Northeastern Association
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Contents

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Forensic Toxicology Abstracts

Academy Standards Board (ASB) Toxicology Consensus Body Update
Sabra Jones, MS, MA, D-ABFT

The Academy Standards Board (ASB) is an ANSI-accredited Standards Developing Organization with the purpose of providing accessible, high-quality science-based consensus forensic standards. The ASB was established in 2015 and accredited by the American National Standards Institute (ANSI) in 2016. The ASB consists of Consensus Bodies (CB), which are open to all materially interested and affected individuals, companies, and organizations; a Board of Directors; and Staff. The ASB works closely with the Organization of Scientific Area Committees for Forensic Science and its subcommittees which are dedicated to creating a national registry of forensic standards. This presentation will provide an update on the Toxicology Consensus Body activities, published standards, and updates on standards that are in the standard development process.

Breath: A Bodily Fluid
Dennis Hillard, MS and Gil Sapir, JD, MSc

Employers have developed drug free workplace policies. Nationally 46 states have laws imposing drug-testing restrictions specifying testing methodologies, use of test results and privacy issues. (1) Statutory workplace testing of controlled substances permit urine, blood, tissues, or other bodily fluids (2) and may include alcohol. Analysis is by a federally certified laboratory. Frequently, blood is initially analyzed using the enzyme multiplied immunoassay technique (EMIT) and UV analysis. Positive test results require confirmation testing employing gas chromatography/mass spectrometry (GC/MS) or technology recognized as being at least as scientifically accurate. (e.g., LC/MS or UV/IR spectroscopy). Labor statutes usually entail "controlled substances" and may include alcohol. (3) A creative interpretation for workplace alcohol intoxication testing, absent designated statutory or contractual language, seeks to define breath as a "bodily fluid" for statutory testing compliance.

Breath is a derivative factor of blood. The air people breathe is a gas. It is compartmentalized in the lungs for an exchange of oxygen and carbon dioxide which is essential for life. The breath/gas expired from the respiratory system should be considered a bodily fluid - a technical distinction due to practical methods of measurement. However, common sense is paramount. Bodily liquids cannot replace breath and breath cannot replace bodily liquids in living organisms. Creatively contrived attempts to circumvent and redefine basic fundamental science are specious and problematic. If legislators or employers want to employ breath alcohol intoxication for termination under a drug free workplace policy, then labor statutes or contracts should be amended specifying the use of breath as a testing specimen.

Semantics, syntax, syllogism, and euphemisms are irrelevant to breath alcohol as a bodily fluid. Common sense should prevail - statutory construction and the novel interpretation of breath alcohol analysis for workplace termination is at issue, not the science.

Key Words: Breath Alcohol, Bodily Fluids, Workplace, Blood

References:
1. US Department of Labor's Drug-Free Workplace Act of 1988, 41 U.S.C. 81.
2. Common body fluids are: saliva (oral fluid), vitreous humor, cerebral spinal fluid, synovial fluid, urine, blood, bile, semen etc.
3. Schedule of Controlled Drugs, 21 U.S. Code Sect.802, et seq (2002); See, each state's-controlled substance act. A workplace argument is controlled substances include alcohol due to control by age, intoxication laws, state owned liquor stores etc. Approximately half of the states include breath alcohol sample testing.

Utilizing BIO-SPME and DART-MS to Detect Drugs in Human Breast Milk
Emily Woods, MS and Adam B. Hall, PhD

Human breast milk is a biofluid produced by a woman's body during pregnancy. Breast milk contains necessary nutrition to a growing infant as well as xenobiotics--including drugs of abuse—consumed by the woman which diffuse into the breast milk from the bloodstream. Since breast milk is recommended to be part of all infants' diets, being able to detect any toxic components--such as drugs--in the matrix is critical. However, despite the ease and noninvasive nature of collection, human breast milk is a difficult matrix to analyze due to its high fat and protein content. Thus far, no literature has been published on the analysis of breast milk through direct analysis in real time mass spectrometry (DART-MS). Adapting DART-MS to detect drugs of abuse in human breast milk will allow for quick and timely identification of drugs present in an individual's breast milk, as well as aid in research regarding the potential harmful effects of drugs--both licit and illicit--on an infant who is breastfeeding. Forensically, this method could potentially allow toxicologists to use breast milk as a matrix to determine if drugs played a role in a woman's or breastfed child's death. Using both C18 biocompatible solid phase microextraction (BIO-SPME) fibers and QuickStrip™ cards, a DARTMS method was developed to be able to detect drugs of abuse in human breast milk. Four drugs of abuse (cocaine, codeine, morphine, and delta-9-tetrahydrocannabinol (Δ9-THC)) --all of which are either commonly abused during the postpartum period or are of particular danger to breast feeding women--were chosen to be studied. The drugs of abuse were extracted from either whole or prefiltered human breast milk using either liquid-liquid extraction or C18 BIO-SPME fibers and detected with DART-MS using parameters suggested by IonSense, Incorporation (Inc.). Mass spectral results indicated that macromolecules in whole breast milk did not hinder extraction or detection and that a larger amount of the analytes were ionized/desorbed when using the BIO-SPME fibers. Thus, a BIO-SPME method adopted from IonSense, Inc. utilizing C18 fibers and SPME DART-MS parameters (with temperature and rail time adjustments) can be used to quickly detect cocaine, codeine, morphine, and Δ9-THC in human breast milk, indicating that this method may be used for the detection of other drugs of abuse in breast milk. In addition, BIO-SPME fibers can be used to quantify the concentration of cocaine in breast milk between a range of 50 and 200 nanograms per milliliter as demonstrated by a matrix matched calibration curve created using various concentrations of cocaine. Despite its benefits, the BIO-SPME and DART method cannot be used on samples containing more than one drug of abuse (based upon the drug concentrations utilized in this study) due to competitive adsorption and competitive ionization, respectively, as not all drugs could be detected when this method was applied to breast milk samples containing numerous drugs.

