid samples were collected from subjects prior to the consumption of alcohol. Blood samples were collected every 15 minutes over a 3-hour period; oral fluid and breath samples were collected every five minutes for the first 30 minutes post-consumption and every 15 minutes following for three hours. Blood and oral fluid samples were prepared using 3 mL of internal standard (0.016% n-propanol), 300 μL of sample, and 1⁄4 teaspoon of NaF/NaCl salt mix. Breath samples were measured with a portable breath-testing device. Results showed the ethanol concentration profiles correlated well between blood and oral fluid. The Pearson correlation values between samples of oral fluid and blood were 0.92 – 0.97.

Detection of fentanyl and related synthetic opioids in biological matrices

Robert M. Lockwood, Ph.D., Connecticut Division of Scientific Services, Michael Parks, BSc, Shimadzu Scientific Instruments, Katie Pryor, MS, Shimadzu Scientific Instruments

Synthetic opioids are currently a major focus in the forensic toxicology community as they have been responsible for a number of deaths in recent months across the country. Of particular concern is the drug, fentanyl and its related analogues. Fentanyl, originally a prescription analgesic, is now frequently observed in combination with other drugs of abuse, including heroin and cocaine, leading to increasing death rates. Based on current data from the Office of the Chief Medical Examiner, in Connecticut, it is projected that there will be over 600 accidental drug intoxication deaths involving fentanyl compared to 75 reported in 2014. The potency of these compounds result in small concentrations being detected in real-world samples, thus requiring methods utilized by a forensic or clinical laboratory to be very sensitive. Hyphenated techniques such liquid chromatography-tandem mass spectrometry (LCMS/MS) can achieve this needed sensitivity through multiple reaction monitoring (MRM). The Shimadzu LCMS-8060 features heated electrospray ionization (ESI) source along with Ultrafast MRM Acquisition software and polarity switching for increased accuracy, sensitivity and robustness. The objective of this study was to develop a single LC-MS/MS method to identify and determine a limit of detection (LOD) for several synthetic opioids including fentanyl, several fentanyl analogues as well as a few of their major metabolites in blood and urine matrices. This method was then applied to a real-world sample of a suspected fentanyl user.

Method: Standards of fentanyl, fentanyl analogues, as well as other synthetic opioids were acquired from Cerilliant (Round Rock, TX) and Lipomed (Cambridge, MA) and spiked into validated drug-free blood and urine. Serial dilutions were performed to create calibrators starting from 1 ng/mL. Fentanyl-D5 was added as an internal standard. All calibrators (each 0.5 mL) were then subjected to a solid phase extraction, using a mixed-mode sorbent. The extracts were reconstituted in 250 μL of starting mobile phase conditions of 20% methanol in water (v/v) and analyzed using a Shimadzu X2 Nexera UHPLC combined with a Shimadzu LCMS-8060 triple-quad mass spectrometer. Brief LC parameters are as follows: Mobile Phase A was 95:5 (v/v) water: methanol while Mobile Phase B was methanol; both mobile phases had 0.1 % Formic acid added. The method operated at 0.5 mL/min flow rate, using a gradient from 20 to 80% B in 3.5 minutes. The method utilized a Restek (Bellefonte, PA) Biphenyl column (100 x 2.1 mm, 2.7 μm) to complete the chromatographic separation in five minutes. The LCMS-8060 mass spectrometer was operated with positive mode dual source ionization. All opioid analytes were successfully resolved with good acceptable peak shape and width.

SPE in LC: Using Automation and Chromatographic Principles to achieve High performance SPE (SmartSPE) Mark Hayward, ITSP Solutions Inc., Jonathan Ho, ITSP Solutions Inc., Tom Moran, Shimadzu Scientific Instruments, Kim Gamble, Shimadzu Scientific Instruments

Despite >40 yrs. of SPE using LC sorbents, LC principles have been ignored. At the root of this is the lack of flow control in the SPE devices in use today. Whether using vacuum or pneumatic pressure, the changing weight of liquid above the sorbent changes the flow rate through the sorbent. When SPE is performed in parallel, this effect is exacerbated because each SPE device (or well) has a different resistance to flow. The result of variable flow is considerable variation in results. Internal standards must be used to achieve meaningful results with all single use SPE devices and overall data must be judged based on the worst case scenario (flow far from optimal). Furthermore, achieving high absolute recovery against external standards, the gold standard in demonstrating the absence of matrix effects, seems to be lost. With a new SPE device, all of this is changed. It uses a syringe to achieve both automation & accurate flow. With PAL autosamplers, SPE & LC/MS/MS is automated in a single parallel workflow, & bar code reading ensures chain of custody. Van Deemter curves are measured & SPE performed at flow achieving >99% absolute recovery. As a micro device, sample dry down isn't needed for enrichment up to 200x. SPE is performed efficiently, economically, & with performance matching all LC knowledge of the last 50 yr. Examples of drug measurements using reverse phase & ion exchange SPE are provided.

Recommendations for the determination of matrix suppression in biological samples by UPLC-ESI-MS/MS: Extending Quality Measures in Forensic Toxicology

Jamie Foss, PerkinElmer, Courtney McGowen, Boston University School of Medicine, Sabra Botch-Jones MS, MA. D-ABFT., Boston University School of Medicine, Kacey Kilburn, FAA; Frank Kero, PerkinElmer

Ion suppression or enhancement of analytes due to incomplete removal of matrix components can negatively impact analytical methods. Based on the Scientific Working Group for Forensic Toxicology Standard Practices for Method Validation in Forensic Toxicology, suppression/enhancement can be evaluated using two approaches: post-column infusion or post-extraction addition. By using one of these two approaches, laboratories can assess extraction efficacy to gauge impact on crucial validation parameters. This study demonstrates the use of post-column infusion as a tool to evaluate the effectiveness of different sample preparation methods to reduce such matrix effects. De-identified, drug-free pooled human blood (n=8) was extracted at Boston University. Extracts were shipped to PerkinElmer (Shelton,

CT) for analysis. The analytes morphine, BZE, THC-COOH, Fentanyl, 6MAM, and hydrocodone (Cerilliant Corporation, Round Rock, TX) were selected to capture the effect of matrix suppression on analytes that elute at different time points during a chromatographic run. Different sample preparation techniques were compared. Protein precipitation, phospholipid depletion, and solid phase extraction were evaluated separately and in combination. The instrument used was a PerkinElmer QSight™ 220 MS/MS coupled to a PerkinElmer UHPLC. The post-column infusion approach was effective in mapping the suppression events versus retention time to help predict future issues with quantitation for specific analytes. It was suspected that early eluting analytes would be most affected by salts. It was determined that later eluting analytes were most affected by phospholipids via MRM monitoring of known lipid and phospholipid transitions. Further comparison of human blood from six different sources was further evaluated.

See What You Have Been Missing-The Synthetic Screening Conundrum

Matthew Clabaugh, SCIEX, Mass Spec Systems

Over the past decade the influx of synthetic cannabinoids, and other synthetic drugs of abuse, has gone from a curious irritant to a major issue within the forensic community. The need for a methodology to be able to keep up with these constantly changing compounds is of critical nature to the forensic community along with public health. Today we will discuss technology/ workflow that allows for high speed routine screening of the "normal" drugs of abuse as well as screening for new synthetics on the fly.

A comprehensive clinical research method employing simplified, mixed-mode sample preparation and rapid LC/MS/MS analysis for urinary pain management

Jonathan Danaceau, Waters Corporation, Timothy P. Foley, Waters Corporation, Kenneth Fountain, Waters Corporation

Background: As the opioid addiction crisis grows in the United States, pain management continues to play a crucial role in fighting this crisis. Some of the key workflow considerations for pain management labs include the use of fewer, more comprehensive analyte panels and rapid sample preparation and analytical techniques, all of which must be balanced against the need for sample integrity and data quality. We have developed a comprehensive clinical research strategy using a simplified solid phase extraction (SPE) protocol incorporating in-well hydrolysis and pretreatment, eliminating the need for any sample transfer steps. This is combined with UPLC/MS/MS analysis resulting in a rapid method that accurately quantifies 80 compounds from 22 drug classes in 3 minutes.

Methods: Urine samples were extracted using mixed-mode cation exchange polymeric SPE plates. All hydrolysis and pretreatment steps were conducted within the SPE plate wells. Analytes were then extracted using a modified procedure designed to extract basic, neutral and acidic components in a single protocol. LC/MS/MS analysis was conducted on a Waters ACQUITY I-class UPLC system coupled to a Waters Xevo TQ-S micro mass spectrometer under reversed-phase conditions.

Results and Conclusions: The method was validated for extraction recovery, matrix effects, linearity, accuracy, precision, and limits of quantification (LOQ). All analytes eluted within 3.1 minutes while maintaining baseline separation of all isobaric compounds. Recoveries were high and consistent for all compounds, averaging 80% with % RSDs under 15% for all analytes. Calibration curves were tailored to individual compounds, and ranged from 2 ng/mL for 6-MAM and fentanyl, to 2,500 ng/mL for many opiates and amines. Calibration curves and QC results were

accurate and precise for all compounds over the entire calibration ranges, with LOQs confirmed for the lowest calibrators. This clinical research method enables the rapid extraction and analysis of a large panel of drugs for pain management analysis. A single, rapid SPE method is used to extract 80 compounds with high efficiency. The use of 96-well plates, combined with in-well sample pretreatment eliminates sample transfer steps, minimizing the risk of cross-contamination or sample transfer errors. The rapid chromatography maintains baseline separation of all relevant compounds, enabling accurate and precise quantification with an analysis time of just over 3 minutes. The combination of sample preparation, chromatography and tandem MS analysis results in a complete, comprehensive workflow for a wide variety of relevant compounds.

Prevalence of dihydrocodeine in hydrocodone-positive blood samples collected for DUID investigations
Gregory Sarris, New York State Police Forensic Investigation Center

A retrospective data mining study was performed to determine the prevalence of dihydrocodeine in hydrocodone-positive blood samples collected for impaired driving investigations. Data from 77 hydrocodone-positive antemortem blood samples was evaluated for the presence of the primary metabolites including dihydrocodeine, norhydrocodone, and hydromorphone. Factors such as dose, route of administration, age, gender, and health status were not controlled or accounted for in this study. This was intended to provide a more realistic depiction of hydrocodone metabolite prevalence in blood from a wide sample population where the drug is used for various reasons at different doses intervals. Dihydrocodeine was positively identified through qualitative methods in 45.4% of cases (n=35), norhydrocodone in 6.4% of cases (n=5), and hydromorphone was not identified in any samples.

Results of this study and current literature suggest that human performance toxicology laboratories should consider including dihydrocodeine in standard opioid blood confirmation panels. These results are consistent with the limited prevalence data that exists, and with the knowledge that phase I reduction (the process by which hydrocodone is converted to dihydrocodeine) is a more consistent metabolic process. Published recommendations for DUID testing currently do not include dihydrocodeine and the drug is not included in opioid panels at many laboratories. Routine detection as a metabolite and as a parent drug may provide relevant interpretive information, as the compound itself is also a controlled substance with a high potential for abuse. From an analytical standpoint, implementation in both screening and confirmation procedures is relatively simple, and well – established testing methods are available.

Prevalence rates found in this study also present a topic for further investigation. Limited data exists on prevalence and levels of dihydrocodeine as a metabolite in blood, as well as any potential implications on pharmacological activity. Current literature mainly focuses on postmortem prevalence and controlled pharmacokinetic studies, which provide data that has limited utility for results interpretation in DUID testing. Additional research is warranted to determine the pharmacological and interpretive significance of dihydrocodeine presence in blood as a result of hydrocodone use.

Trace, Arson & Explosives Abstracts

Determining the Difference Between Blunt and Sharp Force Traumas in Human Head Hair

Amy E. Evans and Carol Ritter, Cedar Crest College

After attending this presentation, viewers will understand how hair can be utilized as a reconstructive tool at crime scenes to assist in narrowing down the cause of death when the victim's body is absent. Attendees will be informed about specific breakage patterns that are transferred to hair and are indicative of particular types of trauma. This talk will impact the forensic science community by creating standards for microscopic hair analysis, thus allowing cases to be solved more efficiently.

Hair has been proven to be a useful specimen for evaluating past drug use, and distinguishing animals and humans via the measurement of the medulla.[1] In addition; numerous studies have been published on traumatic deaths of the sharp and blunt force varieties. Recently, research was completed to show that patterns are exhibited in the hair post- trauma; however, this study was not able to define specific characteristics nor was it able to link statistics to this pattern-based analysis.[2]

Trauma recreation is completed for both sharp and blunt force trauma with the use of fifteen weapons on hair in an attempt to define traits that are unique to each type of trauma. Statistics will then be applied to provide measures of consistency and repeatability of this experiment. Each hair is photographed and evaluated under crossed polars and is taped on a wooden circular post to mimic a human skull.[3] Based on the makeup of these weapons, it is expected that sharp force implements will leave behind a smooth straight pattern, while blunt force weapons will exhibit a jagged pattern due to multiple, forceful blows needed to break the hair. This procedure is repeated for the three common race categories of hair studied in forensic science: Caucasoid, Mongoloid and Negroid.

Based on the 90 hairs that were evaluated so far, sharp force cuts showed mostly smooth characteristics with the exception of the serrated knife, which was jagged due to the uneven cutting edge of the knife. The blunt force weapons displayed much more variability in appearance, and lastly the screwdrivers exhibited the most combination traits, which is not surprising since they have both a sharp edge for cutting and a blunt force component due to compression. Additionally, damage was evident only away from the break in blunt force trauma and in this case, lacked color changes under the polarized light microscope, which provided an important way to differentiate between the two traumas. Comparison across trials of the same race showed much more consistency within the same weapon for Negroid and Mongoloid rather than Caucasoid.

Future research will attempt to study influences from hair dyes, shampoos, and age on breakage characteristics. Furthermore, hair samples will be acquired from actual traumatic fatalities to compare to the above recreations and

then blind samples will be given to volunteers to see if they can differentiate sharp and blunt force trauma via the established pattern types.

Reference(s):

1. Buffoli, Barbara, Fabio Rinaldi, Mauro Labanca, Elisabetta Sorbellini, Anna Trink, Elena Guanziroli, Rita Rezzani, and Luigi F. Rodella. "The human hair: from anatomy to physiology." International Journal of Dermatology (2013): 1-11. doi:10.1111/ijd.12362.
2. Mazzarelli, Debora, Stefano Vanin, Daniele Gibelli, Lara Maistrello, Davide Porta, Agostino Rizzi, and Cristina Cattaneo. "Splitting hairs: differentiating between entomological activity, taphonomy, and sharp force trauma on hair." Forensic Science Medical Pathology (2014). doi: 10.1007/s12024-014-9639-6.
3. Raymond, David E, and Cynthia A. Bir. "A Biomechanical Evaluation of Skull-Brain Surrogates to Blunt High-Rate Impacts to Postmortem Human Subjects." Journal of Forensic Sciences 60 (2015): 370-3. doi: 10.1111/1556-4029.12693.

Investigating Fundamental Factors Affecting the Formation of In Vitro PMRB-Like Features in Antemortem Head Hair

Katherine Ferrari and Jack Hietpas, Penn State University

A postmortem root band (PMRB) is defined by the Scientific Working Group for Material Analysis (SWGMAT) as "the appearance of an opaque microscopic band near the root area of hairs from a decomposing body". The microstructure of PMRBs has been characterized through light microscopy and scanning electron microscopy (Hietpas et al. 2016). This study revealed that PMRBs may be caused by degradation of the intermacrofibrillar matrix (IMM) within the cortex of anagen and catagen growth-phase hairs. The degradation is limited to the cortex in the pre-keratin region of the hair shaft, while the layers of the cuticle are unaffected. There is uncertainty as to whether PMRBs are caused by external factors (bacteria, proteases, other enzymes) or by an internal break down (autolysis). To gain insight into the complex phenomenon of PMRB formation, our study was performed which investigated the effects of temperature and pH (buffer solutions) on the in vitro formation PMRBlike features (dark elliptical-shaped bands) in antemortem human head hairs. The results from this study show the optimum pH for PMRB-like features ranges between 7.8 and 8.0. PMRBs were not observed at pH levels below 7 or above 9.

Furthermore, results from this study demonstrated that a minimum temperature of approximately 12 °C was necessary for PMRB-like characteristics to form. Additional analyses will be performed using transmission electron microscopy and x-ray microscopy on both hair samples displaying PMRB-like features that were produced in vitro as well as those derived from cadavers that have true PMRBs. The results from this study may provide valuable information for better understanding the mechanism of PMRB formation.

Differentiation of Fibers Using Polarized Light Microscopy and the Digital Recording of Color

Victoria Ngo and Lawrence Quarino, Cedar Crest College

Polarized light microscopy uses colors of retardation to differentiate birefringent materials such as fibers through the use of Newton colors cataloged on the Michel-Levy chart. The question posed in this research is whether colors on the Michel-Levy chart can be converted to numerical values on the RGB (red, green, blue) color scale and if subsequently

those values can be used to discriminate fibers between and within generic classes. Research has been done previously using RGB format to characterize hair; however, there has never been research done on using the RGB format to discriminate fibers under crossed-polars using a polarized light microscope. This project is designed to be a way to objectively measure the retardation of fibers by converting the colors of the MichelLevy chart to a numerical format using the RGB color scale. Prior to examining fibers, RGB values were recorded for each color in a 4-order quartz wedge. The percentage of red, green, and blue at each color were graphed separately on a line graph to serve as a comparative reference for values obtained with test fibers.

Eight examples of polyester, rayon, nylon, cotton, and acrylic fibers were mounted in Cargille Meltmount® (n=1.539) and examined under crossed-polars using an Olympus BX53 Polarizing Light Microscope at 400x. Images were digitally captured using cellSens® Entry software. RGB values were recorded at five points on the highest order color found on each fiber (typically in the center of the fiber) and the percentage of each color recorded. The percentage of each color for each fiber tested was then compared to reference colors on the line graph generated from the 4-order quartz wedge. Generic classes of fibers were easily differentiated as placement of values for each class was found at different parts of the reference graph. Samples of nylon mounted in different refractive index media showed negligible change in RGB values.

Given that the birefringence of fibers is affected by thickness and cross-sectional shape, studies have begun to see if fibers within the same class but from different sources could be differentiated by this method. To date, ten nylon fibers from two separate sources were examined using this method. Reproducible data was produced with replicates of nylon fibers from each source but both sources could be clearly differentiated. Further study on nylon fibers from other sources are on-going as well as statistical evaluation.

The Evidentiary Significance of Automotive Paints From the Northeast: A Study of Red Paint

Kaitlin Kruglak, University of New Haven, John A. Reffner, John Jay College of Criminal Justice, Virginia Maxwell and Brooke W. Kammrath, University of New Haven

This research was completed to provide data relating to the significance of automotive paint chips found in a specific population. Research has previously been conducted regarding Midwestern automotive paint populations (1) as well as populations regarding the layer chemistry of the paints (2). But to date, no research has been conducted on automotive paints from the Northeast. This research looks at paint samples from the Northeastern portion of the United States, and uses common techniques in addition to emerging techniques for automotive paint analysis. The populations of automotive paints are constantly changing, and thus need to be thoroughly monitored. By investigating these populations, forensic scientists can begin to understand what significance each individual automotive paint may hold. In order to do this, the physical appearance, layer structure and layer chemistry can be analyzed which can then be used to give strength to a conclusion made during an automotive paint examination. This population study involved the discrimination of red automotive paints using a comparative analysis approach and data analysis. The red samples were chosen as a target group from a larger automotive paint population based on popularity among consumers and manufacturers. The first portion of the analysis used stereomicroscopy, brightfield and polarized light microscopy to analyze all samples collected in the population. This study has analyzed the paint samples from approximately 200 automobiles ranging from the years 1989-2017. The macroscopic and microscopic characteristics of each sample analyzed included: relative surface color, presence of effect pigments, relative size of effect pigments, number of different pigments, number of layers, layer color, layer texture and relative thickness of the layers. The population data obtained varied from the previously released reports from a Midwestern (1) and North American (3) automotive paint

populations. The Midwestern study analyzed 300 samples and the North American study was conducted on a much larger scale but each one demonstrates the importance of doing this type of study. For example, the present research had a 20% grey colored frequency which differed from the less than 10% obtained in the Midwestern study and 16% in the North American study. The target color of red had a 13% frequency in the current study, as compared to the 15% in the Midwest and 10% in North America. Next, only the red automotive paints were further analyzed using a comprehensive sequence. This helped to determine the differentiating power of the analytical sequence as well as analyze the chemical properties of similarly colored paints. Current laboratory methods were used to analyze the red automotive paints, and included ultra-violet-visible microspectrophotometry (UV-Vis MSP), scanning electron microscopy with electron dispersive x-ray spectroscopy (SEM-EDX), and Fourier-transform infrared (FT-IR) microspectroscopy. In addition, this research used Raman microspectroscopy, an emerging technique for automotive paint analysis that has been demonstrated to provide valuable pigment information (1). This study was conducted to highlight the significance of automotive paint comparisons and the characteristics each sample possesses. The frequency data and the degree of differentiation is important information as it can provide a foundation for determining the significance of indistinguishable samples.

References:
(1) Palenik, Christopher S., Skip Palenik, Ethan Groves, and Jennifer Herb. "Raman Spectroscopy of Automotive and Architectural Paints: Insitu Pigment Identification and Evidentiary Significance." Microtrace LLC (n.d.): n. pag. 2016.
(2) Zięba-Palus, J., & Borusiewicz, R. (2006). Examination of multilayer paint coats by the use of infrared, Raman and XRF spectroscopy for forensic purposes. Journal of Molecular Structure, 792- 793, 286-292. doi:10.1016/j.molstruc.2006.03.072
(3) Global Automotive 2016 Color Popularity Report - Axalta. (n.d.). Retrieved from http://www.axaltacs.com/content/dam/New%20Axalta%20Corporate%20Website/Documents/Brochures/Axalta%202016%20Color%20Popularity%20Report.pdf

Stepping into the Future of Gunshot Residue Analysis: An Overview and Examination of GSR in Light of the SWGGSR Guide

Stephanie Horner and Tarah Helsel, RJ Lee Group

Gunshot residue (GSR) has long been collected during criminal investigations. Currently, the standard method for collecting primer GSR is using adhesive stubs, which can then be analyzed via scanning electron microscopy (SEM). SEM analysis is a confirmatory technique because it allows scientists to view the elemental composition of individual particles and determine whether or not all important primer metals are present in one particle. The SEM also allows the scientist to confirm the shape, or morphology, of each particle. The particles should look rounded or molten, as is the result of high heat reaction from the firearm's discharge. This type of analysis is both quantitative and qualitative, meaning the SEM determines how many particles are present and of what they are composed. The development of standard instrumentation has resulted in the formation of more efficient collection procedures, quality protocols, improved report wording, and result interpretations. On November 29, 2011, the Scientific Working Group for Gunshot residue (SWGGSR), sponsored by the National Institute of Justice, released a comprehensive guide to the forensic community, in regards to gunshot residue. This guide makes recommendations concerning training, contamination issues, testimony, report writing, and above all else, analytical interpretation. More recently the National Institute of Standards and Technology (NIST) assisted in forming the Organization of Scientific Area Committees (OSAC) for Forensic Science. Members of the OSAC are from government agencies, private sector and academic institutions. The OSAC for GSR will work to develop standards and promote those standards in the forensic science

community. This presentation will present a brief overview and history of Gunshot Residue (GSR), as well as, explore the specifics set forth by the Scientific Working Group for Gunshot Residue (SWGGSR) guide allowing for an open discussion of the interpretation of Scientific Working Group for Gunshot Residue (SWGGSR) standards. We will also look towards the future of Gunshot Residue.

Determination of Gunshot Residue Settling Velocity

Cassidy Schultheis, Stephanie Wetzel, Allison Laneve and Stephanie Horner, Duquesne University Forensic Science and Law Program

Gunshot residue (GSR) is a type of trace evidence that can be used in any type of forensic case involving a discharged firearm. Gunshot residue consists of all particulate expelled from a firearm during discharge. This presentation focuses on primer gunshot residue. During primer GSR analysis the three main elements of interest are barium, lead, and antimony. Most gunshot residue particles have smooth spherical morphologies and are micron sized. Gunshot residue is produced when the firing pin hits the cartridge, which activates the primer. The primer then ignites the gunpowder (propellant) that causes a pressure build-up. This pressure then pushes the bullet down the barrel at a high velocity and the particles are released as a vapor through various openings in the firearm. This research studied the rate at which GSR particles settled to the ground once the firearm was discharged. GSR samples were collected onto 0.4-micron polycarbonate membrane filters that were held above the ground by metal stands inside a closed shooting room. The filters located inside the shooting room were connected to air pumps that were located outside the room; the ability to control all the filters from outside the room limits particle contamination. Once the firearm was discharged, the filters were turned on at different time intervals to collect GSR from the air and to determine the settling velocity. The filters were then transferred onto SEM stubs and analyzed using the Aspect 75 Scanning Electron Microscope and Zeppelin Energy Dispersive X-ray Spectroscopy Software with a RJ Lee Group Detector (RJ Lee Group, Inc. Monroeville, PA). Determination of Gunshot Residue Settling Velocity Gunshot Residue (GSR), Forensic Science, Scanning Electron Microscopy Energy Dispersive X-Ray Spectroscopy (SEM/EDS) The preliminary data has shown that the particles fall at considerably lower rates than expected. The results showed that a substantial amount of particles were still in the air after three hours.

Assessing the Potential for Secondary Transfer of Gunshot Residue to Bystanders Through Contact With Surfaces That Were Near the Discharge of a Firearm

Ryan Schonert, Peter Diaczuk and Jack Hietpas, Penn State University

Gunshot residue (GSR) refers to the residues ejected from a firearm due to the discharge of a cartridge. Primer-derived GSR, or pGSR, consists of unique particles that are commonly identified by scanning electron microscopy/energy-dispersive X-ray spectroscopy (SEM-EDS). Although the primary transfer of pGSR is a well-established phenomenon, limited research has been published that investigates the potential for secondary transfer of pGSR to individuals not present during the firearm discharge event. Secondary transfer of pGSR may have significant implications for the evidence interpretation in casework if bystanders (not involved with the firearm discharge) can have pGSR transferred to them (i.e. hands or clothing) by simply touch or handling objects within a scene.

The goal of the presented research is to investigate the potential of secondary pGSR transfer in several realistic shooting incident scenarios. Four mock indoor shooting scenarios were designed such that a firearm was discharged near one of four different surfaces (wooden tabletop, glass bottle, sink faucet, and doorknob). After approximately three minutes, a bystander (whom did not discharge or handle a firearm) made a natural contact with the surface using the palm surface of the hand.

Five replicates were performed for each shooting scenario. To assess the influence of firearm type (semi-automatic vs. revolver), we shot .45 ACP ammunition in both a .45 S&W Victory revolver and a .45 Kimber 1911 semi-automatic pistol. After each shooting scenario replicate, carbon adhesive SEM stubs were collected from the surface of the hands of the shooter and the hands of the bystander. Samples were collected from the palm and back of hand separately for both shooter and bystander. Following sampling, the surfaces, shooter's hands, and bystander's hands were thoroughly cleaned. To ensure the cleaning process was sufficient, several negative controls were collected.

All samples are to be analyzed using a Zeiss Signa field-emission SEM equipped with an Oxford XMax 80 EDS system. In addition, we will utilize the Oxford GSR software package (which follows the ASTM-E1588 protocol) for GSR identification and classification. The results from this study will assess the likelihood of secondary transfer of pGSR, which will have important implications for evidence interpretation.

On-Site Screening of Volatile Accelerants Using a Portable Gas Chromatograph Ion Trap Mass Spectrometer
Zachary E. Lawton, Ashley Tolzmann, PerkinElmer, Inc., Richard Jones, Forensic Investigations Group, LLC, David Matthew, International Association of Fire Chiefs, John DeHaan, Fire-Ex Forensics, Inc.

In the field of forensic science fire investigations are critical for determining the origin, cause and development of fire. To properly evaluate whether a fire was an accident or an act of arson requires a thorough analysis of fire debris to detect and identify the presence of ignitable liquid residues (ILR). In a routine investigation, fire debris samples are collected on-site and transported to a central laboratory where gas chromatography – mass spectrometry (GC-MS) instrumentation is employed to separate and identify volatile organic compounds following the classification guidelines of ASTM E1618-14.1 For decades the time between sample collection and GC-MS identification can take days to months depending on the backlog of evidence at central laboratories, prompting the need for field portable GC-MS systems capable of identifying the accelerant on-site to increase the effectiveness of fire investigations and reducing the time for identification.

In this study, fire debris samples were collected and analyzed by fire investigators equipped with a Torion T9 Portable GC-MS (PerkinElmer Inc., Shelton, CT). ILRs were extracted from fire debris using a retractable solid phase microextraction (SPME) syringe consisting of a PDMS/DVB SPME fiber and injected directly into the portable GC-MS. The portable GC-MS equipped with a low thermal mass (LTM) gas chromatograph column and toroidal ion trap mass spectrometer provided rapid identification of compounds through automated NIST library matching in <5 minutes. An automated method library was developed to provide a diagnostic tool to aid in ILR classification as outlined by ASTM E1618-14.1

An overview of portable LTM GC-MS instrumentation, rapid sample collection techniques and the diagnostic capabilities of the automated NIST GC-MS library matching for this application will be presented.

References:
1. ASTM Standard E1618-14. Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry. ASTM International, West Conshohocken, PA, 2014.

Explosives Analysis with Portable Ion-Trap Gas Chromatography-Mass Spectrometry (GCMS) for Battlefield Forensics

Kayla M. Moquin, Brooke W. Kammrath, University of New Haven, Pauline E. Leary, Smiths Detection

Recent advancements in portable GC-MS instrumentation have enabled the field analysis of explosives and explosive residues by emergency responders, the military and law-enforcement organizations. For battlefield forensics, portable GC-MS instruments are used for the detection and confirmatory identification of threats pre-and post-explosion to provide intelligence, investigation, and adjudication information. Traditional analysis for explosives involves the sample selection, collection, packaging, transport, laboratory analysis and data interpretation. However there are many challenges to this type of analysis, and if there are any errors in the process, such as incorrect sampling or packaging, the results may be meaningless. Time is also important. The ability of a commander to make decisions in near-real time with data collected and interpreted at the scene can be critical. Additionally, because many explosives readily decompose or evaporate it is imperative that analysis be completed in a short time frame, which is not always possible due to remote military locations or laboratory backlog.

Although the on-scene detection and analysis of explosives by portable ion mobility spectrometry (IMS) has been used for years, this is a presumptive test that suffers from both false-positive and falsenegative results. Using modern portable GC-MS instruments in the area of battlefield forensics is crucial as it provides an easy user interface that gives clear confirmatory results in a short time frame, which is important when the chemical being searched for may endanger lives.

In this research, twelve different explosives covering both military (e.g., PETN, RDX) and homemade (e.g., TATP, HMTD) explosives were deposited onto the SPME fiber and separately injected into the GC-MS. Various parameters (e.g. injection port temperature, column temperature and ramp rate) were adjusted to determine an optimal method for detection and identification of the explosive. This method development is important because there is a need for a cooler-temperature method than the standard test method employed to test for dangerous toxic industrial chemicals and chemical warfare agents. Many explosives degrade at higher temperatures which can make their analysis challenging. The current standard method for the portable GC-MS employs an inlet temperature of 270^{o}C, a column start temperature of 50^{o}C with a hold time of 10 seconds, and a ramp rate of 2^{o}C/second, resulting in a total run time of 180 seconds. Eleven of the twelve explosives were detected and identified at amounts of 200 ng. Explosive compounds that have traditionally been difficult to identify, such as TATP, were readily detected using all methods tested. RDX was the only nonidentified explosive, which was not surprising given that it is notoriously difficult to detect by GC-MS due to its rapid thermal decomposition. It was concluded that a lower temperature method than what is currently implemented is a superior alternative for the detection of explosives in the field by portable GC-MS because it yields less degraded results.

Examining the Stability of Explosive Residues on Multiple Surfaces and Time Intervals

Jessica Shiffert and Stephanie Wetzel, Duquesne University

As a result of the increased terrorism occurring around the world, the attacks by Improvised Explosive Devices (IEDs) are rising, which leaves them as a constant threat. These devices are not produced for traditional use and are synthesized with homemade components, which make them unstable and unpredictable. Therefore, additional investigative and analytical efforts are required to identify the explosive elements in IEDs. Both pre- and post-blast residues can be used to identify the explosive element of the IED. Determining the explosive element can aid in the

identification of a suspect, which is vital to investigations. These residues can be can be crucial to an investigation. Additionally, this information can allow analysts to prioritize the analysis of evidence that is more likely to yield an identification. During this research, an alcohol wipe was used as a universal swabbing method to collect explosive residues from multiple surfaces that could be found on an IED or around a clandestine laboratory. These surfaces included galvanized steel, poly vinyl chloride (PVC), and packing tape. Common explosive residues, Royal Demolition eXplosive (RDX), Trinitrotoluene (TNT), and Pentaerythrite Tetranitrate (PETN) were deposited on these surfaces in the form of a liquid standard. These samples were prepared in triplicate to ensure reproducibility and then stored in air-tight containers for the following time intervals: immediate, found at both clandestine laboratories and explosion sites on and around the IED, and various swabbing techniques can be employed to collect them from these locations. It is known that explosive residues will degrade over time; however, it is unknown how the time before collection and different surface compositions affect this degradation. Understanding the degradation curve of explosive residues 3 days, 1 week, 2 weeks, 3 weeks, and 4 weeks. Overall, 84 samples per residue were analyzed, generating a total of 252 samples. An optimized method was developed for liquid chromatography with triple quadrupole mass spectrometry (LC-QQQ-MS) in atmospheric pressure chemical ionization (APCI) negative ion mode to identify the explosive compounds. All samples were compared to the immediate time interval to calculate a percent recovery of the explosive residue, and then the data was analyzed to see if a trend was observable in the degradation rates. The results of this experiment show the degradation effects of delayed collection and different surface composition.

Criminalistics/Crime Scene Abstracts

Investigating novel methods for estimating time since deposition (TSD) of bloodstains in forensic samples
Amanda Adams and Claire Glynn, Department of Forensic Science, University of New Haven.

Blood is the most commonly encountered biological fluid found at violent crimes, and provides probative evidence in terms of DNA profiles and pattern analysis. However, these stains can also reveal previously untapped potential in determining the time of deposition of a bloodstain resulting from a trauma or event. The ability to estimate Time Since Deposition (TSD) of bloodstains has been researched utilizing many different methods, yet, results varied with no complete agreement on one method for implementation into real casework. The aim of this study was to investigate a variety of methods which exhibit potential for TSD estimation. These include investigating over time; enzyme activity, the quantification and spectrophotometric observation of total protein, and the degradation of two RNA species.

Following Institutional Review Board (IRB) approval, venous blood was collected from volunteers with informed consent into sterile EDTA vacutainer tubes. 100µL of blood was deposited on white cotton cloth, in triplicate, and allowed to age in a cool, dark environment for 24 hours to 6 months. Enzyme activity of Alkaline Phosphatase (ALP) was determined by a colorimetric reaction measured using a Nanodrop® OneC UV-Vis spectrophotometer. Total protein was extracted, quantified, and viewed spectrophotometrically using the UV-Vis spectrophotometer. Total RNA was extracted using the RNeasy mini kit, quantified, and expression analysis was performed using RT-PCR targeting betaactin and 18 S RNA.