Evaluating the Efficacy of Three Beta- Glucuronidase Enzymes for the Detection of Opioids for Forensic Toxicology Urine Testing in Drug Facilitated Crime Investigations

Reshma Gheevarghese, MS, Traci Reese, MS, Kelsey McManus, MS, Collin Hill, MS, Jamie Foss, BS, and Sabra Botch-Jones, MS, MA, D-ABFT

Drug-facilitated sexual assaults (DFSA) are a severe public health and safety concern. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is an analytical technique that has been previously shown to be effective for detecting and quantifying drugs in human biological samples in DFSA cases. Urine is the preferred sample of choice in these cases, as the detection window for certain analyte metabolites can be detected up to four days after the alleged incident. Opioids are of particular concern due to the central nervous system depressant effects and are often excreted in the urine as glucuronidated metabolites. By incorporating an enzymatic glucuronide hydrolysis step in sample preparation, the parent drug of these compounds can be targeted.

The main objective of this study is to evaluate the efficacy of three enzymes for the recovery of parent drugs using enzymatic hydrolysis in sample preparation. Four opioid metabolites were utilized in this research: codeine-6-β-D-glucuronide, dihydrocodeine-6-β-D-glucuronide, hydromorphone-3-β-Dglucuronide, and morphine-3-β-D-glucuronide (Cerilliant, RoundRock, TX, USA). Enzymatic recovery was evaluated for three enzymes: B-OneTM shelf-stable β-Glucuronidase for high-throughput analysis from Finden by Kura (Puerto Varas, Los Lagos, Chile), BGTurboR Glycerol Free High-Efficiency Recombinant β-Glucuronidase from Finden by Kura, and Fast β-Glucuronidase, Recombinant from Sigma (St. Louis, MO, USA). Enzyme hydrolysis was conducted according to the incubation conditions provided

in Table 1. Sample extraction was performed using supported liquid extraction (SLE) using ISOLUTER SLE+ (Biotage, Charlotte, NC, USA). Following sample preparation, including hydrolysis, and supported liquid extraction, urine case samples were analyzed using the QSightR 220 CR laminar flow tandem mass spectrometer with electrospray ionization, which was operated in positive ion mode (PerkinElmer, Waltham, MA, USA). Chromatographic separation was achieved using a 50 x 4.6 mm pore size 100 A˚, 2.6 µm core-shell KinetexR phenyl-hexyl HPLC column from PhenomenexR (Torrance, CA, USA). The column was kept at 40°C for the duration of the run. The aqueous mobile phase A was 0.1% formic acid in Millipore water, and the organic mobile phase B was 0.1% formic acid in methanol. The flow rate was kept constant at 0.600 mL/min for the entirety of the run. The total run time for the method was 11 minutes.

A linear dynamic range of 5-200.0 ng/mL was established for all four analytes. Based on the established calibration model, the limit of detection (LOD) was 2.5 ng/mL and the limit of quantitation (LOQ) for all analytes was 5 ng/mL. Analytes displayed an acceptable bias of +/-20%. Precision was analyzed concurrently and determined to be within +/-20% for all analytes. All analytes were determined to be free from significant carryover. No matrix interference peaks were observed in blank urine samples, which fell within 2% of a known analyte retention time and had a signal intensity greater than the calculated LOD. Recovery of the analytes was conducted in triplicates, and the results are reported in Table 2. Based on the study results, B-OneTM and BGTurboR from Finden by Kura are user-friendly, with explicit instructions for enzyme hydrolysis mix formulation and incubation steps, thus facilitating the integration of enzymatic hydrolysis in sample preparation. Further optimizations of the hydrolysis parameters are required for Fast β-Glucuronidase, a recombinant enzyme from Sigma, to be integrated into sample preparation.