After spectrophotometrically observing the enzymatic activity of ALP, the concentration was determined using a standard curve. From fresh blood to 6 month blood the concentration dropped from 178.9 U to 32.36 U. When looking at quantified total protein, it decreased from 5.912 mg/ mL to 4.981 mg/mL in the same 6 month period. When each sample is spectrophotometrically observed, three specific peaks are seen at 412 nm, 541 nm, and 576 nm. These peaks have historically shown correspondence to the derivatives of hemoglobin and decrease in parallel with the conformational changes that correspond to hemoglobin's degradation. The most specific change is found between 541 nm and 576 nm. These two separate peaks begin to fuse into one smaller peak, relating the eventual conformational change of hemoglobin into hemichrome. The most pronounced peak, found at 412 nm, remains present over time, however diminishes from an absorbance of 1.52 to 0.50, over the 6 month period. Quantifiable amounts of total RNA were extracted from all samples ranging from 22.159 ng/uL to 7.000 ng/uL, with no real trend observed with an increase or decrease over time. Both Beta-actin and 18S RNA were detected in all samples and have shown a general trend to decrease in expression over time.

This study has shown, using several different methods, the great potential present in the ability to estimate the TSD of bloodstains. Further research is yet to be conducted, however the results obtained thus far show great promise for the future. It is the authors belief that not one single method will provide the answer, more so the utilization of multiple methods in concert with each other to ultimately provide the investigator with greater accuracy.

A study of error in the estimation of the origin of a radial spatter pattern

Jordan Deppisch, The Pennsylvania State University Forensic Science Program

The origin of a radial spatter pattern of blood is estimated by the analysis of discrete blood deposits within the pattern, which are produced by individual airborne blood drops. The major (D) and minor (d) dimensions of these typically ovate deposits are measured and used to calculate the approximate angle at which each airborne blood drop has impacted the substrate. A geometric model, the foundation of the so-called impact angle calculation, is based upon the oblique projection of a sphere onto a plane. Alternatively, the cross-sectional dimensions of a cylindric section, formed by the intersection of a plane and right circular cylinder, is another geometric model that produces an equivalent result. The arcsine of the width/length (d/D) ratio of the dimensions of the cylindric section or spherical projection, is equal to the angle (θ) of intersection or projection; i.e., $\theta = \sin^{-1}(d/D)$. These models are the basis for the approximation of an airborne blood drop trajectory at impact with a flat surface. The ratio of the dimensions of the resultant elliptical deposit are used to calculate the so-called impact angle, the acute angle between the tangent of the trajectory at impact and the substrate. Several assumptions are required for use of the model; these are outlined and assessed in this study. Defibrinated ovine blood was used to create blood deposits on various surfaces; experimental variables include incident angle, height of fall, and blood drop mass. In this study, common surfaces found at crime scenes were used including clothing fabrics, household textiles, and other surfaces (painted drywall, glass, etc.). The dimensions of each deposit were measured by hand (estimation with the unaided eye) and digitally using a "best-fit" ellipse in Microsoft® Excel or similar software. The calculated ratios of both measurement techniques were compared to the expected ratio for each variable. Error was assessed with a Student's t-test (two-sample, unpaired and one-sample) to determine statistical significance.

Analysis of biomarkers via bioaffinity-based cascades and chemical assays for forensic applications

Jan Halamek, Juliana Agudelo, Erica Brunelle, Mindy Hair, Crystal Huynh, Lenka Halámková, Leif McGoldrick, and Elizabeth Weiss, University at Albany – SUNY.

The analysis of biomarkers has been used in the field of forensics for many years in the form of DNA. The process of matching DNA samples, however, is very time consuming and has caused backlogs in many states. While this is a useful tool, it may not be the best method of analysis during an active criminal investigation. There are many biomarkers present in blood, as well as other body fluids, that can be analyzed in a much shorter amount of time by utilizing bioaffinity-based enzyme cascades or chemical assays. Our lab has developed and is in the process of

developing more systems for the purpose of identifying personal attributes from individuals such as age, biological sex, and general health. These cascades have been developed for both blood and fingerprint analysis.

The research presented here introduces the use of bioaffinity-based and chemical assays for quick and straightforward on-site analyses of blood and fingerprint samples, specifically. These systems use substrates that are naturally present in the samples – enzymes or amino acids – in order to identify various traits of the sample originators such as age, biological sex, ethnicity, and general health conditions. The fingerprint patterns traditionally used for identification are created by sweat/sebum emulsions excreted from the fingertips. Like all bodily fluids, including blood, the emulsions have their own unique chemical composition meaning there are biomarkers present for analysis. Specifically, 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, where the systems for fingerprints have focused on biological sex. There is also ongoing research aimed at the development of a larger variety of cascades and chemical assays to be able to determine additional attributes and sample properties from blood and fingerprint samples.

Unscrambling the egg: Species identification of necrophagous insect eggs by amino acid profiling

Justine Giffen, University at Albany-SUNY; Jennifer Y. Rosati, John Jay College of Criminal Justice; Cameron M. Longo and Rabi A. Musah, University at Albany – SUNY

In forensic entomology, the exploitation of the colonization of decomposing remains by necrophagous insects for the purpose of extracting information about the circumstances under which a death occurred is well-established. Blow flies in particular are of significance in death investigations because they are usually the earliest colonizers, and their presence and stage of development can be correlated to time since death, or post mortem interval (PMI). Since the length of time associated with a given developmental stage (i.e. egg, larva, pupa and adult) varies as a function of species, accurate identification of the species of entomological evidence that is found is essential to PMI estimation. Although species identification of adults is readily accomplished through visual examination, this task is much more challenging for the juvenile life stages because the eggs, larvae and even pupae of multiple species are visually very similar. It is for this reason that definitive species identification is often accomplished by rearing retrieved eggs and/or larvae to adulthood so that the species can be more readily ascertained. This approach not only is time consuming, but also requires the expertise of an entomologist. Thus, it would be highly advantageous if methods were available to permit more efficient identification of the juvenile life stages.

We report here the development of an efficient method for determination of the species identity of blow fly eggs based on their chemical fingerprint acquired by direct analysis in real time-high resolution mass spectrometry (DART-HRMS). Fresh eggs were collected from four species representing the Calliphoridae family (i.e. Calliphora vicina, Lucilia sericata, L. coeruleiviridis and Phormia regina) as well as members of the Phoridae and Sarcophagidae families. The eggs were transferred to aqueous ethanol for storage, as is typically done at a crime scene. DART-HRMS analyses were performed directly on these ethanol-egg suspensions, and no other sample preparation steps were required. The DART-HRMS analysis furnished chemical fingerprints that were highly similar for samples representing the same species, but markedly different between species. The interspecies variance was a consequence of differences between the amino acid profiles of the represented species. These results were further confirmed by MALDI-MS analyses. The rapidity of the DART-HRMS analysis approach enabled the rapid generation of the volume of spectral replicates required to subject the data to multivariate statistical analysis. The performance of linear discriminant analysis (LDA) confirmed that the data exhibited consistent intraspecies similarities and interspecies differences that could be exploited for rapid species identification of the earliest blow fly life stage (i.e. eggs). The results provide a foundation upon which

to build a blow fly egg chemical fingerprint database that can be used to rapidly identify blow fly species and circumvent some of the challenges associated with insect species identification by conventional approaches.

Comparison of RSID, Hematrace and Seratec Biological Testing Cards

Beth SaucierGoodspeed, Massachusetts State Police Crime Laboratory

The Criminalistics Unit of the Massachusetts State Police Crime Laboratory is responsible for performing biological fluid identification. The Unit currently uses the Abacus' Hematrace blood card for the confirmation of blood, which indicates human blood, and Independent Forensics' RSID cards as a screening test for the detection of amylase and semenogelin, components of saliva and semen, respectively. The Criminalistics Unit has noted artifacts in the test strip as well as inconsistencies with the control lines of the current cards. Consequently, a study was conducted to compare the sensitivity of the biological testing cards across manufacturers and determine if another card could be implemented as a replacement and/or a secondary option for use. Independent Forensics' RSID Semenogelin and Amylase cards were compared to Seratec's PSA and Amylase cards and Abacus' Hematrace blood card was compared to Seratec's blood card. Additionally, the Unit's current protocol allows for extracts to be frozen for two weeks. Therefore, the stability of the semen and saliva proteins was also evaluated over time to determine how long extracts can be tested after the initial extraction process. The results of the performance check will be presented as well as the current recommendations for the Criminalistics Unit of the Massachusetts State Police Crime Laboratory.

Raman spectroscopy of semen traces to differentiate races

Lenka Halamkova, Claire K. Muro and Igor K. Lednev, Chemistry Department, University at Albany – SUNY

Ability to determine the physical appearance of an individual on the basis of trace evidence could have a big impact on forensic investigation. Determining the race of a body fluid sample can be an important part of casework analyses. To develop a universal, nondestructive, approach that can be used to differentiate race, we combined the specificity of Raman spectroscopy with the analytical power of statistical modeling.

We characterize semen samples from the viewpoint of donor's race using Raman spectroscopy, coupled with artificial neural network (ANN). We hypothesize that detectable differences could be seen in the biochemical make up of semen. In this study Raman spectra were acquired from human semen samples, from 36 donors of three different races (Caucasian, Black, and Hispanic). Their spectra were preprocessed and analyzed with ANN to investigate the efficacy of predicting race from dried semen sample. This advanced computerized approach developed in R language was applied for sample classification resulting from pattern learning of race-related spectral features. The method and its predictive performance was validated using cross-validation and with independent split data set, thus the model was verified on data samples not used for training. The cross-validated and externally validated predictions classified correctly 99 % and 87% of the spectra, respectively. When the overall donor' race classification was then determined, 100% accuracy for cross-validated and externally validated predictions was achieved.

We have shown that ANN can be used to leverage the complex biochemical compositions of semen and their detailed Raman spectra to discriminate race of donors. The ability of ANN to classify all three races suggests that ANN of Raman spectra is able to integrate several minor changes into a classification structure. We suggest that this approach can have great utility in forensic application but can also provide a new method for classification of a broad range of

features of Raman spectra that may vary as a consequence of race, age, health status, gender, and environmental response.

A comparative analysis of globally used forensic semen detection methods and their differing applications
Megan Peters, University of New Haven

The ability to detect and identify the presence of seminal fluid can be crucial for an investigation. Methods used to detect seminal fluid are common across the world; however, the application of these methods can vary across the globe. The AP press test is common in Europe, however there is a lack of research into how this method is applied, with varying approaches taken in different laboratories. Further, for the confirmatory identification of semen, the most commonly utilized method is microscopic visualization of spermatozoa. It is essential however, to extract potential stains from substrates such as fabric, in order to perform microscopic examination. A number of different methods of extraction have been identified, however not compared. Therefore, the aim of this study was to first investigate the differing methods of applying AP for presumptive testing, and second to compare the differing extraction methods from a variety of substrates.

Following Institutional Review Board (IRB) approval, semen was collected from healthy volunteers with informed consent. For each experiment 100µL of semen was deposited onto white cotton fabric in dilutions from neat to 1:1,000. The differing AP application methods examined include: 1. Wetting both the substrate and test paper versus just the test paper, including examination of the potential transfer of spermatozoa to the test paper, 2. The application of the two AP reagents (sodium α-napthol phosphate and Fast Blue B) as a combined formulation versus sequential application, 3. The application of the AP reagents directly onto the substrate versus indirect application using test paper, and finally 4. The evaluation of the reliability of the 2-minute cutoff for the AP reaction. The differing extraction methods examined include 5 methods used globally and were performed on 5 substrates (cotton, denim, polyester, wool, and cotton swabs) for each of 5 dilutions. The stain extract was seeded on a microscope slide, stained with Christmas tree stain, examined under a microscope, and scored. The results of this study showed 1. Wetting both the test paper and the substrate greatly enhances the positive AP reactions obtained, with no observable transfer of spermatozoa to the test papers, 2. The sequential application of the AP reagents provides stronger and faster color reactions, 3. Similarly, the direct application of the reagents onto the substrate provides greater sensitivity and faster/stronger reactions, when compared to the indirect application, finally 4. The 2-minute cutoff for the AP reaction was insufficient time for positive reactions to be observed with dilutions above 1:5,000. The results of investigating the differing extraction methods showed one method, utilizing two stacked eppendorf tubes, to extract the most spermatozoa from 4 of the 5 substrates, across all dilutions.

This research highlights the potential impact on results obtained when using differing semen screening and identification tests identified across the globe. These results emphasize the need for more research into the varying application methods. It is crucial for forensic laboratories to be aware of the variety of these methods, and the potential to improve the effectiveness and sensitivity of their testing.

Wet-vacuum forensic DNA sampling dramatically increases capabilities to collect essential DNA material and solve more crime

Jared Bradley, M-Vac Systems

When investigators are processing a crime scene or reviewing items in the lab for biological evidence, choosing the most effective and appropriate collection method is essential. Common methods typically available include swabbing, cutting, scraping and taping, and these techniques have been the mainstay of forensic DNA collection. In recent years, the importance of DNA evidence in solving both active and cold cases has increased dramatically, and much of society expects technological advances in all areas of obtaining viable profiles. As a result, billions of dollars have been invested to improve the technologies and processes that contribute to a better DNA profile. However, the front end of that process, the collection of DNA material, is still a frontier with room for significant improvement, especially the collection of materials from large, rough and/or porous surfaces. The M-Vac System, a wet-vacuum collection system, focuses on that area.

M-Vac Systems®, Inc. is helping investigators solve more crime through providing a more sensitive and scalable forensic DNA collection method. As a major innovation in surface sample collection, M-Vac System's advanced wet-vacuum sampling device enables experts to better collect DNA material, often leading to stronger DNA profiles and more cases being solved. Effective micro-particle testing is based on the three principles: collection, amplification and detection, and each is equally critical. The M-Vac System raises the collection standard, and has potential to dramatically improve surface collection capabilities in nearly every scenario. In both the research setting and in actual casework, the M-Vac has differentiated itself from other methods, proving its value to law enforcement, the forensic sciences and society.

This presentation will cover the basics of wet-vacuum DNA collection and why it is so effective, validation research including comparisons to taping, swabbing and cuttings, casework examples and acceptance by the courts of this new collection method.

The novel use of an enzyme panel for identification purposes

Crystal Huynh, Leif McGoldrick, Erica Brunelle, Lenka Halamkova and Jan Halamek, University at Albany – SUNY

Individuals are made up of different combinations of physiological characteristics that set them apart from one another. This area of study is generally focused on traits such as facial features as well as fingerprint and iris patterns. The research presented here will introduce the use of enzyme levels for the purpose of differentiating between individuals. More specifically, the levels of CK (creatine kinase), LDHA (lactate dehydrogenase), and ALP (alkaline phosphatase) will be tested as a tri-enzyme panel for biometrics. Each of the enzymes listed have been studied in various medical contexts: elevated CK levels are known marker for muscle injuries, high levels of LDHA has been found to indicate malignancy, and ALP is an indicator of liver or bone disease. The levels of each of these enzymes will, therefore, be linked to an individual's general health condition and genetic makeup. This panel of enzyme levels will work as an identification method since these conditions should not be identical in any two individuals. Future work will lead to the investigation of other enzymes for a more comprehensive identification panel as well as improvements for a more easily useable, on-site method.

Transforming fingerprint analysis: The transition from multi-analyte to single-analyte chemical assays for the identification of originator attributes

Erica Brunelle, University at Albany – SUNY

In the past century, fingerprint analysis relied solely on visualization and pictorial comparisons. However, in the last 10 years fingerprint analysis has begun to focus not only on the image itself, but also on the components that make up the fingerprint image. Early studies concentrated on exogenous compounds such as drug metabolites that are secreted from the body via sweat while others have looked for the presence of explosive residues. This work was the first real step into utilizing the traditional fingerprint for more than just identification.

Using these studies as a background, our group decided to look not at the exogenous compounds, but rather the endogenous compounds. Many studies have indicated that the contents of fingerprints are produced by multiple hormone-based control mechanisms and are thus, a function of physical characteristics such as age, ethnicity, or biological sex. While traditional fingerprint analysis is undoubtedly accurate and dependable in most cases, there are many situations where a fingerprint is rendered exclusionary or practically useless. One example of such an instance is if a matching fingerprint is not saved in a database or if the person of interest is not physically present for comparison; the same can be said about DNA. Additionally, if the fingerprint is smudged and the ridges cannot be seen then it cannot be used for any sort of visual comparison. The research presented here investigates the use of endogenous compounds – particularly amino acids – that are excreted in the sweat content of a fingerprint for the purpose of identifying the biological sex of the originator. In the future, it is anticipated that one amino acid (or metabolite) can be correlated to one originator attribute. Having such a correlation would allow for the possibility of identifying multiple originator attributes from a single fingerprint. Here we demonstrate the progression from multi-analyte to singleanalyte via chemical assays for the identification of biological sex. The intention of this progression was to determine if biological sex identification via a single analyte was possible without compromising the integrity of the assay or concept. This is not to say that the amino acid chosen is the only amino acid that is indicative of biological sex. Ultimately, this progression was successfully accomplished using the ninhydrin assay which targets 23 amino acids, the Bradford assay which targets six (6) amino acids, and the Sakaguchi test which targets only one (1) amino acid.