Table 1. Enzymatic Hydrolysis Conditions

Enzyme	Enzymatic Activity	Temperature (°C)	pH	Incubation Time (mins)
Kura Biotech- B-One™	12,000 PS-U/mL	~22 (room temperature)	6.8	5
Kura Biotech-BGTurbo®	200,000 U/mL	55	6.8	15
Fast β-Glucuronidase, Recombinant from limpets (*P. vulgata*)	300,000-400,000 U/mL	70	5.2	15

Table 2. Average Hydrolysis Recovery Data

Analyte	Kura Biotech – B-OneTM	Kura Biotech – BGTurbo®	Fast β-Glucuronidase, Recombinant
Morphine	103.10	96.07	49.80
Codeine	99.76	99.67	49.19
Dihydrocodeine	92.61	96.34	41.64
Hydromorphone	97.73	100.7	50.54

Bladder Wash: A (Not-So) Alternative Specimen for Postmortem Toxicology. An Update Including Survey Results

Karen Scott, PhD, Kylie E. Candela, MSFS, Amy P Hart, MD, Luke N Rodda, PhD

Bladder washes can serve as an efficient and accurate alternative specimen when there is no urine available to analyze. They are also a reliable alternative specimen to analyze when other specimens, such as blood and vitreous humor, cannot be collected and toxicologically analyzed.

Urine is the preferred sample in postmortem forensic toxicology to provide evidence of antemortem drug use. However, there are many scenarios in which the bladder is voided or dehydrated prior to their autopsy. In these cases, it is possible to wash the bladder with saline and collect the bladder wash and any residual urine for drug screening and confirmation. The San Francisco Office of the Chief Medical Examiner (OCME) has made the collection of bladder washes at autopsy an option when urine is not available. While bladder washes are not conventional, this study aims to determine its use as an alternative specimen in postmortem forensic toxicology.

Data from analysis of bladder wash samples collected at the OCME were analyzed to assess the efficiency of this alternative sample in comparison to urine from the same individual by determining the identities of individual analytes and their metabolites. Following laboratory analysis of drugs by two LC-MS/MS methods and alcohol by HS-GC-FID, authentic case results showed that bladder wash drug analyses have the good sensitivity and selectivity to serve as an alternative specimen when urine is not available. These results are even more so if both individual analytes and metabolites comparisons are considered during data analysis. The bladder wash drug analysis data was also compared to blood analysis data from the OCME to determine if the two were complementary. While bladder wash and blood drug analyses have compatible selectivities, the sensitivity of bladder wash analyses are lower than their blood counterparts. Finally, in cases where only a bladder wash is analyzed, the detected drugs may provide crucial information to a forensic pathologist about the cause and manner of death that would have otherwise not been obtained.

Simultaneous, to this study, a two-part survey was sent via the NAME list serve to evaluate pathologists' views of the use of bladders washes before and after reviewing the results of the bladder wash data. Overall, pathologists were responsive to adopting the practice of collecting bladder washes for toxicological analysis.

This study appears to indicate that standardizing the collection and analysis of bladder washes in postmortem toxicology will provide forensic pathologists with a comprehensive toxicological profile in cases where urine and/or other biological specimens are not available for collection and subsequent analysis.

Keywords: Bladder wash, urine, blood, alternative specimen, postmortem toxicology

The Effects of Adulterants on the Detection of Delta-9-Tetrahydrocannabinol and Methamphetamine in Oral Fluid Immunoassay Testing

Alicia Huerta, MS, Joseph Jones, MS, Shanna Cawley, MS and Sabra Botch-Jones MS, MA, DABFT

Introduction: Drug screening is widespread in contexts such as the criminal justice system, employment, and substance abuse treatment centers. Traditionally, drug testing methods have targeted urine matrices. Depending on the nature of sample collection, urine specimens may be tampered or adulterated in efforts to invalidate or pass the drug test. For various reasons, including the effects of adulteration, time, and costs associated with urine drug testing, oral fluid (OF) has become an increasingly important alternative matrix for screening for drugs of abuse (DOA). It offers distinct advantages since tests can be administered noninvasively, quickly, and under observation, thus reducing the risk of adulteration. Despite mandatory guidelines by Substance Abuse and Mental Health Service Administration procedures controlling for OF specimen adulteration, it is recognized that manufacturers will continue to develop and market new products to avoid drug detection, just as with urine drug tests.