Factors influencing the identification of gunshot residue (GSR) and powder tattooing patterns on decomposed skin

Meaghan C. Dougher, Ralph R. Ristenbatt III, Jason W. Brooks, and Peter Diaczuk, The Pennsylvania State University

Gunshot residue (GSR) powder patterns can prove significant as forensic evidence because they can be used in shooting reconstruction in firearm-related incidents. A specific type of GSR powder pattern is powder tattooing, which results when unconsumed and partially consumed gunpowder kernels impact live skin and become embedded in the skin. The condition of the target material, however, can impact the persistence of these powder patterns over time. Decomposition is a dynamic, complex process than can potentially alter any physical evidence, such as GSR, present on a body; however, there is little information available on the subject. In this study, two types of animal skin were shot, subjected to decomposition, and evaluated for the presence of GSR. In one experiment, fresh pig skin was shot with several different firearm and ammunition combinations to create GSR powder patterns and then subjected to decomposition, fully exposed. Eleven anesthetized bull calves were also shot with a handgun at a distance of approximately 2 inches to generate powder tattooing patterns, and the calves were immediately euthanized. The bull calves were subjected to decomposition either fully exposed, covered in vegetation, or covered in insect repellant. Identification of GSR was assessed through photography, stereo light microscopy, modified Griess tests, and sodium rhodizonate tests. Results of this study indicate that the best method to identify GSR on decomposed skin will depend on the stage of decomposition and condition of exposure, type of skin, and type of firearm and ammunition used. From these experiments, the sodium rhodizonate test appears to be the more sensitive chemical method to identify

GSR on decomposed skin. Visual observation of the bull calf decomposition trial has also indicated that the presence of GSR may limit the insect activity around the entry wound.

Cryptography using bioaffinity-based assays

Leif McGoldrick and Jan Halamek, University at Albany-SUNY

Since IBM and the NSA introduced the Data Encryption Standard to the general public, the need and use of encryption of important personal data has been exponentially increasing. This has led to more dynamic and varied methods of encryption to be researched. Recently, it was seen that one can use chemicals reactions in order to build a cypher for encrypting data. In this presentation, biochemical assays (bioassays) using enzyme cascades will be used in order to encrypt data based off of the WWII enigma machine used by German cryptographers. By using bioassays, one is able to encrypt their data using the output data from the instrument used, according to the very specific parameters. Multiple enzyme cascades are used in order to build an encryption key, which is then applied to the message text or data of interest which makes a cypher text. This cypher text can only be decrypted using the same exact parameters and instrument as the one who encrypted the message. These parameters include, but are not limited to, enzyme units, substrate concentration, model of instrument, wavelengths chosen, and instrument setup. This data would then be applied to the cipher text, resulting in the one receiving the message to produce the original, or plaintext, message.

In the realm of forensics, cryptography is also important since people use various methods in order to hide their data and communication. This methodology may be applicable in multiple facets of forensic investigation involving illegal activities where data is encrypted. It has been seen that many instances of encryption in forensic cases ranging from the Zodiac Killer to illegal business deals such as illegal gambling and money laundering.

Enzymatic Cascades for Biochemical Identification from Sweat

Mindy Hair, Adrianna Mathis, Erica Brunelle, Lenka Halámková, and Jan Halámek, University at Albany-SUNY

Biomarker analysis is a well-established discipline in forensic science that involves the analysis of biological samples for the presence of various substances indicative of personal attributes, such as the identification of an individual through DNA in blood. Although the forensic science field has developed rapidly over the years, the investigation processes are lengthy and majority of the routinely used forensic science techniques require proper sample collection at the crime scene followed by transportation to a laboratory facility before performing any informative analyses. The research presented here addresses this situation by introducing the use of bioaffinity-based assays for quick and straightforward analyses of sweat capable of serving as an alternative to the time-expensive DNA identification processes via the bio-affinity detection of small molecules in sweat. Sweat is a non-invasive, biological fluid that contains various amino acids and other low molecular weight compounds. The concentrations of the biochemical content within an individual's sweat are controlled by hormone metabolism processes that fluctuate regularly based on factors such as age, gender, and activity levels. Since no two individuals will have the same hormone levels at a given time, the concentrations of these sweat components are specific to each individual. Instrumental detection limitations prevent the determination of an individual based on a single analyte. However, monitoring multiple analytes increases the probability of correctly identifying a person based on these metabolic analyte concentrations.

Analysis was first performed on 50-mimicked sweat samples that were created and tested based on the physiological concentrations of amino acids and small molecules known to be present in sweat. Three of these compounds were

studied using three separate single-analyte enzymatic assays and ultraviolet-visible spectrophotometry to determine the concentrations of each compound within the sweat of the sample originator. Additionally, a collection and extraction method was successfully developed in order to collect and test authentic sweat samples from volunteers. The use of similar enzymatic assays has been previously demonstrated when analyzing fingerprints. The application of sweat collection decreases the sampling error compared to fingerprint collection methods and increases the amount of sweat collected to aid in analysis of small molecules at low concentrations. This work provides a new method and purpose for sweat analysis that is capable of differentiating metabolic compounds inherent in each individual's sweat. By utilizing re-programmable bioaffinitybased cascades, this approach has the potential to create a new method for identification, which can decrease crimes through increased investigation speeds by moving the strictly laboratory-based analyses to rapid on-site analyses that do not require specialized laboratory training. This novel sweat analysis approach only requires miniscule amounts of enzymes, substrates, and samples, which allows it to be developed into a field kit for on-site forensic testing. This could lead to the revolution of the field of forensic science and result in the acceleration of many criminal investigations.

An investigation of the effect of varying impact force on the formation of three-dimensional fabric impressions in automotive finishes

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In some vehicle-pedestrian collisions, patterns from clothing fabric may be impressed into the surface coatings of the hood, front fascia, bumper, and fenders. Occasionally, patterns are found in headlamp assemblies and other polymeric vehicle components. Generally, the focus in these cases is the association of physical evidence collected from the scene, victim, and a suspect vehicle. Threedimensional (3D) fabric patterns often contained embedded fibers; the fibers and patterns may assist in providing important associative evidence. The force required to produce these patterns, which could yield important information regarding vehicle velocity at impact, has received little attention.

The aim of this study is the elucidation of the various factors involved in the production of 3D patterns. Variables studied include the type of fabric (structure and composition), surface coating, angle of impact, and impact force. This study utilizes two impact devices: a pendulum (6-foot arm) and a falling weight device (11-foot drop height).

The pendulum utilizes a dome-shaped weld cap mounted to the front of the arm. It is covered with a layer of foam and the test fabric to simulate a human kneecap. The pendulum is permitted to impact the vehicle components that are cut to an approximate 10 cm by 10 cm square and bolted to a sturdy metal backer plate. To vary impact force, the pendulum arm is raised or lowered in 5 inch increments. The falling weight device consists of a cylindrical weight suspended in a guide tube by a pulley. A layer of fabric is placed over a 10 cm by 10 cm section of vehicle component positioned at the base of the guide tube. To vary impact force, the weight is dropped from varying heights.

With fabric type, surface coating, and impact angle held consistent, preliminary results show repeatable fabric imprint pattern formation, provided a narrow range of pendulum heights is reached or exceeded. Precise impact force is calculated through the analysis of high-speed video of the impact. This permits determination of impact force and

assessment of pattern variability as other factors are varied. Initial evaluation of the patterns has been conducting using stereo microscopy and micro-level terrain mapping. Other variables, including angle of impact, surface coating, and fabric type, will be examined in future studies.

Detection of gunshot residue in nasal mucosa via laser induced breakdown spectroscopy

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During and after the discharge of a firearm, a gunshot residue (GSR) cloud forms and dwells in the area where the gun had been fired. Some of this residue then resides on the hands and clothes of the shooter, which can then be lost in a short period of time via washing, sweating, or transfer issues. Some of the GSR is also breathed in through the nasal cavity of the shooter. Classic detection methods of gunshot residue include multiple chemical and instrumental tests which can be costly and time consuming. Laser Induced Breakdown Spectroscopy can provide a practically instantaneous and economic method for the detection of GSR on shooters. It is an ideal method of choice for the analysis of nasal swabs as no sample prep may be required. In a preliminary study using LIBS to detect GSR in the nasal mucosa of shooter, the nasal cavity samples from three shooters were collected using nylon swabs. A negative swab (prior to shooting) was collected along with sampling after 1 shot, and then 10 shots from a 9mm Glock. Samples collected after 10 shots from both shooters 1 and 3 yielded pronounced peaks of Ba and minor peaks of Pb and Sb. Ba, Sb, and Pb were not detected in the negative swabs of these shooters. In shooter 3, a pronounced Ba peak as well as a minor Pb peak was detected after only 1 shot. We were unable to detect GSR in shooter 1 after 1 shot due to possible background interference from the type of collection swabs used. Shooter 2 worked at the gun range where collection took place which lead to his negative samples yielding peaks of Ba, Pb and Sb. Immediate future studies include optimizing the type of collection swab to be used along with the collection method as well as sampling of more shooters to address the ability of this analysis method to detect GSR in the nasal mucosa of shooters.

Predicting the time of the crime: Bloodstain aging estimation for up to two years

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The chemistry of blood and the biomolecular changes that occur as a bloodstain ages are both inherently intricate, but can be monitored using specific analytical techniques. It has been shown that bloodstains are a rich source of information, which can be used to help in solving crimes. Particularly, the time since deposition (TSD) can be estimated by analyzing bloodstains and extracting information related to the natural chemical processes that occur as bloodstains age. This work summarizes a proofof-concept study demonstrating the effectiveness of using Raman spectroscopy to nondestructively analyze bloodstains, and probe for specific kinetic changes in aged bloodstains for up to two years. As an initial step, bloodstains were identified as blood, and not a different body fluid, using a recently developed classification modeling technique. An overall success rate of 89% was demonstrated for predicting the identity of all stains as blood, with 100% correct blood identification for stains aged up to one month. The observed changes in the spectra over time were consistent with the known biochemical processes occurring as blood ages naturally, and those variations were sufficient enough to allow for differentiation and TSD predictions on the scale of hours to years. Specifically, TSD predictions were performed using partial least squares regression (PLSR) and principal component regression (PCR) analyses; where root mean squared errors of prediction of 0.29 and 0.31, respectively, were obtained. These errors correspond to an overall accuracy of about 70% for the models to correctly predict the TSD at each time point.

Remembering the murder you did not commit

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Beatrice is a small town in Nebraska. In the early morning hours in February 1985, the nude body of an elderly woman was found in her apartment. Five individuals, three women and two men, confessed to the crime. They described in vivid detail how she was sexually assaulted, and how all of them helped to smother her. They were told by their psychologist, some of these five individuals have been his patients prior to 1985. He insisted that they were repressing the memory of a heinous crime. They were threatened with death sentence. The sixth, Joseph White, never confessed, and was found guilty and sentenced to life. All five testified against him and it was their testimony, which convicted him. The district attorney falsified the reports on body fluids analyzed in the Nebraska State Patrol Criminalistics Laboratory by Reena Roy. She was never asked to testify during the original trial, which took place in 1989. Upon the insistence of Joseph White STR DNA testing was performed eventually and all were in 2008. In June 2016, Reena Roy was asked to testify on this case in Federal court. She testified in 2016 that even in 1989, the evidence and the results did not point to the six individuals. In June 2017, this case was written up in the New Yorker.

Poster Session Abstracts

Development and validation of a liquid chromatography tandem mass spectrometry method for the determination of pharmaceutical coumpounds in the Brandywine River

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Occurrences of pharmaceutical compounds in the environment, classified as "emerging contaminants" have become a new public health concern among scientists worldwide. Even though a large variety of drugs are found in very low concentrations, there is still lack of knowledge about long-term health risks that they may pose for non-target organisms as well as for human health. The emergence of ever new drugs is an ongoing challenge for the field of analytical toxicology, because these drugs may cause long term toxicity.

The Brandywine Creek watershed is located in Christina Basin and forms part of the Delaware River Basin in Pennsylvania. Land use patterns along the creek continue to vary as the river cuts through rural/suburban communities in Chester County, PA to Christina River in Wilmington, Delaware. Due to variabilities in land use along the river, we sought to understand how it correlates with the excessive chemical pollution emanating from human drug use and agricultural activities. Understanding the correlates would provide informed strategic decisions in controlling the pollution at source.

The aim of the present study was to develop and validate a liquid chromatography tandem mass spectrometry (LCMS/MS) method for the determination of codeine, fluoxetine and acetaminophen in river samples of the East Branch of Brandywine River at Downingtown, PA in order to study their occurrence and fate. Efficient separation and ionization is important for the successful determination of these compounds at very low levels in environmental matrices, so different analytical columns, mobile phase composition and MS parameters were tested and optimized for their successful detection. A systematic optimization of mobile phase, solid phase extraction and MS response was also performed to cope with different polarities and charge of compounds. The method involves clean-up by solid phase extraction (SPE), followed by LC-MS/MS, using a Poroshell EC-C18 2.1 x 100 mm 2.7µm reversed-phase column and electrospray ionization (ESI) in positive mode. Mobile phase consisted a gradient program using acetonitrile and ammonium formate 10 mM/formic acid 0.05%. Codeine was detected using 300.14>165, 300>52 MRM transitions, fluoxetine was detected using 310.14>148, 310.14>44 MRM transitions and acetaminophen was detected using 152.07>93, 152.07>65.1 MRM transitions. The method was validated and proved to be accurate, precise, selective and specific with satisfactory linearity within the calibration range.

Anticipating the Admissibility of Forensic Evidence Screened On-Site via Ambient Sampling, Portable Mass Spectrometry

Sara Bell and Chase Deberry, Illinois State University; Angelica Traub and Christopher C. Mulligan, Dept. of Chemistry, Illinois State University

Ambient sampling, portable mass spectrometric (MS) systems have the potential to assist in crime scene investigation and law enforcement activities by providing rapid and discriminate chemical identification. But with any new method or technology that is utilized in legal proceedings, the burden of admissibility of both collected data and corresponding expert testimony in placed on the court. To compensate for the lack of scientific literacy judges may carry, established standards become extremely pertinent to establishing validity of new techniques and ensuring an individual's right to due process. In this work, the admissibility of this new MS technology is preemptively considered under the rigors set forth by the Daubert standard. The technology considered in the interdisciplinary work was paper spray ionization (PSI) employed on a field-portable, ruggedized mass spectrometer (MS), specifically the FLIR Systems AI-MS 1.2 cylindrical ion trap MS. Both instrumental data and pertinent review of scientific/legal literature was utilized to fulfill core requirements of the Daubert standard, such as the refutability of the method, level of peer review and acceptance in the scientific community, and established error rate. An overview of these findings will be presented.

Evaluation of the Booz Allen Hamilton Tactical Forensic Device: Vampire

Jennifer Busk, University of New Haven; Peter Massey

Fingerprints have proven to be valuable in the investigation of crimes by placing an individual at a specific location and/or providing insight into whether that individual handled specific items. The Vampire is capable of capturing both developed and undeveloped latent prints through a simple "point and shoot" method. Once captured, the image is compared to both the onboard collection history and/or a watch list of individuals the operator generates from known persons of interest. The Vampire's algorithm provides a list of possible identifications leading to future verification. No previous research or validation of the Vampire has been conducted to date as the device is relatively new. By comparing the Vampire captured images with the images more commonly acquired by a DSLR camera on the image quality, image clarity, identified number of minutiae and image quality map percentages it will be possible to show if the Vampire is a valid tool for field and lab based forensic work. To evaluate the Vampire a trio of programs were utilized1: the FBI Criminal Justice Information Services Division's Universal Latent Workstation version 6.6 (ULW), GNU Image Manipulation Program version 2.8 (GIMP), and Wolfram Mathematica version 11.1.1. Thirtytwo friction ridge impressions from the same individual were placed on fifteen surfaces of varying textures and porosities. Three magnetic powders (blue, black, and white) and one dyestain (cyanoacrylate fuming and Ardrox) were used to develop the latent impressions. Each of the developed impressions were captured by both the Vampire and a Canon EOS Rebel XSi camera and introduced to the ULW where the image quality, image clarity, and number of minutiae identified were recorded. The ULW allowed for application of an image quality mask. An image quality mask provided a more standardized analysis of the size and clarity of areas within a given image2. Once the image quality mask was applied, it was 'darkened'1 within the ULW and exported. GIMP was used to brighten the image. The image was imported into Mathematica where a series of commands generated the percentage of the total image that corresponded to the specific colors present. The colors of the quality map range from red (questionable detail) to the observed royal blue (certain detail). The comparison of assigned color percentages as well as earlier recorded values between the Vampire images and the DSLR images allowed for any differences between the methods to be established. Preliminary statistical analysis utilizing three of the fifteen total surfaces (aluminum foil, Styrofoam, and white painted wood) indicated that there was a significant difference between the two capture methods for the number of total minutiae identified in each image. The Vampire images had a significantly larger number of minutiae identified than the DSLR images. No significant differences were found between the two methods when comparing the latent quality score and the overall clarity of the images.

Further research is underway to analyze the remaining twelve surfaces. The conclusion will provide further insight into the capabilities and limitations of the Vampire as compared to the more commonly used DSLR.

Development of Synthetic Blood for Use in Crime Scene Reconstruction

Karla Chun, Syracuse University, Alfred State College

The type of pattern created when an external force is applied to a blood mass is determined in part by the physical properties of the blood. In order to analyze these patterns to determine a precipitating event, a blood substitute is often used. While animal and human bloods have both biological and ethical concerns, synthetic blood avoids these concerns. This experiment tested a variety of synthetic blood substitutes (SBSs), none of which contained biological material, against a reference of human blood to determine the similarity of the substitutes to the physical characteristics of human blood. One of the SBSs tested was a commercial stage blood, Vampire Blood. The other SBSs examined were created in the lab and were comprised of various mixtures which were water and corn syrup based, using flour

and cornstarch as thickeners, and glycerin as a thinner to help alter the surface tension of the water. These SBSs were selected because they contain non-hazardous components, by-pass ethics approval, are cost effective, and have long-term stability. The SBSs were compared to human blood in three tests: a viscosity test, a density test, and a pattern test, which included impact spatter, knife spatter, and droplets. The mixture containing 50mL water, 1mL glycerin, and 12.5g flour, had the most similarities in these tests with human blood. The commercial stage blood, Vampire Blood, was found to not accurately represent the physical properties of human blood. The Vampire Blood was more viscous and less dense than the human blood reference. This method can be used to test other commercially available SBSs to determine if they are an accurate substitute for human blood in crime scene reconstruction.

Balancing the Utility and Legality of Employing Portable MS-Based Screening Methods in Traffic Stops

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Advanced, portable technologies, when provided to law enforcement and firstresponders, can serve as tool to offset the ingenuity of criminals. Chemical instrumentation, if made both field-portable and rugged for non-technical operators, could be employed for routine drug evidence analysis, but it also has the potential to be implemented as a flexible investigative tool in law enforcement activities. Ambient sampling, portable mass spectrometers featuring "ambient" ionization methods like paper spray ionization (PSI) have been demonstrated as applicable to broad screening of drug evidence types. However, the underlying legal implications of evidentiary data require the discretion of potential users to ensure both lawful and ethical usage. In this work, we investigate practical usage modes of a portable MS system employed in routine traffic stop enforcement, such as the screening of trace level drug residues from interior/exterior automotive surfaces. Novel applications that are more nebulous in terms of legality, particularly the screening of latent fingerprints for contraband residues to establish "probable cause" searching, are also demonstrated. In these scenarios, the current state of U.S. search and seizure law is examined to postulate potential violation of Fourth Amendment protections.

Another usage mode examined was the screening of abandoned materials and trash pulls (e.g. latent fingerprint and drug residue screening from discarded materials such as Solo cups and storage media), providing rapid, chemical information. To demonstrate utility in traffic stops after probable cause has been established, direct screening of emerging paraphernalia types was demonstrated, such as residues stemming from beverages spiked with codeinebased cough syrups and electronic cigarette liquids adulterated with illicit substances like α-PVP.

Estimating Bullet Angle using a Trigonometric Method

Peter Diaczuk, Pennsylvania State University; Jeanine Day, Linda Rourke, Nicholas Petraco, John Jay College of Criminal Justice, CUNY

Estimating the angle at which a bullet was fired can be pertinent to the reconstruction of a shooting incident. Unfortunately, fired bullets exhibiting deformation from impacting a hard unyielding surface are not always recovered, or can sustain additional damage at subsequent impacts before exhausting their kinetic energy and coming to rest. It was hypothesized that a trigonometric function, similar to that used by bloodstain pattern analysts to determine the

trajectory of a blood droplet, may be used to estimate the incidence angle of a fired bullet by measuring the major and minor diameters of the ricochet mark left on the substrate. The correlation between the trajectory of a blood droplet and a fired bullet was established because of shape similarities between an angular blood droplet and a bullet ricochet mark. Using several different types of ammunition, over 100 ricochet marks have been recovered and examined from shots fired at 5°, 10°, 15° and 20° onto marble and steel substrates. The same arcsine equation used by blood pattern analysts was applied to the ricochet marks, after measuring the lesser ("little d") diameters and greater ("big D") diameters of the ellipses created by the bullet impacts. These calculated incidence angles were then compared to the actual angles of firing using the Analysis of Covariance (A.N.C.O.V.A.) statistical linear regression model to fit data consisting of discrete and continuous variables. It was determined that the incidence angle can be predicted using the trigonometric equation within plus or minus 4 degrees.

Validation Study of the SERATEC® PMB Forensic Identification Assay for Human Menstrual Blood

Ariana Dindial, Pennsylvania State University; Dr. Reena Roy, Penn State University

Blood is one of the most common fluids found at a crime scene. This makes the determination of human peripheral blood versus menstrual blood a valuable source of identification in many forensic investigation scenarios, especially when concerning the issue of consent in sexual assault cases. This study tests the sensitivity and robustness of SERATEC® PMB, a chromatographic immunoassay, to distinguish between human peripheral and menstrual blood, as well as to identify the minimum amount of menstrual blood detected by the assay. A single, female volunteer with a regular menstrual cycle was recruited for the study. Samples were collected from the first day of the menstrual flow until the end of the menstrual flow on sanitary pads and tampons. The SERATEC® PMB test is designed to qualitatively detect human hemoglobin and human D-dimer. D-dimer is a small protein that is present in the blood after a blood clot is degraded by fibrinolysis. This makes D-dimer suitable for the detection of menstrual blood, as during the menstruation period coagulation and fibrinolytic pathways are activated. The samples collected on the sanitary pads and tampons were cut into .25x1 cm, .50x2 cm, and 1x3 cm. Additionally, cross-reactivity was tested using horse, bull, and human sperm, as well as saliva and human breast milk samples. 10µL of each possible crossreactive samples were tested separately. The results of this study showed that the SERATEC® PMB test was able to reliably distinguish human menstrual blood from human peripheral blood. Visibility of positive results for known menstrual blood on the test cassettes varied with the amount of sample used. All sperm, saliva, and breast milk samples did not have any cross-reactivity with the testing kit. In future research, a variety of volunteers should be used to evaluate the effects of human variation in D-dimer levels on the effectiveness of the SERATEC® PMB kit.

A Comparison of Spectral Overlap in the Applied Biosystems 3100 and 3500 Series Genetic Analyzers

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The interpretation of DNA profiles in a forensic setting can be greatly impacted by the sensitivity and limit of detection of the capillary electrophoresis platform being used for analyses. The Applied Biosystems 3100 and 3500 series differ in the sensitivity (peak size) and more so in the limit of detection[1]. In addition, the 3500 series instruments have higher overall baselines which may lead to interpretation challenges. This study compares the spectral overlap, or "pull-up" associated with the Applied Biosystems 3100 and 3500 Genetic Analyzer Series. Specifically, we compare the

rates of pull-up across samples and quantify the amount of pull-up relative to the allelic peaks present. Preliminary data suggests the rates of pull-up were not statistically significant between the instruments. However, it was also found that there is a higher rate of occurrence of pull-up per sample with the Applied Biosystems 3500 than the 3100 series Genetic Analyzers.

[1]J.-A. Bright, S. Neville, J. M. Curran, and J. S. Buckleton, "Variability of mixed DNA profiles separated on a 3130 and 3500 capillary electrophoresis instrument," Australian Journal of Forensic Sciences, vol. 46, no. 3, pp. 304–312, 2014.

Qualitative and Quantitative Analysis of Minute Level of Saliva in Expirated Blood

Mackenzie Whiting, University of New Haven; Dr. Claire Glynn, PhD, University of New Haven

A major challenge that comes with Bloodstain Pattern Analysis (BPA) is the differentiation of expirated and impact blood spatter stains. As expirated blood is expelled from the mouth, it is logical to assume there would be trace amounts of saliva mixed with the resultant blood droplets. To date however, a method has not yet been identified which is adequately sensitive or specific enough to detect minute traces of saliva in expirated bloodstains. The aim of this research is to investigate the ability of SALIgAE® to accurately detect the presence of, and quantity of, trace amounts of saliva within expirated bloodstain patterns.

Following Institutional Review Board (IRB) approval, and informed consent, venous blood and saliva was collected from the volunteer. The sensitivity of the SALIgAE® solution was first tested with dilutions of saliva:ddH20, and saliva:venous blood, ranging from 1:1 to 1:1,000,000. Expirated bloodstains were created by placing 1mL of blood into the volunteer's mouth for 30 seconds, followed by coughing the blood onto white butcher paper approximately 12 inches in front and below the volunteers' mouth. Two stains were created on separate days where the volunteer had not drank or consumed any food for at least one hour. Individual blood spots/stains were chosen to be tested from both the vertical and horizontal planes, totaling 42 stains. Each sample to be tested was incubated in ddH20 for 30 minutes before addition to the SALIgAE® solution. Both a visual color change test and a spectrophotometric reading using the Nanodrop™ OneC UV-Vis Spectrophotometer were used to determine the color change and change in absorption of salivary amylase and SALIgAE®.

The sensitivity of SALIgAE® with dilutions of saliva:ddH20 produced the required positive color change up to 1:1,000, as previously reported, with absorbance values ranging from 1.28 to 10.0, and salivary amylase concentrations ranging from 0.12µg/mL to 1.33µg/mL. The sensitivity with dilutions of saliva:venous blood produced the same required positive color change up to 1:1,000, however, the red color of the blood made a distinct color change difficult to observe. The first expirated stain created a large dispersed pattern on the vertical plane, with less abundant but larger drops on the horizontal plane. Of the 42 stains, 8 produced a positive color change, with absorbance values ranging from 0.08 to 1.05, and salivary amylase concentrations ranging 0.00µg/mL to 0.09µg/mL. Of the 42 stains on the second pattern, 9 produced a positive color change, with absorbance values ranging from 0.05 to 1.46, and salivary amylase concentrations ranging from 0.00µg/mL to 0.15µg/mL. While the concentrations obtain from the expirated stains are low, a visible color change did occur. The results of this study highlight the ability of SALIgAE® to detect the presence of minute quantities of saliva when mixed with blood.

This reveals the SALIgAE® method to be an ideal candidate for the differentiation of expirated spatter and impact spatter, thereby overcoming a significant challenge facing bloodstain pattern analysts. This information will ultimately help guide forensic professionals to develop more effective strategies in their processing and analysis techniques.

Out of Thin Air: Detecting the Presence of Plant-based Legal Highs in Air

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There are over 400 species of plants that are revered in various societies around the world because of their ability to promote access to altered states of consciousness. Although in many cultures the use of these psychoactive plants is restricted to religious ceremonies or medical applications, they have found their way in recent years into Western societies where they are used recreationally. Twenty of these plants have been designated by the United Nations Office on Drugs and Crime (UNODC) as "plants of concern", because of the dramatic rise in their abuse, and the consequent health and law enforcement challenges that this has imposed. Of these 20 plants, 18 are classified as "legal highs" because they are as yet unscheduled, and thus their possession, sale and abuse are not prosecutable. Although ingestion of these materials has been implicated in poisonings and fatalities, legislating their use is hampered by the absence of routine means by which to identify them. Unlike plant-derived drugs such as marijuana, these plants and their products generally have a non-characteristic appearance that makes it difficult to distinguish them from innocuous plant materials such as spices and condiments, or dietary supplements. The challenge of not recognizing these substances based on their bulk morphological features makes their importation easy, since it is usually impossible to confirm their identity at ports of entry around the U.S. Since these products are imported from international locations, an important step towards their eventual control is to be able to identify their presence in cargo containers. There are currently no procedures to accomplish this task.

We report here the development of a method to enable identification of these products in cargo containers. It is based on the finding that the ambient ionization mass spectrometry-derived chemical signature of the headspace volatiles of plant species, including those identified by the UNODC as plants of concern, is unique and diagnostic. In this approach, the headspace volatiles are concentrated on polydimethylsiloxane (PDMS) solid phase micro-extraction (SPME) fibers, which are then analyzed by direct analysis real time-high resolution mass spectrometry (DARTHRMS). The observed chemical signatures are then subjected to multivariate statistical analysis methods to enable rapid classification and identification of the plant material. Species represented in this study include Althaea officinalis, Calea zacatechichi, Echinopsis pachanoi, Lactuca virosa, Leonotis leonurus, Mitragyna speciosa, Nymphaea caerulea, Ocimum basilicum, Origanum vulgare, Piper methysticum, Psychotria viridis, Salvia divinorum, Turnera diffusa, and Voacanga africana. Kernel discriminant analysis (KDA) of the DART-HRMS data showed that the headspace signature could be used to accurately identify the bulk material. The reliability of these results was tested through external validation which was 100% accurate in all cases. These outcomes establish proof-of-concept for the development of a database against which DART-HRMS-derived chemical signatures of headspace can be screened for the detection and identification of plant-based legal highs. This work was supported in part by the United States National Institute of Justice (grant 2015-DN-BX-K057).

Principles of a Laminar Flow Based Interface for MS/MS Instrumentation: Improved Robustness for the Analysis of Samples with Complex Matrices

Jamie Foss, PerkinElmer; Sabra Botch-Jones, Boston University School of Medicine; Frank Kero, Josh Ye, Sharanya Reddy, and Feng Qin, PerkinElmer

The emergence of liquid chromatography tandem mass spectrometry (LC-MS/MS) as a gold standard analytical platform for quantitative method development in high throughput toxicology, environmental surveillance and food safety laboratories has been well documented. Recent trends in practical considerations for improvements towards laboratory implementation focus on reduced downtime to facilitate testing methods for large sample populations as well as samples with difficult matrices. This poster will report on MS/MS instrumentation using a heated coaxial flowbased dual spray ionization source and a Hot Surface Induced Desolvation (HSID) interface. Ions are extracted at atmospheric pressure and focused through a series of heated multi-orthogonal channels and turns entrained in a laminar flow of hot gas (different than traditional mass spectrometry instrumentation). The advantages for this HSID platform include high sensitivity and signal stability due to an inherent reduction in chemical background through improved desolvation and de-clustering. The orthogonal sampling and laminar flow keeps the ion path free of contamination resulting in a robust, low maintenance instrument. This technology lends itself to the efficient analysis of complex biological samples without constant downtime due to instrument cleaning compared to traditional interfaces. Because laminar flow does not require axial electrical fields for the introduction of ions into the mass spectrometer, there is no mass discrimination resulting in fewer parameters to set, simplifying the method development process. Demonstration of this technology for MS/MS applied to relevant clinical and forensic analysis will be presented.

The Analysis of Semen Stains Using ATR FTIR Spectroscopy and the Investigation Into the Spectral Differences Based on Protein Degradation Over Time

Lina Guindy, University of New Haven; Alyssa L. M. Marsico, Ph. D. Principal Investigator

Bodily fluids are one of the more common types of evidence found at crime scenes, and they contain valuable DNA information. However, in some cases, DNA is not as useful unless the time in which it was deposited can be determined. Human semen has an abundance of proteins that can degrade over time and when exposed to the environment, the degradation of these proteins could be used to establish a biological clock to determine the age since deposition of a semen stain. This information could be used in crime scene reconstruction, corroboration, and supply time intervals for investigators.

While there are numerous methods to positively identify and detect bodily fluids, many of these techniques can be destructive or require a great amount of sample. Instrumentation that can positively identify semen non-destructively and in miniscule amounts allows DNA analysis to be done after identification, or determining age since deposition in this case. Attenuated total reflectance fourier-transform infrared (ATR FTIR) spectroscopy is one of the few instruments that is non-destructive and requires miniscule amounts for analysis. It has been previously proven that ATR FTIR can in fact be used in the identification of bodily fluids, including semen.

In this study, various samples were deposited and left to age, ranging from 1 day to 30 days. The IR spectra were then collected and chemical shifts references in a human semen sample that are attributed to proteins were observed. These shifts were then used to calculate the ratios of all chemical shifts observed to each other. The relationship between the age of the sample and the ratios were shown graphically to determine a relationship. There was an observed decrease between days 1 and 2, which can be attributed to the protein degradation. After 2 days, there were slight changes in the ratios but the significance of these changes would have to be determined with further research. Samples were also left to age in various conditions such as in sunlight and in complete darkness and were analyzed the same way as the aged samples. This study showed that sunlight promotes protein degradation resulting in a lesser percent transmittance value than human semen that was to degrade in complete darkness. The protein degradation exhibited in both the aged and environmental studies show promise that a biological time clock may be determined with further research.

The Use of WHATMAN FTA Elute to Simplify Storage and Reanalysis of Extracted DNA from Forensic Samples

Tom Hansen, GE Healthcare Life Sciences; P. Patel, K. Reams, and M. Schwandt, GE Healthcare

Forensic laboratories and their submitting agencies often have an obligation to maintain forensic DNA samples and extracts for use in future testing. Required by statute or laboratory policy, many of these samples are stored as liquid extracts in freezers that are expensive and take up precious laboratory space. Therefore, the need for a room temperature storage solution was recognized and thus became the premise of this study.

For many years, Whatman™ FTA™ Elute cards have been recommended for biological sample collection, room temperature storage and isolation of DNA for downstream applications. Like FTA, FTA Elute cards are comprised of a special matrix that lyse cells on contact, denature proteins, and protect DNA from degradation. FTA Elute technology allows for long-term DNA preservation at room temperature, in addition to, the release of DNA into solution with a simple water and heat elution step. Eluted DNA can be used for many applications including STR analysis, sequencing, and real-time PCR. An optimized elution protocol was specifically developed for storage of extracted DNA which may contain limited quantities when compared to biological samples. This presentation will demonstrate the ability to recover and profile extracted DNA stored on FTA Elute cards.

DNA extract, isolated from whole human blood, was diluted into starting concentrations of 10ng/µl, 1ng/µl, 500pg/µl, 100pg/µl, and 50pg/µl. The extracts were applied to FTA Elute cards and allowed to completely dry. Samples were then stored in sealed pouches with desiccant. Punches were removed from the cards and the DNA was released into solution using the newly optimized elution protocol. Finally, the samples were quantified using a forensic quantitation kit and STR profiles developed. The results were evaluated to compare DNA recovery across various starting concentrations, and assess STR profile quality. The data presented will demonstrate that the DNA recovered from FTA Elute cards was of sufficient quality and quantity to develop full STR profiles from all samples tested following elution.