Methods: This experiment investigated the effects of the commercially available drug testing subversion products High Voltage Saliva Cleanse Mouthwash (High Voltage Detox, Las Vegas, Nevada, USA) and Stinger Detox Mouthwash (Stinger Detox, Phoenix, Arizona, USA) on immunoassay testing for tetrahydrocannabinol (THC) and methamphetamine (MET) in OF. The High Voltage Saliva Cleanse was designated adulterant "A" and Stinger Detox was designated adulterant "B". Two separate immunoassay kits, Discover™ (American Screening Corporation, Inc., Shreveport, Louisiana, USA) and Orawell® (Jiangsu Well Biotech Co., Ltd, Changzhou, Jiangsu, China), were assessed to investigate the differences in performance of the current available testing devices in addition to the effects of the subversion products. Using Discover™, samples were spiked according to 0.5 times (x), 1x, and 2x the cutoff concentrations of 50 ng/mL THC and 50 ng/mL MET without adulterant, with Adulterant A, and with Adulterant B. Testing with Orawell® devices was initially conducted at 1x and 2x the cutoff concentrations of 40 ng/mL THC and 50 ng/mL of MET. Additional testing was conducted at 1.5x and 3x the cutoff concentrations without adulterant, with Adulterant A, and with Adulterant B.

Results: In using the Discover™ kits, 83% (n=6) of the negative controls produced a positive result for THC. Twenty-six results (~96%) from the THC tests were found to be positive at each concentration level, even in samples with adulterants present. Twenty-six results (~96%) from the MET-containing tests were found to be negative. In the Orawell® kits, 32 (~97%) samples tested containing THC at any concentration level were found to be negative, regardless of the presence of adulterants. Samples containing less than 2.5x the expected cutoff concentration of MET were found to be negative, while samples containing more than 2.5x the cutoff were found to be positive.

Conclusions: There were no significant observed effects of adulterants on testing of either DOA on the Discover™ immunoassay. It was concluded that the adulterants affected the test results in the Orawell® device, by producing false positives for DOA not present in the sample for 17 (56.7%) of the 30 tests containing adulterants. Additionally, it was concluded that both immunoassay tests assessed were lacking in analytical sensitivity and reproducibility.

An Evaluation of Weak Anion Exchange Solid Phase Extraction Cartridges for the Quantitation of PFAS Compounds in Human Biological Matrices

Halia Haynes, BS, Reshma Gheevarghese, MS, Peyton Tierney, BS, Audrey Ozga, Abderrahim Abdelkaoui, Joyce Wong, PhD, Jason Weisenseel, PhD, Sabra Botch-Jones, MS, MA, D-ABFT

Introduction: Per- and polyfluoroalkyl substances (PFAS) encompass a large group of manufactured compounds that have been used in various production processes such as food packaging, commercial products, workplaces, homes, water supplies, and in food. PFAS are persistent, resistant to degradation, and can bioaccumulate. The CDC's 2015-16 health survey found average blood levels of 4.72 ng/ml for PFOS and 1.56 ng/ml for PFOA, although an exposure limit that predicts adverse health effects has yet to be determined.

Objectives: Sample preparation and analytical methods are necessary to detect and quantitate these compounds in human biological matrices and help us fully understand how they relate to a variety of health outcomes such as pre- and postnatal health, immunity, cancer, and hormone disruption. Materials and Methods: All samples and quality controls were prepared by spiking certified reference material into pooled human serum. A laminar flow ultra-high pressure QSightR220 LC-MS/MS was equipped with a Selectra C18 column. Extraction was accomplished using a Weak Anion Exchange (WAX) solid phase extraction (SPE) column (UCT, ECWAX053) by first conditioning the columns with 1 mL of methanol followed by 1 mL of 100 mM pH 7 phosphate buffer. Samples were loaded onto the column at a rate of 1-2 mL/min. The SPE cartridges were washed with 1 mL of 100 mM pH 7 phosphate buffer and 1 mL of DI water (Millipore Milli-Q Ultrapure Type 1 water system), then dried under full vacuum for 5 minutes. Elution was carried out with 2.5mL of a 98:2 methanol:ammonium hydroxide solution. The eluted samples were then evaporated to dryness using a nitrogen system at 55°C and 5 psi. All samples were reconstituted in 100 µL of a 96:4 methanol:water solution. Parameters assessed followed ASB 036 standard for method validation, including matrix recovery, limit of detection (LOD), limit of quantitation (LOQ), and calibration model.

Results: The results of the study were gathered from the following eleven analytes: PFBA, PFBS, PFHxA, PFHpA, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoA. Depending on the analyte, a lower limit of quantitation was established at 0.12 - 0.69 ng/mL and an upper limit of quantitation at 44.25 - 50 ng/mL. Based on the established linear calibration model an LOD and LOQ in the range of 0.12 - 0.69 ng/mL were achieved. All eleven PFAS analytes showed an acceptable bias of +/-20%. All analytes showed a between-run precision (%CV) in an acceptable range of +/-20%. The average recovery for SPE ranges from 77.64-104.73% with recovery of ~77% for PFBS, ~83% for PFBA, and ~95-105% for PFHxA, PFHpA, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoA.

Conclusions: Utilizing the UCT WAX SPE column, we were able to demonstrate good recovery for the majority of the PFAS compounds. Further, the extraction technique was efficient for high throughput analysis with the extraction time comparable to other traditional SPE methods. The total analytical run time using the UCT Selectra C18 column, 11 minutes, allowed for adequate reequilibration and system washes to prevent carryover and contamination of these persistent pollutants with excellent chromatography. Accurate quantitation of PFAS compounds in biological matrices will allow for better understanding of prevalence, bioaccumulation in biological matrices, and how these concentrations relate to various health outcomes.

Drugs, Alcohol, and the COVID pandemic: A four year look at New York State trends

Amanda Cadau, M.S., D-ABFT- FT

Retrospective toxicology trends from New York will be discussed including any changes recognized during the COVID pandemic. The New York State Police receives ~3100 cases per year for DUID, for which the primary evidentiary sample is blood. The scope encompasses 14 classes of drugs as well as alcohol testing. The scope meets or exceeds the DUID Tier I recommendations and ANSI/ASB Standard 120: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations for all drug classes. The laboratory doesn't have any administrative cutoffs for alcohol testing. Therefore, if a case is submitted for alcohol and drug testing, it will be tested for both, irrespective of the BAC level.

Since 2018, the most prevalent drugs identified in blood were Cannabinoids, Fentanyl, Cocaine/Benzoylecgonine, Alprazolam, Amphetamine/Methamphetamine, 7-aminoclonazalom, and Buprenorphine. Buprenorphine was added to the scope of testing in 2019. The most prevalent drugs remained consistent over four years. One notable increase in prevalence was for Fentanyl identifications. Fentanyl remains a popular identified drug, showing a 60% increase from 2019-2020.

Blood alcohol levels were examined for any correlation to gender. Since 2018, the average BAC level has increased for all subjects from 0.156% to 0.167%. There are twice as many blood alcohol requests received from males than females, but average BAC levels are higher in females. Data also show age/gender differences in positive alcohol results. For 2021, the most prevalent age bracket for a positive alcohol result is 21-29 for males, and both 21-29 and 30-39 for females.

During the height of the pandemic (March-April 2020), there was a 40% decrease in monthly DUID submissions compared to the previous year. The amount of blood submissions for the entire year of 2020 was comparable to 2019. While the laboratory had more submissions (~8%) requesting alcohol analysis in 2020, there was a 12% decline in positive alcohol cases compared to 2019. The average BAC level increased to 0.165% from 0.158% the previous year. For most of the drug classes, the drug identifications remained consistent in comparison to the previous year. Two notable trends were a 11% decrease in cannabinoid identifications and a 20% increase in methamphetamine cases. Despite business closures during the pandemic, our submissions for driving while under the influence of alcohol and/or drug were still comparable to previous years.

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Trace Evidence/Fire Debris & Explosives Abstracts

The American Board of Criminalistics: Looking to the Future
Amy Duhaime, Rhode Island State Crime Laboratory

During my time as the NEAFS representative to the American Board of Criminalistics (ABC) Examination Committee, I have been witness to much growth and change as we sought to make the certification examinations the best they could be. But it was the ABC's decision to pursue ISO Accreditation that has brought about the biggest changes, particularly for the Examination Committee. In addition to amending policies and procedures, the ABC decided to go through the process of developing new certification examinations that aligned with the accreditation standards to which we hope to someday be accredited. During my presentation, I will update you on the ABC's quest for accreditation and give you some information on our new certification examinations.

Identification of Inorganic Residues Using Microcrystalline tests and Raman Microspectroscopy
Caitlyn Kresge, Lawrence Quarino, Ph. D., ABC-GKE, and Lindsey Welch Ph. D., of Cedar Crest College, and Matthew Quinn, M.S., of Waters Corporation

While the combination of microcrystalline tests and Raman spectroscopy have been used in the detection and identification in forensic drug analysis[1,2], no such method has been attempted with inorganic explosive analysis. Many other techniques have been used in the analysis of components of explosives, but none are as rapid, reproducible, and require trace amounts as the method to be described. In this robust method, Raman microspectroscopy is paired with microcrystalline tests to identify inorganic ions commonly found in explosives, specifically, those found in fertilizers, fireworks, and pyrotechnics. Raman spectra were generated from microcrystals produced in aqueous test samples and reacted with five test reagents respectively: squaric acid, nitron, silver nitrate, methylene blue, and chloroplatinic acid. The combination of microcrystal shape and Raman spectra was used to create a flow chart to identify urea and fifteen ions commonly found in these types of homemade explosives: nitrate, nitrite, sulfate, phosphate, oxalate, tartrate, chloride, ammonium, potassium, sodium, calcium, strontium, chlorate, perchlorate, and silver. The method is suitable for the identification of explosive residues found on evidentiary materials.