Investigating the Impact of Protein and Peroxidase Blood Enhancement Reagents on DNA Recovery from Laundered Clothing

Gabrielle Hartley, University of New Haven; Dr. Claire Glynn, University of New Haven

Blood is a commonly encountered biological fluid in criminal investigations, and visual traces of the fluid on a suspect's clothing can be diminished through laundering. Despite the potential to help reconstruct a crime, the mere presence of blood on laundered clothing is not often sufficient enough to make conclusions about a specific person's involvement in a crime. Because of this, it is essential that a method exists that both enhances a forensic examiner's ability to visualize a dilute bloodstain on a piece of laundered fabric while maintaining and preserving the quality of the DNA evidence present. This study aims to analyze the effects of laundering and the application of commercially available blood enhancement reagents commonly used to improve visualization of dilute bloodstains on DNA recovery. Following IRB approval and informed consent from volunteers, venous blood was collected in sterile vacutainer EDTA vials. Six commonly used and commercially available enhancement reagents were chosen, including Hungarian Red, Coomassie Blue, Amido Black, luminol, Bluestar® Forensic Magnum, and aqueous leuco crystal violet (LCV). 100 µL of human blood was deposited onto cotton, polyester, denim, and wool in triplicate, and these samples were laundered under standard washing conditions with blank controls. Following laundering, a selection of samples from each fabric type were enhanced with each of the six reagents. DNA was extracted from these samples using a QIAamp® DNA Investigator Mini Kit and quantified using a NanoDrop™ OneC UV-Vis spectrophotometer. DNA, blood, laundered.

Following laundering and enhancement, quantifiable amounts of DNA originating from bloodstains were obtained from all fabric types. Although often lower than unwashed blood samples, all untreated washed samples had a suitable DNA recovery, ranging from 0.9ng/mL to 38.2ng/mL. Similarly, samples treated with all enhancement reagents except Amido Black had DNA yields higher than the washed blank samples that ranged from 1.1ng/mL to 23.0 ng/mL. Despite this, measurements indicated that the application of some blood enhancement reagents, particularly Amido Black, may affect DNA recovery. Across all fabric types, samples treated with Amido Black had a low DNA recovery comparable to blank samples, returning yields as low as 0.7ng/mL on cotton samples. Across the board, washed blank fabric samples had a higher amount of recovered DNA than the unwashed blank samples; however, these samples were treated exactly the same with the exception of laundering. While unwashed blank samples had an average DNA yield of 3.57 ng/mL, washed blank samples had an average DNA yield of 8.59 ng/mL. Because of this, it is suggested that cross transfer of DNA between samples during the laundering process is possible. Despite these results, the NanoDrop™ OneC UV-Vis spectrophotometer does not provide differentiation between human specific DNA and DNA from other sources. Although unlikely, it remains possible that not all of the quantified DNA yield is representative of probative human specific DNA.

Hyperspectral Imaging: Proof of Concept for Field Analysis of Select Opioids and Salvia Divinorum

Ani Kazaryan, Boston University School of Medicine; Courtney McGowan, Sabra Botch-Jones, Boston University; Janette H. Wilson and James T. Daly,Bodkin Design & Engineering

Hyperspectral imaging systems operate in wavelengths ranging from the ultraviolet through long-wave infrared in a variety of spatial and spectral resolution combinations. Historically hyperspectral imaging has been used in the classification of ecstasy tablets, explosives, bloodstains, and fingerprint analysis, typically using longer wavelength instruments. The purpose of this evaluation is to establish whether visible near-infrared (VNIR) hyperspectral imaging can be utilized to determine the presence/type of controlled and non-controlled substances for portable field

applications. Here, we study Salvia divinorum, a plant species native to Oaxaca, Mexico. The leaves of this plant contain the active compound Salvinorin A which, when smoked, causes the user to experience hallucinogenic effects. Currently Salvia divinorum is not listed as a scheduled drug under the United States' Controlled Substances Act, though some states such as Ohio and Texas have passed laws to prohibit its use. In addition, prescription opioids from various manufacturers were evaluated to identify any spectral differences.

Current Trends of Opioid Abuse

Ada Kong, US DOJ Northeast Laboratory

Deaths related to drug overdoses in the United States are dramatically increasing, largely due to the abuse of opioids. Counterfeit oxycodone tablets appear to be the latest trend in the opioid crisis sweeping the nation. With recent efforts to regulate the availability of prescription opioids, illegal suppliers have turned to counterfeit tablets to meet drug abusers' demands. As such, forensic laboratories have been receiving an increasing number of counterfeit oxycodone tablets for analysis.

Counterfeit oxycodone tablets are a particularly insidious threat. They can be easily manufactured in a private residence with little specialized equipment beyond a pill press and some raw material, and then circulated through decentralized drug distribution networks. Some of the tablets appear indistinguishable from the pharmaceutical drug, yet can contain a fetal dosage of highly-potent opioids or designer drugs. The current trend is that suppliers are producing counterfeit oxycodone tablets with fentanyl and its analogues. The sheer number and diversity of fentanylrelated compounds and designer drugs make identification by forensic laboratories a challenge.

The aim of this poster is to show the fentanyl-related compounds and designer drugs that were identified in exhibits analyzed by our laboratory. The instrumentation and techniques used to analyze these drugs and/or the isomers will also be presented.

The Investigation of Ancestral Origins Using Human Cranial Hair

Sirena Lam, University of New Haven; Robert H. Powers, PhD. and Alyssa L. M. Marisco, PhD. Principal Investigator

Human hair is often collected at a crime scene, and microscopic hair comparison (MHC) is typically performed, along with DNA analysis if possible. Unknown samples are microscopically assessed and compared to those of known origin to identify distinct morphological characteristics. Although MHC is a useful screening tool, it relies heavily on the experience and knowledge of the examiner. Hence, it is more subjective and the significance of the results can easily be overstated, leading to flawed testimonies. On the other hand, DNA analysis is a confirmatory test but results from hair are not always consistent. This is because nuclear DNA degrades rapidly during hair keratinization, and is only present in the hair root. Alternatively, mitochondrial DNA (mtDNA) can be used to generate genetic profiles from samples with no root attached as it is found on the hair shaft. However, the amount of mtDNA recovered varies in each case, and depends on the individual. Thus, DNA analysis using hair may not be possible in certain cases. Unfortunately, MHC and DNA analyses are limited options that could serve as roadblocks, and potentially reduce the evidentiary value of hair. Therefore, additional studies are necessary to explore other comprehensive techniques. This research utilizes a more objective approach for hair analysis, notably to assist in the differentiation of ethnic origins without the

heavy scrutiny and subjective nature of MHC. Hair is mostly composed of proteins, and therefore, contains a large amount of amino acids that can provide information specific to the donor. Considering this, ratios of three amino acids (serine, phenylalanine and threonine) were utilized to investigate differences in hair from donors of different ancestral origins.

With consent, hair fibers from four regions of the head were collected from three individuals of the Mongoloid, Caucasoid, and Negroid anthropological groups. All samples were washed to remove surface contaminants, ground, and digested to break proteins down into amino acids. Finally, samples were derivatized to increase sample volatility, and analyzed by gas chromatography/mass spectrometry (GC/MS). Chromatographic data was obtained using scan mode, the integrated peak areas were measured and various amino acid ratios were calculated for all individuals. Results indicate that two ratios, serine to phenylalanine and threonine to phenylalanine, are different in all three samples. The greatest difference in both ratios is between individuals of Mongoloid and Negroid descent. These results imply that certain amino acid ratios may vary among people of different ethnic origins, and that this method is potentially useful in distinguishing individuals of different ethnic backgrounds in cases where MHC is insufficient or DNA analysis cannot be performed.

Simultaneous quantification of synthetic cathinones and metabolites in urine by gas chromatography- mass spectrometry

Mei-Chih Lin, Division of Research and Analysis, Food and Drug Administration, Ministry of Health and Welfare, Taiwan

In recent years, synthetic cathinones have emerged and grown to be popular drugs of abuse. In Taiwan, Mephedrone is one of the most popular drugs. Some criminals and drug users began to synthesize structural analogs of cathinone to circumvent the rules. To protect people's health and to prevent drug abuse, we developed an analytical method using gas chromatography mass spectrometry (GC/MS) for 15 synthetic cathinones and 5 metabolites including Mephedrone, 4-Fluoromethcathinone, 4-Bromomethcathinone, 4-Chloromethcathinone, 3,4- Dimethylmethcathinone, Methedrone and 4-Methylethcathinone quantification in urine samples. The urine samples Northeastern Association of Forensic Scientists Annual Meeting 2017 Mount Pocono, PA were extracted with the solid phase extraction (SPE) following the derivatization and injected to GC/MS. Separation was carried out using a HP-5MS capillary column. The selective ion monitoring (SIM) mode was applied and whole analysis could be achieved within 30 minutes. The linearity from 50 to 2000 ng/mL ($r>0.995$). The limit of detection (LOD) and limit of quantification (LOQ) of this method were from 5 ng/mL to 20 ng/mL and from 20 ng/mL to 50 ng/mL respectively. The extraction recovery of SPE was 86.3% to 104.6%. This developed method also applied to 6 real urine samples and one of them was found to contain 4-Fluoromethcathinone metabolites. The method for the determination of 15 synthetic cathinones and 5 metabolites in urine has been validated by assessing the limits of detection and quantification, linearity, repeatability, and accuracy.

Accelerating the Analysis of Ignitable Liquids and Ignitable Liquid Residues on the Agilent Technologies GCMS 5977B/9000 Intuvo System

Kirk E. Lokits, Ph.D., Agilent Technologies; Raymond J. Kuk, Alcohol, Tobacco, Firearms, and Explosives; Michelle Clarke Ph.D., Alcohol, Tobacco, Firearms, and Explosives; Graham Robinett, Agilent Technologies; Eric Pavlich, Agilent Technologies

Routine analysis of ignitable liquids (ILs) and ignitable liquid residues (ILRs) by the fire debris analyst, has routinely utilized capillary chromatography with mass selective detectors (MSD). The total ion chromatogram (TIC) pattern of peaks representing components found in various ILs and can be compared to patterns from known IL references. The MSD provides additional selectivity and permits structural identification of the specific compounds found in (IL) and (ILRs). The purpose of this research is to demonstrate that several recent advances in gas chromatography, found in the Agilent Technologies 9000 Intuvo GC, can be successfully incorporated into the current proven methods of fire debris analysis. This work seeks to show that this can be done with minimal disruption to the established practices of data acquisition and analysis while demonstrating the improvements that can be derived from recent developments in GC technology. Method translation software was used in this study to convert an existing fire debris GC method with a runtime of 38 minutes to a fast GC method with a runtime of 19 minutes. The fast GC method is achieved without changing the peak elution or negatively affecting peak resolution.

Analysis of explosive materials and explosive residue on contaminated matrices on the Agilent Technologies GCMS 5977B/9000 Intuvo System

Kirk E. Lokits, Ph.D., Agilent Technologies; Graham Robinett

Analysis of explosives and explosive residues, has routinely utilized capillary chromatography with mass selective detectors (MSD). The MSD provides sensitivity, selectivity, and permits structural identification of the specific compounds found in explosives and residue matrices. The purpose of this research is to demonstrate that several recent advances in gas chromatography, found in the Agilent Technologies 9000 Intuvo GC, can be successfully incorporated into the current proven methods of explosive analysis. This work seeks to show that this can be done with minimal disruption to the established practices of data acquisition and analysis while demonstrating the Northeastern Association of Forensic Scientists Annual Meeting 2017 Mount Pocono, PA improvements that can be derived from recent developments in GC technology. This study utilized an existing conventional explosives GC method without changing the peak elution or negatively affecting peak resolution. The advancements in GC design facilitates the Intuvo to be the solution for a field mobile GCMS solution, utilizing Hydrogen as the carrier gas, and generating a custom library for explosive compounds.

Reading the Fine Print: Connecting Perpetrators to Crimes by Imaging of Forensically-relevant Molecules in Fingerprints by Matrix-assisted Laser Desorption Ionization Mass Spectrometry Imaging

Cameron Longo, University at Albany-SUNY; Professor Rabi A. Musah, University at Albany- SUNY

Fingerprint analysis has been in use since the early 20th century in investigating crimes, and it continues to constitute important evidence in many court cases. Usually unique to an individual, fingerprints can, beyond a reasonable doubt, place a particular person at the scene of a crime. In addition, more than simply providing identifying information, fingerprints have a high information content that remains vastly underutilized in forensic science. Examples of

information that can potentially be determined through the analysis of fingerprint residues include exposure to chemical markers associated with prescription medications or illicit drugs, gunshot residue, or explosive materials components among others. The identification of diagnostic molecules in fingerprint residues has already been accomplished and in some cases, is used in routine screening such as that which is conducted at some airports for explosive materials. Nevertheless, the revelation of fingerprint traces that are based not on the presence of endogenous biomarkers such as fatty acids or amino acids, but in addition, molecules that may be directly related to a crime, would provide an important dimension to the observed evidence. For example, while detection of a fingerprint based on the presence of human-derived biomarkers can indicate that a particular individual was at the crime scene, the presence of a print trace based on molecules indicative of exposure of drugs may provide insight into the state of mind of the individual when they were at the scene. Thus, access to such information could provide vital clues in forensic investigations.

We demonstrate here that biomarkers demonstrative of events that may be relevant to forensic investigations are detectable in fingerprints by matrix-assisted laser desorption ionization high-resolution mass spectrometry (MALDIHRMS) analysis. Furthermore, the spatial distribution of these molecules can be mapped by using imaging mass spectrometry techniques to reveal fingerprints that provide a direct connection between the small-molecule markers in question and an individual. Fingerprints containing markers of interest such as those indicative of exposure to such varied products as psychoactive plants (e.g. San Pedro cactus, Mimosa hostilis, Picralima nitida), gunshot residue, tobacco products and illicit drugs among others, were deposited onto conductive ITO slides, sprayed with a matrix solution, and interrogated by SpiralTOF MALDI-HRMS. Subsequently, ion images derived from determination of the spatial distributions of m/z values representative of molecules of interest revealed the fingerprints of the individuals who handled the product, thereby providing information on the history of the individual prior to the deposition of the print. Importantly, the images generated from the markers of interest were identical to those created by endogenous lipids such as oleic acid. This method provides not only information about a person's lifestyle or recent actions, but furnishes it in a way that is directly linked to the identity of the individual. Such information may have significant evidentiary value.

An Investigation into the Intermediates Produced in the Synthesis of 3,4-Dichloromethcathinone

Zachary Lutz, Cedar Crest College; Thomas A. Brettell, Ph.D, D-ABC and Marianne Staretz, Ph.D, Cedar Crest College

Increased activity of clandestine chemists led to the creation of numerous designer drugs. These designer drugs vary slightly from the large families of drugs, such as cannabinoids, cathinones, amphetamines; all drugs mentioned previously are psychoactive. This can lead to some challenges in regards to identification, such as mislabeling or accidental misidentification. The rise of designer drugs leads to the growing need to identify these new compounds and their occurring intermediates during the synthetic process. Intermediates produced during the synthesis of 3,4-dichloromethcathinone can be analyzed using GC/MS, ATR-IR and NMR. (1,2)

In conclusion, providing information regarding the synthesis and identification of the intermediates during the synthesis of 3,4-dichloromethcathinone was performed to help reduce the mislabeling or accidental misidentifications of these compounds. Forensic laboratories would benefit from this information by applying this information to their libraries, which will help improve the accuracy of forensic chemistry casework.

Cryptography Using Bioaffinity-Based Assays

Leif McGoldrick, University at Albany-SUNY; Jan Halámek, University at Albany-SUNY

Since IBM and the NSA introduced the Data Encryption Standard to the general public, the need and use of encryption of important personal data has been exponentially increasing. This has led to more dynamic and varied methods of encryption to be researched. Recently, it was seen that one can use chemicals reactions in order to build a cypher for encrypting data. In this presentation, biochemical assays (bioassays) using enzyme cascades will be used in order to encrypt data based off of the WWII enigma machine used by German cryptographers. By using bioassays, one is able to encrypt their data using the output data from the instrument used, according to the very specific parameters. Multiple enzyme cascades are used in order to build an encryption key, which is then applied to the message text or data of interest which makes a cypher text. This cypher text can only be decrypted using the same exact parameters and instrument as the one who encrypted the message. These parameters include, but are not limited to, enzyme units, substrate concentration, model of instrument, wavelengths chosen, and instrument setup. This data would then be applied to the cipher text, resulting in the one receiving the message to produce the original, or plaintext, message.

In the realm of forensics, cryptography is also important since people use various methods in order to hide their data and communication. This methodology may be applicable in multiple facets of forensic investigation involving illegal activities where data is encrypted. It has been seen that many instances of encryption in forensic cases ranging from the Zodiac Killer to illegal business deals such as illegal gambling and money laundering.

Recommendation for the determination of matrix suppression in biological samples by UPLC-ESI- MS/MS: Extending Quality Measures in Forensic Toxicology

Courtney McGowan, Boston University School of Medicine; Sabra Botch-Jones, Boston University School of Medicine; Kacey Kliburn, FAA; Frank Kero, PerkinElmer

Ion suppression or enhancement of analytes due to incomplete removal of matrix components can negatively impact analytical methods. Based on the Scientific Working Group for Forensic Toxicology Standard Practices for Method Validation in Forensic Toxicology, suppression/enhancement can be evaluated using two approaches: post-column infusion or post-extraction addition. By using one of these two approaches, laboratories can assess extraction efficacy to gauge impact on crucial validation parameters. This study demonstrates the use of post-column infusion as a tool to

evaluate the effectiveness of different sample preparation methods to reduce such matrix effects. De-identified, drug-free pooled human blood (n=8) was extracted at Boston University. Extracts were shipped to PerkinElmer (Shelton, CT) for analysis. The analytes morphine, BZE, THC-COOH, Fentanyl, 6MAM, and hydrocodone (Cerilliant Corporation, Round Rock, TX) were selected to capture the effect of matrix suppression on analytes that elute at different time points during a chromatographic run. Different sample preparation techniques were compared. Protein precipitation, phospholipid depletion, and solid phase extraction were evaluated separately and in combination. The instrument used was a PerkinElmer QSight™ 220 MS/MS coupled to a PerkinElmer UHPLC. The post-column infusion approach was effective in mapping the suppression events versus retention time to help predict future issues with quantitation for specific analytes. It was suspected that early eluting analytes would be most affected by salts. It was determined that later eluting analytes were most affected by phospholipids via MRM monitoring of known lipid and phospholipid transitions. Further comparison of human blood from six different sources was further evaluated.