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Determining Minimum Size of Soil Samples for Forensic Geological Analysis
Brittany Claassen, Emma Redman, Thomas A. Brettell, Ph. D., ABC-GKE, and Lawrence Quarino, Ph. D., ABC-GKE of Cedar Crest College, and Ted R. Schwartz, M.S., ABC-GKE of Westchester County Forensic Laboratory, NY

Although not widely practiced in most forensic laboratories, numerous case studies exist in literature showing the evidentiary value of the forensic comparative analysis of soil1. Soil in a forensic context can be of limited size and although numerous comparative techniques exist, the minimum sample size required for reproducible and accurate data in each of these techniques is not well known. In fact, determining the minimum sample size for visual color determination of soil samples has been identified as an OSAC research need. In this study, 15 surface layer soil samples from different geologic areas were collected and homogenized for analysis. Various techniques including digital color determination, particle size distribution, d-space ordering by x-ray diffraction (XRD), and loss on ignition (organic content) were applied to sample sizes ranging from 2 to 0.25 grams. Differences in color between samples was measured using the Nix Color Pro QC Sensor and calculated using Delta E. It was found that color differences between samples and sample sizes was highly dependent on particle size components within samples. Locations that consisted of higher portions of silt and clay fractions reported low Delta E values between sample sizes and among sample sizes, compared

to samples with higher course fractions which reported low Delta E values between sizes and among samples sizes. Descriptive statistics were utilized on particle size distribution and loss on ignition data. The use of correlation analysis and Kolmogorov–Smirnov D statistic suggests that samples below 1 gram begin to show diverging results. Analysis of variance performed on loss on ignition determined that samples with lower carbon content lost showed statistical differences between sample sizes. In addition, confidence intervals indicate that 0.25-gram samples do not often overlap with other sample sizes within each location. D-space ordering via x-ray diffraction was done on 2- and 0.25-gram samples of each location and it was determined that both were visually different. To determine where the differences in sample sizes begin to diverge via XRD, further analysis will be performed on 1- and 0.5-gram samples. Overall, based on preliminary data findings and analysis, samples smaller than 0.5 grams begin to give divergent results compared to larger sample sizes.

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The Effect of Washing on the Transfer and Persistence of Fiber Evidence

Madison Carter, Dr. Brooke W. Kammrath, and Dr. Virginia Maxwell of University of New Haven, and Dr. John A. Reffner of Jon Jay College of Criminal Justice

Learning Overview: After attending this presentation, attendees will gain an understanding of (1) primary, secondary, and tertiary transfer of fiber evidence via washing and drying; and (2) persistence of fibers on clothing after washing and drying.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insight as to how washing affects the transfer and persistence of the two most common fiber types, cotton, and polyester, which can be used to inform the evidentiary significant of fiber evidence.

Fiber evidence has proven to be valuable forensic evidence in a plethora of cases by providing associations between a suspect, victim, and/or location. This can most readily be seen in the Wayne Williams murder trial of 1984, where unique fibers served as linkages between victims and the suspect's home1. Specific characteristics of fibers such as shedability, cross-sectional shape2, colorant (dye or pigment), and fiber type (natural versus synthetic3) can be observed in order to determine the rarity of the fiber, which can be used to assess the evidentiary significance of an association.

A gap in the literature has been identified regarding the effect of different variables, specifically washing, on the transfer and persistence of fibers. This research has been based on the underlying assumption that a perpetrator will wash clothing that was worn during the crime in an attempt to remove any evidence. Four different scenarios were displayed in this research, with target fibers on the donor garments being fluorescently dyed for means of easy identification. These scenarios included washing a single donor garment, washing the donor garment with a recipient garment, washing the donor garment with two recipient garments, and finally washing a single donor garment, taking it out, and washing a single recipient garment. These sets of washes were run for both cotton and polyester donor fibers. After washings were complete, the individual garments were bagged, labeled, and stored for examination using fluorescent photography.

Whether it be a primary, secondary, or tertiary transfer, after examination and documentation, fibers were observed to have been transferred in all scenarios from donor to recipient garments as well as secondary locations such as the inside of the washer and/or dryer. There were also major differences noted in the quantity, location, and size of the recovered fibers based on the number of garments washed as well as the donor fiber type. By understanding how washing affects transfer and persistence of different fibers, forensic scientists can be better informed of the recovery potential and location, as well as evidentiary significance of fibers from washed garments.

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Forensic Discrimination of copper metal by laser induced breakdown spectroscopy

Chase Notari, Brooke W. Kammrath, Ph. D., ABC-GKE, Henry C. Lee Institute of Forensic Science, University of New Haven

Copper metal has great potential as forensic evidence due to its presence in a range of cases from thefts of copper wiring and pipes, the use of copper wiring in IEDs, and its common function as bullet jackets. Excellent discrimination of copper metal has been demonstrated through trace element profiles collected using solution-based ICP-MS. Although ICP-MS has many advantages for elemental analysis, including its low detection limits, high accuracy and excellent precision, alternative methods that are faster, require less (or no) sample preparation, and require smaller sample sizes are being investigated for a range of forensic samples (e.g., glass, polymers, paint, tape, geological materials, etc.).

LIBS is an analytical technique that has gained prominence as a valuable tool for elemental profiling and continues to grow in acceptance in numerous industries. LIBS is an advantageous method due to the fact that it is rapid, requires no sample preparation, is able to simultaneously provide information on multiple elements at once, and is less expensive than other instruments used for elemental analysis. LIBS has proven value for the analysis of glass, paint, soil, ink, and other samples of forensic interest.

The purpose of this research was to evaluate LIBS to determine if it has the ability to perform comparative analysis of copper, specifically the jacketed metal on different bullets. This study first explored the detection capabilities of LIBS, determined an appropriate element menu, and outlined the optimal parameters for LIBS such as laser pulse energy, spot size, pattern size, gate delay, number of pulses per spot, and repetition rate using a copper density block. These optimal parameters were then applied to the analysis of the copper used in jacketed bullets, and the discrimination ability was explored using multivariate statistical methods. The ability of LIBS to perform comparative elemental analysis on copper-jacketed bullets has the potential to provide a novel method for forensic scientists to use in comparing ballistic evidence. The results of this research can be extended to other sources of copper, such as pipes and wiring, thus expanding the utility of LIBS instrumentation in forensic laboratories to alternative evidence items.

Vortex

Pete Diaczuk, John Jay College

Propellant patterns on a garment or on skin can provide useful information about muzzle to target distance in shooting scene reconstructions. Pattern density and pattern size on the evidence are assessed and compared to standards. The standards are created in the laboratory by shooting at targets at fixed distances, ideally with the same firearm and same ammunition suspected of being used in the shooting incident.

Until recently, these propellant patterns were predictable; at close range the pattern was dense and relatively small, with increasing diameter and decreasing density as the distance increased. Historically, the two most common types of bullets used in handguns are the full metal jacket and the jacketed hollow point. In these types of bullets, the lead cores are mostly enclosed with a copper jacket. The copper jacket varies somewhat in thickness but on average is approximately a half millimeter. The newcomers on the block are total metal jacket bullets, which unlike full metal jackets and jacketed

hollow points, have a jacket that is substantially thinner than their predecessors. Average jacket thickness of a total metal jacket bullet is merely a fifth to a tenth as thick as its full metal jacket counterpart. This thin jacket can be compromised by the rifling from some barrels, exposing the lead core. When the rifling cuts through the thin plated-on jackets of these bullets, the exposed lead can be expelled along with the unburned and partially burned propellant, to create an interesting pattern that looks like a swirl. Under certain conditions, it is possible to predict not only the direction of twist of the barrel but even the number of lands and grooves. Of the names associated to this phenomenon "spiral galaxy", "comet tail" and "vortex", I like *vortex* the best!

Fireworks and GSR
Pete Diaczuk, John Jay College

Gunshot residue (GSR) has long been characterized as spherical particles composed of two/ three components of molten lead, barium, and/ or antimony, due to the combination of compounds used in the chemical primer: Presence of these particles typically serve as linkage of a person to a shooting event, whether he/she was holding the firearm or within close proximity to the firearm when discharged. The integrity of GSR is questioned, however, when GSR- like particles are reported to be found through environmental and occupational exposure that do not involve a firearm. One of these exposures is fireworks. Due to the use of different compounds to uphold a chemical reaction and color effects, as advertised in various firework products, identifying the GSR metals lead, barium, and antimony, can possibly be found. The purpose of this research was to use a series of instrumental methods to investigate the potential for GSR-like particles to be formed and be detected from fireworks. Scanning electron microscopy, along with the Oxford AZtecGSR software, was used to identify GSR-like particles, elementally and morphologically from firework residues, while energy dispersive x-ray spectroscopy was used in attempts to identify chemical compounds used in commercial samples. Preliminary samples collected showed that classifiable components of firework residue were largely composed of environmental elements. Although an abundance of particles was identified as 'Consistent with GSR' and 'Consistent with Lead-Free/ Non-Toxic', 'Characteristic' particles were rarely found. Commercial firework products analyzed in this study showed the absence of lead and antimony, which are essential components in GSR particles. Results from *this* study suggested that the potential for GSR-like particles to form from the firework samples tested is low.