Quantification of 11-Nor-9-carboxy-Δ9 -tetrahydrocannabinol in Urinary Samples via GCMS

Ebrar Mohammad, Syracuse University; Dr. Ulrich Englich and Omar Salem, Syracuse University

Cannabis is one of the most commonly abused drugs. Its consumption is associated with legal applications as medical marijuana and as a recreational drug. The drug is legalized in some parts of the United States and in several countries, though federally marijuana and all cannabis products are still listed as a Schedule I drug. Upon use of cannabis, the active ingredient tetrahydrocannabinol (THC) can be identified in blood and saliva samples. However, blood levels drop as THC is quickly metabolized in the body to its oxidation products 11-hydroxy-THC (hydroxy-THC) and 11-Nor-9-carboxy-Δ9 -tetrahydrocannabinol (carboxy-THC). Therefore, in forensic and clinical applications instead of detecting THC in blood, the abundance of the carboxy metabolite in urinary samples is usually determined to identify recent as well as continued or chronic cannabis consumption. This compound remains detectable for several hours and in some cases days or weeks after consumption.

In a collaborative study with the Psychology Department at Syracuse University, we were tasked to develop an analytical method to determine the amount of carboxy-THC in urine samples of participants. We developed an improved protocol for the detection and quantitation of carboxy-THC in urine samples based on solid phase extraction methods and subsequent GC-MS analysis. The goal was to achieve a sensitive, reliable and reproducible method for the detection of carboxy-THC in the range from 1000 ng/mL down to 10 ng/mL or less. Linearity, detection limits and limit of quantitation will be presented, and some of the interferences in the analysis that we encountered will be discussed. The acquired data will be compared with the consumption habits of some of the study participants.

Spray Solvent Dependence Observed During the Analysis of Synthetic Cannabinoids via Paper Spray Ionization-Mass Spectrometry

Shahnaz Mukta, Illinois State University; Chase Deberry, Sara Bell, and Christopher C. Mulligan, Dept. of Chemistry, Illinois State University

Paper spray ionization (PSI), a newer ambient mass spectrometric ionization method, employs a paper substrate to allow the direct analysis of deposited or swabbed samples through simple addition of high voltage and an appropriate

spray solvent. During PSI investigation, analyte ions are generated through an electrospray-like process directly from the paper substrate itself. To date, PSI-MS has been shown highly applicable to forensic evidence screening, particularly for drugs of abuse. While a majority of drug classes have been shown broadly robust to the spray solvent system employed for PSI analysis, recent research has shown that successful analysis of synthetic cannabinoid and related evidence is highly dependent on the solvent employed. In this presentation, a systematic study of spray solvent composition and its effect on broad synthetic cannabinoid screening will be discussed. For comparison, Δ9-THC and several synthetic cannabinoids of diverse classification were investigated. Solvent systems examined included variable ratios of methanol (MeOH), acetonitrile (ACN) and water and acidification via addition of dilute formic acid (0.1% v/v). For every solvent combination, each cannabinoid was analyzed via PSI-MS using a 2 μg deposited mass, and the maximum signal intensity was collected. Using the entire dataset, a heat map was produced that allows the visual inspection of relative performance of each spray solvent system in regards to obtained signal intensity, allowing a user to optimize their intended experiment. Authentic samples of synthetic marijuana evidence were analyzed through interactions with local law enforcement agencies to show similar solvent constraints for plant-based samples. Results obtained show that polarity and solubility of the target analyte can dramatically affect experimental results, and care must be taken in regards to establishing PSI-MS method protocols for synthetic cannabinoid/marijuana evidence.

An Assessment of the PreCR® Repair Mix as a Viable Repair Method for Soil-Degraded DNA

Olivia Negron, University of New Haven; David San Pietro, PhD., University of New Haven

Detection of a DNA profile from a piece of evidence can be crucial in a forensic investigation. Buried evidence containing human DNA such as bloodstains can pose a challenge for profiling due to degradation processes in soil by components such humic acid (HA) which has also been determined to be a PCR inhibitor. Therefore, the aim of this study was to determine if PreCR® Repair Mix can be used for the repair of soil-degraded DNA, potentially aiding casework by providing potential suspect and/or victim leads in violent crimes where evidence containing human blood was buried.

Venous blood was obtained from a single source volunteer. Both 50μL and 150μL aliquots of blood were deposited in quadruplicate on 2in.x2in. polyester swatches, for a total of 30 stained fabric swatches, and dried beneath a hood for 24 hours. 18 of the stained swatches and 6 non-stained swatches were buried 1.5inches deep in a 10-gallon fish tank containing approximately 3inches of soil. Over the course of 4 weeks, 4 stained samples from each blood amount, along with 2 non-stained swatches that were used as negative controls, and 2 bloodstained swatches from the table were extracted with the QIAmp DNA Mini® Kit (Qiagen,USA), quantified with the Investigator Quantiplex ®Kit (Qiagen, USA), amplified using the Powerplex Fusion® Kit (Promega, USA) and capillary electrophoresis on the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, USA), and analyzed using the GeneMarker® HID software (Softgenetics, USA), in order to assess the level of degradation in the donors' original profile. Portions of the degraded samples from each blood-stained group were repaired using the PreCR® repair mix (New England Biolabs, USA) prior to their Fusion® amplification. The results of each extraction group were evaluated based on the extent of allelic dropout/recovery and any changes to the corresponding peak height ratios.

After one week, the 50μL blood samples showed minimal (1 to 2 alleles) allelic activity. Every PreCR® treated sample showed a recovery of 2 to 4 alleles out of the 43 total alleles in the known persons' profile. In weeks 2-4 none of the 50μL samples did not show any allelic activity prior to being treated with PreCR®. None of the nine bloodstained

samples showed any improvement when treated with PreCR®. Studies are currently being conducted with larger quantities of blood (150µL) to examine repair possibilities when larger amounts of DNA are present. Also, direct HA inhibition using various amounts of HA are being assessed in an effort to study its effect on DNA degradation and repair.

This research has highlighted the challenges that come with severely degraded human DNA, but also brings attention to the possibility of repairing soil-degraded DNA using commercially available methods such as PreCR®. Studies remain to be conducted prior to laboratory casework application. It is hoped that extensions of this work will enable the forensic science community the ability to explore DNA repair treatment as an option for what were once thought of as unusable samples.

LC-MS/MS Analysis of Ketamine and its Metabolites in Urine

Jacob Samuel, Boston University School of Medicine; Paul D'aloise and Elizabeth Stapleton from Aegis Sciences Corporation

Ketamine, a dissociative anesthetic, "club" drug and pain management aid, has recently found another role as an acute antidepressant. The excitement surrounding ketamine and the influx of corroborating studies make it likely that ketamine prescription will increase and as such, the need for compliance testing for ketamine will increase. While 96-98% of a ketamine dose excreted in urine are metabolites, Aegis's current GC-MS method only monitors parent Northeastern Association of Forensic Scientists Annual Meeting 2017 Mount Pocono, PA ketamine. To enhance the current method in the face of this opportunity, a faster, more sensitive LC-MS/MS method for detecting ketamine (KET), norketamine (NK) and dehydronorketamine (DHNK) in urine has been developed and validated. The extraction step has moved from three liquid-liquid extractions to one supported liquid extraction saving both time and money in solvents and consumables. The lower limit of quantitation (LLOQ) has been reduced from 4 ng/mL to 1 ng/mL and the run time was been reduced by 82%. All three analytes met internal validation criteria for linearity, precision and accuracy, stability, specificity, LLOQ, upper limit of quantitation (ULOQ), carryover and matrix interferences. Additionally, 60 patient samples, previously analyzed by the current method, were analyzed by the new method. Of those samples, four were positive for KET and had concentrations within 20% of the original reported concentration. Additionally all four samples were positive for NK and DHNK on the new method. While concentrations of metabolites NK and DHNK in patient samples varied, they were consistently higher than that of KET with DHNK being roughly 3x higher than NK.

Assessing the Viability to Detect Decomposition Gases Using Sensors on UAVs

Jason R. Sanderson, Pennsylvania State University; Jack Hietpas, Pennsylvania State University

The identification and location of the remains of missing individuals is costly in terms of both time and manpower. In an attempt to address this issue, we constructed an ammonia sensor that could be attached to an unmanned aerial vehicle (UAV) or drone. We chose ammonia as the analyte of interest because it is released (at times in significant quantities) during several stages of decomposition. The premise of our study was to investigate whether there is a

correlation between ammonia gas sensor signal and the location of a decomposing body. Thus this method may help to constrain the location of potential human remains.

The operation of the ammonia gas sensor (model MQ137) was performed through customized coding of an Arduino Uno R3 micro-controller. Once rudimentary laboratory experimentation and operation of the sensor was complete, we tested the instrument in a staged outdoor scene. The scene was a grass open field. Six calves in the active and dry stages of decomposition placed within a wire fence. The temperature, humidity, approximate wind direction, and surrounding environment were monitored and recorded.

Starting in an area upwind from the location of the calves, the gas sensor was energized and was held stationary for approximately sixty seconds. This warm-up time was found to be necessary for the sensor to establish a stable background signal. The sensor was taken around the perimeter of the fence. The sensor was held at approximately two feet above the grass surface. Ammonia readings were captured every 100 milliseconds. The data collection duration was approximately sixty seconds. The sensor was then exposed to volatiles that were potentially being expelled directly from the decomposing calves. This phase of the experiment was performed by taking the sensor into the fenced-in region and separately presenting it to each of the six calf stations. This process lasted for a total of approximately sixty seconds. At the end of the test, the sensor was turned off and the data was transferred to a laptop computing using an SD storage card. The results from this pilot study show a correlation between the position of the sensor and the location of the decomposing calves. The correlation was slightly obscured by the gentle cross winds. Given the encouraging initial results, additional experiments with the sensor attached to a UAV as well as using different decomposition scenes will be performed.

Solvent Evaporation Studies

Melanie A. Schade, Cedar Crest College; Kelly Reading and Thomas A. Brettell, PhD., D-ABC, Cedar Crest College

It is common laboratory practice to store and preserve standards and samples in chromatography vials with caps. An assortment of different vials and caps are available from commercial sources for this purpose. Types of caps include screw, snap, and crimp among others. The caps may also have no septa or septa for needle puncture. Knowledge of the best conditions to preserve a sample is absolutely necessary when handling evidential material. Knowing what environmental conditions are best and which type of vials best preserves and retains the sample the longest is extremely important and helpful. Solvents evaporating from vials, specifically when the analyst is handling small quantities, is problematic in many circumstances. Solvent evaporation can cause concentration changes or worse, loss of sample altogether. All of this information is especially useful when having to store evidence that may potentially need to be reexamined for the case at a later date.

Solvent evaporation studies were performed in screw, snap, and crimp cap vials with septa. One milliliter of each of three commonly used solvents (methanol, acetonitrile, and chloroform) was pipetted into ten of each type of vial. The vials were then weighed weekly using an analytical balance to determine the amount of solvent that evaporated over a two-month period. These 90 vials were stored at room temperature for the duration of the study. Crimp and screw cap vials lost 10% over the duration of the study. The solvent was completely evaporated in some of the snap cap vials in less than a two month span and most snap cap vials lost over half.

This study was then expanded to include various temperature conditions including at room temperature, in the refrigerator, and in the freezer. Results from these studies showed similar results to the room temperature study; however, a higher rate of evaporation was observed for crimp cap vials stored in the freezer. This data and more results will be reported in the presentation.

Development of a Spectroscopic Method for the Forensic Analysis of Fingernail Polishes and Gels

Alyssa Smale, Lebanon Valley College; Dr. Donald Dahlberg, Lebanon Valley College; Nicole Bois and Dr. Brooke Kammrath, The University of New Haven

Fragments of fingernails are a common type of trace evidence found at crime scenes or on suspects, especially in cases where the victim utilized self-defense. In today's society, billions of dollars are spent on nail polish each year, and wearing nail polish is very common. Five replicate FTIR spectra of 98 different shades of pink and red fingernail polishes and gels were recorded using three different infrared spectrometers. Several classification methods and data pretreatments were used to classify the 490 samples in a leave-one-out cross validation. KNN coupled with Multiplicative Signal Correction and Generalized Least Squares Weighting was able to correctly classify 98% of the samples. In no case was more than one replicate misclassified. No two instruments of the same type produce exactly the same spectra, which would require the construction of a KNN model on each instrument throughout the country that is to be used to identify the polishes and gels. Spectral transfer methods require the spectra of a small number of samples taken on the "parent" and each "daughter" spectrometer. The Direct Standardization algorithm was used to create spectral transfer matrices that corrected each spectrum taken on the daughter spectrometers. This allowed the use of the parent model on spectra taken on daughter instruments with 99% correct classification. Blind tests of the model are presently being performed to further evaluate the model and to determine if the transfer model is working correctly. Spectra of new bottles of polish and samples exposed to various environmental conditions such as light, water and soil will also be used to evaluate the model.

Nanoparticle based Detection Techniques for Low Explosives and Related Compounds

Alexandra Sterner, West Chester University; Monica Joshi, PhD.

Nanoparticle based sensors for trace detection of explosives and other illicit substances have been reported in literature. Due to their unique optical and electrochemical properties, these sensors allow for simple and sensitive detection of materials. They have also the potential for field applications. In this presentation, we discuss an effort to apply gold nanoparticles end-capped with various functional groups to the detection of components of organic gunshot residue (OGSR) as well as various other explosives such as TNT and 2,4-DNT. OGSR is a complex mixture of various organic compounds primarily those resulting from the propellant and organic primers. While normally inorganic gunshot residue is the target of forensic science investigations, OGSR has recently gained popularity since lead-free ammunition has become more prevalent. Nanoparticles end-capped with amine functional groups that give a color change in the presence of an explosive, thus producing an optical method for detecting these compounds. In the case of TNT, the color change results from the Meisenheimer complex, a reaction that has been well studied for optical detection of 2,4,6-TNT. We explore the application of similar reactions to develop a sensor array for organic gunshot residue that can be further developed into a field testing method.

A Rapid Screening Method for the Detection of Fentanyl-Laced Heroin By Fourier Transform Infrared Spectroscopy

Doug Townsend, PerkinElmer; Heather Harris and Karen Scott, Arcadia University

A rapid screening method for the detection of fentanyl-laced heroin by Fourier transform infrared spectroscopy The illicit use of fentanyl and fentanyl-related compounds has become a global concern due to their extreme potency and high risk of fatal overdose. The United States has seen a drastic increase in fentanyl-related overdoses over the past few years with 20,100 deaths reported in 2015, a 540 % increase from 2013.

Current identification of fentanyl in street-drugs requires relatively complex sample preparation, lengthy analysis times, high costs and the sample is inevitably destroyed during the course of the analysis. Fourier transform infrared spectroscopy (FTIR) is considered an easier, less expensive and non-destructive technique that requires little to no sample preparation, making it an attractive candidate for rapid analysis of street-drugs.

In this study, we present a screening method for the qualitative analysis of fentanyl-laced heroin samples using FTIR coupled with multivariate analysis. Six different brands of seized heroin were manually adulterated with pure fentanyl citrate resulting in final concentrations ranging from 8.8 to 50 % fentanyl (% w/w). Samples were measured in their adulterated and unadulterated forms and principal component analysis (PCA) was used to extract relevant spectral features associated with fentanyl adulteration. Principal component score plots show clear separation of adulterated and unadulterated heroin samples indicating that fentanyl can be readily detected at concentrations as low as 8.8 % by weight. PCA is also shown to separate heroin samples based on their overall chemical composition relating to cutting and diluting agents, suggesting that FTIR can be used to fingerprint street-brands of heroin. Results demonstrate that FTIR, in conjunction with multivariate analysis, is able to accurately identify fentanyl-laced heroin samples and could be used as a screening method for solid forms of the drug.

Chiral separation and quantitation of the enantiomers of ephedrine, amphetamine, methamphetamine, MDA, MDMA, and phentermine in blood using LC-MS/MS

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Amphetamines are a class of compounds that contain chiral centers, which affect how they interact with the central nervous system. The ability to differentiate between these enantiomers is of forensic importance in determining the legality of amphetamine use. Here, the enantiomers of ephedrine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA), as well as phentermine, were differentiated in whole blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The separation was achieved using the novel Phenomenex Lux AMP chiral column and a gradient method of 5mM ammonium formate (pH 11) and methanol. Sample preparation was performed through the rapid liquid-liquid extraction of 200µL of sample. The method was fully validated to international guidelines through the assessment of its linearity, limits, accuracy and precision, interferences, matrix effect, carryover, and 24-hour stability. The linear range extended from 10 to 1000 ng/mL for all compounds. This method was then applied to 28 cases that had been

previously analyzed using a non-chiral method to demonstrate its applicability to assist in differentiating between illicit and pharmaceutical sourced amphetamine-type enantiomers. This validated method using LC-MS/MS and chiral column technique demonstrates the novel analysis of a range of amphetamine enantiomers in blood in typical forensic casework.

Sequencing Strokes in a Crossed-Line Intersection Using UV-Microspectrometry

Alicia Wagner, University of New Haven; Dr. Virginia Maxwell

Determining the age of a document is essential to a forensic document examiner, but an equally important focus area is the sequence of intersecting lines, which is instrumental in determining authenticity. However, past techniques that have been used for sequencing, both physical and chemical, are not consistently effective and some analyses are destructive towards the evidence. In a recent INTERPOL and AIEED study, the migration distance of a non-visible component of pen ink was used to determine the age of a crossed-line intersection. These components were visualized through luminescence when viewed under alternate light sources. Development of a non-destructive method to sequence two markings in a crossed-line intersection is desirable and a UV-visible microspectrophotometer (UV-Vis MSP) can be used for this purpose through the collection and comparison of fluorescent spectra. This study is based on the hypothesis that the pen that is on top in the intersection will emit greater fluorescence than the pen on the bottom. Specifically, when the intersection spectrum is compared to control spectra, the control for the pen on top should share more peaks with the intersection than the pen on the bottom. These fluorescent spectra are measured in reflectance, which is why the pen on top will fluoresce more and have more shared peaks. However, there are still small amounts of penetration from the pen on the bottom, meaning it will also share peaks, but a lesser amount. This study examined the fluorescent data from intersection samples, which had been stored in heat and humid environments to the control samples, which were stored in an ambient environment. The experimental samples were created using 6 pen types and 5 paper types. The results of this study show that different environments produced varying success of the sequencing.

Characterization of Salvia Divinorum Fortification by Fourier-Transform Infrared Spectroscopy (FTIR)

Robert Walsh, Boston University School of Medicine; Douglas Townsend, PerkinElmer; Heather Harris, Arcadia University; Courtney McGowan, Boston University School of Medicine, Biomedical Forensic Sciences; Sabra Botch-Jones, Boston University School of Medicine

Salvia divinorum is a hallucinogenic herb that is legally available in the United States (US) and world-wide. Salvia products are used recreationally for their ability to induce hallucinations, which are similar to those induced by other Schedule I hallucinogenic substances such as lysergic acid diethylamide. The herb gained popularity in the United States as a legal alternative to marijuana and other schedule I hallucinogens. The psychoactive effects of the plant are due to the compound Salvinorin A, which is a potent and naturally occurring hallucinogen synthesized by the S. divinorum plant. Due to its relatively short-term effects, with reported hallucinations lasting 5 to 10 minutes, manufacturers have begun to sell leaves that are categorized as "fortified extracts" in attempt to prolong the psychoactive effects. Fortified leaf purportedly contains up to 50 times the Salvinorin A content compared to the natural herb, making it 50 times more potent.

Neither S. divinorum nor its active component Salvinorin A are controlled under the US Federal Controlled Substances Act, however several states and local jurisdictions have enacted legislation placing restrictions on both substances. While some states have passed legislation outlawing S. divinorum, no such restrictions have been placed on Salvinorin A. The inconsistent and lack of widespread regulation coupled with the appearance of fortified plant material suggests that Salvia misuse may evolve from a relatively benign issue to an extreme one. Currently, there is no reliable field test for the identification of S. divinorum or Salvinorin A.

To this end, we present the combination of Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) for the selective and confirmatory identification of S. divinorum and Salvinorin A in fortified and un-fortified plant material. A Spectrum Two FTIR Spectrometer with a Universal Attenuated Total Reflectance Accessory (UATR, PerkinElmer, Shelton, CT, USA). The UATR employs a single-bounce monolithic diamond crystal and a pressure arm to provide adequate sample to crystal contact. Scan range was 4000 – 450 cm-1, with 4 cm-1 resolution and 32 scans. The Spectrum Two uses a deuterated triglycine sulfate detector, a silicon carbide mid-infrared source, potassium bromide (KBr) windows and beam splitter. Sample was brushed off and the crystal was cleaned with HPLC grade methanol in-between measurements. Salvia divinorum leaves with zero, 5x, 15x, 35x and 50x fortification were measured by FTIR. Spectral differences between fortified states are primarily due to elevated concentrations of Salvinorin A.

Cartridge Case Ejection Patterns Based on Bullet Weight, Caliber, and Firearm Position

Andrew J. Winter, Middlesex County Prosecutor's Office, New Jersey; Peter Diaczuk, PhD., Pennsylvania State University; Kelly Petersen, Centenary University; Joseph W. Walsh, Jr., Somerset County Prosecutor's Office, New Jersey

Law enforcement regularly comes into contact with two primary types of handguns at shooting scenes -the revolver and the semi-automatic pistol. The semi-automatic pistol can potentially leave additional evidence behind at the scene as the spent case is extracted and ejected from the firearm. These spent cases can become quite significant to investigators in their effort to determine where the shooting occurred, how far the shooter was from his/her target, and sometimes prove or disprove statements from victims, suspects, and witnesses. A total of twelve firearms were used in this research of four different calibers and discharged over two surfaces. A traditional firing method and a non-traditional firing method (firearm canted at 90 degrees counter clockwise) were utilized. This project focuses on the distance the spent cases are capable of traveling from the ejection port of a semi-automatic pistol in an effort to identify the shooter's position.

Bloodstain Pattern on Snow

Charles Kim, Cedar Crest College; Carol Ritter, Cedar Crest College

Bloodstains have been studied on a variety of substrates, for example, the size, shape, and distribution of bloodstains are more affected when deposited onto porous substrates compared to non-porous substrates. One porous substrate with very little published research is snow. In one study conducted by Constable North, snow type was divided into two categories, "hard" and "fluffy". These descriptions are subjective; therefore, in this study average snow densities were calculated for each bloodstain pattern created on snow to add a measurable, more objective component to the appearance of snow. Drip stains, drip patterns, cast-off, transfer, projected, pools, and saturation bloodstain patterns were created on multiple days on both hard, icy snow and soft, fluffy snow as well as on control substrates. The preliminary results will be presented including one observation that bloodstain patterns on hard, icy snow immediately dispersed, losing all fine detail and color. Sequencing of bloodstain patterns, in other words, which pattern was deposited first was also explored in this study. One observation with respect to sequencing was when a bloodstain is deposited on hard, icy snow and then altered by a stepping action. The final conclusion was that the sequencing on snow is not possible.

Rapid Analysis of Pharmaceuticals in Human Tissues using 2D LC-MS/MS

Sabra Botch-Jones, Boston University School of Medicine; Malorie Mella MS[2] , Brendan Schweitzer MS[1], Claude R. Mallet PhD[2], Philip Kemp PhD[3], F-ABFT, Kacey Cliburn MS[3], Dennis Canfield PhD[3], Sabra R. Botch-Jones MS, MA. D-ABFT[1], Boston University School of Medicine[1], Boston, MA, USA; Waters Corporation[2], Milford, MA; Federal Aviation Administration-Civil Aerospace Medical Institute[3], Oklahoma City, OK

Postmortem forensic toxicological analysis involves the analyses on blood or biological tissues of deceased individuals in order to determine cause and manner of death. Validity, reliability, accuracy and precision of the analytical techniques used to perform these analyses are essential. The Forensic Toxicology Research Team at the Federal Aviation Administration (FAA) performs such analyses on samples from victims of fatal aircraft accidents to provide insight on accident causation. Aircraft accidents and crashes are often brutal enough to severely destroy any human remains, which is why toxicologists must rely on more complex biological tissues for analysis. Therefore, there is a need to develop efficient sample preparation methods in order to analyze samples in a timely manner. A robust extraction and clean up methodology, in which a homogenization step precedes, is necessary in order to reach target limits of detection. A micro extraction protocol combined with a multi-dimensional chromatography can decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques

Methods: Based on the chemical structures of the target analytes, a high retention strength sorbent material (Oasis HLB, 40mg, Waters Corp., Milford, MA) was selected for the trap column, while a Bridge Ethyl Hybrid C18 sorbent (Waters Corp.) was chosen for the analytical column. Using ACQUITY Ultra Performance Liquid Chromatography® (Waters Corp.) with 2D technology configured for "Trap & Elute" with At-Column Dilution®. The total run time was 10 minutes, column temperature was set at 60°C, mobile phase A was Millipore waters (MilliporeSigma, Bedford, MA) with 0.5% formic acid (Fisher Scientific, Hampton, NH) and mobile phase B was acetonitrile with 0.5% formic acid (Fisher Scientific). The mass spectrometer was an XEVO TQS (Waters Corp.) operated in positive ionization mode with a source temperature of 150 °C. Tissue samples were provide from fatal aviation accidents for evaluation.

Results: The analytical method included the following analytes: zolpidem, citalopram, norbuprenorphine, oxycodone, normeperidine, dextrorphan, dextromethorphan, diazepam, diltiazem, quetiapine, diphenhydramine, buprenorphine, promethazine, dihydrocodeine, doxylamine, flecainide, hydromorphone, nordiazepam, temazepam,

ndesmethylcitalopram, and oxazepam. The lower limit of detection for all analytes was set at 0.001 ng/mL with a linear dynamic range of 0.01 to 10 ng/mL. The R2 values for all analytes ranged from 0.995 to 0.999 values. Results demonstrated that analytes have recovery values, measured against a post spiked deuterated internal standard (liver ion ratio recovery) ranging from of 50% to 110 %. Of the cases evaluated, case 7 tested positive for dextromethorphan (cough suppressant), case 5 tested positive for flecainide (antiarrythmic agent), and case 2 tested positive for citalopram (antidepressant).

Conclusions: The micro extraction protocol offered the option to evaluate several elution parameters in a short time period and resulted in rapid method development of a 2D LC-MS/MS for the analysis of pharmaceuticals in human tissue samples. The elution optimization was completed within a 4 hrs hands-on work and the 2D LC results were analyzed using an over-night run using a multi-methods sample list (18 hrs). With the extraction protocol optimized, the final protocol produced a clean extract in 30 minutes without any evaporation to dryness and reconstitution into initial mobile phase conditions.

The impact of antioxidant beverages on the chemiluminescent detection of blood stains at crime scenes

Kelsey D Bettex, University of New Haven; Dr. Claire Glynn, University of New Haven

The identification of blood deposited at a crime scene is crucial to the reconstruction of events leading to and following a crime, as well as corroborating or rejecting statements and alibis. Many biochemical reagents are at the scene investigators disposal to both screen for, and confirm, the presence of visible and non-visible bloodstains. One such reagent for the detection of non-visible, or latent bloodstains is a chemiluminescent reagent known as Luminol, which comes in multiple formulations, the most widely utilized being Bluestar Forensic®. It was recently reported,1,2 however not further studied, that the presence of antioxidants in contact with a blood stain may hinder the reaction of such chemiluminescent reagents, giving way to a false negative reaction. Conceivably, blood is the most commonly encountered bodily fluid at crime scenes. Therefore, further investigation of the possibility of antioxidants within the environment potentially masking bloodstains is necessary to address the opportunity for hindered investigations.

This study investigated the potentially negative effects of seven different antioxidant sources on the reaction between chemiluminescent reagents and blood. Methods involved staining both absorbent and non-absorbent surfaces, carpet and tile respectively, with 2mLs of four dilutions of blood: neat, 1:10, 1:100, and 1:1000. Each bloodstain, after a 24-hour drying period, was then treated with 5mLs of one of seven antioxidant sources: orange juice (100%, not from concentrate), green tea (one processed Pure Leaf cold green tea beverage and one unprocessed organic hot green tea leaf beverage), supplement drink (Bai Antioxidant Infusion), red wine (Pinot Noir), coffee (premium roast), or black English breakfast tea. Bloodstains were also treated with Coca-Cola®, which contains no antioxidants, acting as a control. Following a 24-hour drying period, each sample was then treated with one of two chemiluminescent reagents, Luminol or Bluestar Forensic®, and documented for chemiluminescent intensity. A Canon EOS Rebel T3i digital SLR camera was used to document each reaction to later compare to control samples and better approximate the chemiluminescent intensity.

The results of this study revealed red wine and coffee to negatively impact the chemiluminescent reaction of both luminol and Bluestar, creating false negatives on both surfaces, with all dilutions. Samples tested with Coca-Cola® all produced moderate-strong positive chemiluminescence, showing the application of beverages to not act as a barrier for

the reaction, rather potentially the antioxidants present in red wine and coffee inhibiting the reaction. The other five antioxidant drinks produced positive chemiluminescent reactions, however, these reactions were impacted and could lead to misinterpretation. Orange Juice, both green teas, the supplemental drink, and black tea, produced weakmoderate reactions on both surfaces, with Bluestar fading very quickly compared to typical reactions, which could be misinterpreted as a false positive.

This research has highlighted the importance of choice of method and considerations to be taken when screening evidence items/crime scenes for blood, and the possibility of antioxidants in the environment having a negative impact. The results provide a valuable and novel contribution to the forensic science field as the impact of antioxidants has largely been unexplored, yet warrants investigation.

Plenary Session

So whose profession is this anyway? Advancing forensic science in turbulent times.

John Collins, MA, SHRM-SCP
Critical Victories

The profession of forensic science has never been more scrutinized and criticized than it is now. And although the adversarialism that confounds today's forensic science professionals can be frustrating - even demoralizing - all of us can be thankful that we have an opportunity to witness the slow emergence of tomorrow's forensic science profession. The decisions we make today and the examples we set for the newest members of our workforce will determine the kind of future that tomorrow's forensic scientists will experience. In today's environment, it is not good enough to practice forensic science, collect a paycheck, and go home. We must engage the issues and commit to a vision that will allow us to turn over our profession to the next generation. In this keynote address, audience members will:

- Gain an appreciation for the impact we currently have on tomorrow's forensic science professionals
- Understand the threats and opportunities that linger on the horizon
- Learn the single-greatest threat currently facing the forensic science profession
- Appreciate the important differences between science and law

The Pressure from PCAST

David Kaye, Professor
Penn State Law (University Park)

In September 2016, the President's Council of Advisors on Science and Technology issued a Report on Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods. The National Judicial College called it a "shocker" that "rattled the judiciary." The National District Attorney's Association denounced it as "scientifically irresponsible" and protested that adopting "any" of its recommendations would have "a devastating effect on law enforcement." This presentation will describe the contents of the report and discuss its reception in the legal community, the forensic science establishment, and the academic literature. The report's treatment of the terms "validity" and "reliability" will be contrasted to the usual use of these terms in science and statistics, and its proposals regarding the presentation of "error rates" will be explained and compared to some alternatives.

The 2016 PCAST Report, Black Box Testing, and Error Rates – Understanding the Details

JoAnn Buscaglia, PhD

FBI Laboratory Research Chemist

There has been a great deal of recent interest in using "black box and "white box" techniques to evaluate decisions made in a variety of forensic disciplines. In 2016, the President's Council of Advisors on Science and Technology (PCAST) issued a report titled, Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods," which questioned the foundational validity of several forensic science disciplines in part due to the perceived lack of empirical testing of examiner error rates. The PCAST report lauded the black box and white box latent print examiner studies conducted by the FBI Laboratory and Noblis, and provided recommendations for other disciplines to conduct similar evaluations. This talk will help dissect the details of conducting such evaluations, which are not as deceptively simple as they seem.

Black box evaluations are conducted by assessing the examiner's decision without regard to how those decisions are made. Black box evaluations can provide a useful overall understanding of the accuracy, reproducibility, and repeatability of the decisions made in response to a given task. Such evaluations do not attempt to assess how a specific examiner performs on specific data - but black box evaluations are a necessary first step towards such detailed tests. Black box evaluations provide a means of quantifying forensic examinations for which quantitative models do not (yet) exist and, therefore, provide both an interim solution while such models are under development, as well as a means of validating such models.

Conversely, white box evaluations are conducted to gain an understanding of how and why examiners make decisions. White box evaluations are detailed assessments of the bases of examiners' decisions, focused not just on the end decisions but the features and attributes used by the examiners in rendering conclusions. While analyses of black box results deal with the inter-examiner variability of decisions, white box analyses also deal with inter-examiner variability of the detection of features and other attributes.

This presentation will discuss the PCAST report, its recommendations, and topics that should be considered in the design of black and white box evaluations.

Forensic Service Provider Accreditation - The Merger of ANAB and ASCLD/LAB

Laurel Farrell, Senior Accreditation Manager
ANSI-ASQ National Accreditation Board (ANAB)

Accreditation is recognized as an effective mechanism to improve/maintain the quality of work done by a forensic service provider.

Many attending this conference, work for a forensic service provider that is accredited to a program provided by ANAB or ASCLD/LAB. In April of 2016 these two accrediting bodies, both working in the forensic sector, merged. This presentation will address the Why, How, Who, What, Where and When, for the ANAB forensic accreditation programs and provide an overview of the current requirements for testing laboratories accredited based on the ISO/IEC 17025 standard.

- The "Why" behind the merger
- The "How" this merger benefits the forensic science community, as a whole, and your organization individually
- The "Who" is involved in Forensic Accreditation at ANAB and the assessment activities

- The "What" Accreditation Programs are available to you and Overview of the "merged" 2017 ANAB ISO/IEC 17025: 2005 Forensic Science Testing Accreditation Requirements
- The "Where" to go if you want to know more about an accreditation program and forensic training
- The "When" the merged accreditation program and requirements will be mandatory

General Session

Case Study: The 31-Year Investigation of the BTK Serial Killer

From 1974-1991, the BTK Serial Killer murdered 10 people in the Wichita, Kansas area. Hear from a detective who grew up during the attacks, and years later joined the Wichita Police Department's BTK Task Force. The case study will break down the details of one of the most notorious serial killers, his reappearance in 2004, and how he almost got away with it.

Speaker: Detective Timothy Relph, Wichita Police Department

Detective Timothy Relph has been with the Wichita Police Department for 32 years, primarily working in the Homicide Unit, with early work in larceny/embezzlement and robbery. While working in homicide, Detective Relph has been involved in the investigation of several hundred homicides, suicides, and suspicious deaths. His special assignments include a gang drive-by shooting task force, cold case investigations and task force, and the BTK serial killer task force. He has presented the BTK Case Investigation to law enforcement across the country.

He is the recipient of three Distinguished Service Awards, seven Bronze Wreaths of Merit, and was named the 2013 Wichita Police Department Officer of the Year. He has been married for 31 years and is the father of five children.

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Author Index

The presenting author index can provide a quick reference to find when and in what section presenting authors were scheduled to present at the 2017 Annual Meeting. The reference table below assists you in finding the section in which the presentation was given. Letters correspond to the scientific discipline/section in which the presentation was made while the number corresponds to the page number of the abstract in this booklet.

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