ATF – National Response Team
John Pijaca, SACFI, IAAI-CFI, ATF, retired

John Pijaca has spent thirty years in law enforcement, 22 of those years with the ATF and 4 years prior as an ATF Task Force officer. John Pijaca joined ATF's Arson and explosives unit in 2006 and became a Certified Fire Investigator in 2007. SA Pijaca became a member of ATF 's National Response Team in 2012 and responded to numerous callouts throughout the country. SA Pijaca is a Certified Instructor at the Federal Law Enforcement Training Center in Brunswick Georgia and has taught at the National Fire Academy in Emmitsburg MD as well as the International Law Enforcement Academy in Bangkok Thailand. John Pijaca retired from law enforcement in 2020 and currently is a fire investigator in the private sector.

This presentation will discuss ATF's National Response Team and the capabilities it provides to State and local Government. Specific ATF responses will highlight the work done by the team as well as the partnerships between State and local entities.

ATF launched the first National Response Team (NRT) in 1978 as a mobile, rapid response team to investigate large fires, explosions and bombings. ATF now maintains several NRTs strategically located throughout the eastern, central, and western regions of the United States.

NRT members apply their specialized skills to investigate crime scenes, reconstruct fire origins, and identify the cause. They also conduct witness interviews as part of the investigative process.

NRTs are made up of veteran special agents and scientific technicians with expertise in fire origin/cause determination and post-blast analysis, including Certified Fire Investigators, Certified Explosives Specialists (CES), CES Bomb Technicians, Explosives Enforcement Officers, forensic chemists, engineers, medics, and canine handlers. The teams are supported by ATF's intelligence research specialists, forensic auditors, digital media specialists, and other technical and legal experts who work with law enforcement on criminal cases.

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Criminalistics/Crime Scene & Digital Evidence Abstracts

Can Oversight be Done Right? Increasing Transparency Through Collaboration

Lynn Schneeweis, Kristen Sullivan and Darina Griffin, Massachusetts State Police Crime Laboratory, MA

2018 brought criminal justice reform initiatives to the Commonwealth of MA in the form of an "Act Relative to Criminal Justice Reform" (CJR). This act focused on improving several areas of the criminal justice system, and forensic science was no exception. Some forensic science centered reforms in the CJR grabbed national headlines including mandating the inventory and testing of previously unsubmitted SAECK and implementing a progressive 30-day turn-around-time for testing of all investigatory SAECK. A less publicized but ultimately impactful mandate of the CJR included the creation of a Forensic Science Oversight Board (FSOB) for the Commonwealth of MA. The CJR implored the Board have "oversight authority over all commonwealth facilities engaged in forensic services in criminal investigations…" and prescribed the composition of the roles of the individuals on the Board as well as mandated specific actions by the FSOB as they related to forensic science providers within the Commonwealth.

The implementation of forensic oversight boards or commissions is not novel in the United States and is theoretically designed to improve transparency and accountability within the forensic science field. At best, these bodies can positively impact a jurisdiction and help ensure the highest standards of forensic science are maintained. At worst, they can potentially foster a contentious and defensive environment directly contrary to that goal. The MA State Police Crime Laboratory (MSPCL) is the largest forensic service provider in the Commonwealth and as such, stood to be directly impacted by the creation of the MA FSOB. The CJR specifically mandated that the Board, upon its creation, immediately conduct an audit of the MSPCL and its current practices for providing forensic services. As spectators of other jurisdictions implementing these boards across the country with varying degrees of success, there was obvious uncertainty and apprehension as to how this would work in our own state.

The implementation of this board was not without its challenges from the laboratory perspective; however, the challenges were far from contentious. This presentation will discuss the enactment of the FSOB from the perspective of a forensic laboratory. Specifically, the initial audit of the MSPCL will be described as well as ongoing projects the Board is currently engaged in and their potential impact on the laboratory. The goal of this presentation is to provide insight for other laboratories who may see the creation of these types of oversight bodies in their own jurisdictions and demonstrate how with cooperative effort from all parties, a productive professional relationship can, in fact, be fostered between a forensic service provider and an oversight body.

Lab Lawyers, What Are They Good For?

Darina Griffin, Lynn Schneeweis, Samuel Miller and Kristen Sullivan, Massachusetts State Police Crime Laboratory, MA

After attending this presentation, individuals can evaluate the useful contributions a lawyer can make to a laboratory. This presentation affects the forensic and legal community by providing a model for the collaborative work between a laboratory, its attorney, and the criminal justice system. The presentation will also discuss the resources available to laboratory lawyers through the National Association of Forensic Laboratory Counsel.

In 2014, the Massachusetts State Police Crime Laboratory (Massachusetts State Police Crime Laboratory) hired its first ever "Lab Counsel". This lawyer was assigned to the Crime Laboratory and was responsible for providing legal support to the Laboratory Director and the Major of the State Police Crime Laboratory. The attorney's original contribution to MSPCL was focused on providing legal expertise related to a large impact litigation of the reliability of Breath Test

Instruments. This position quickly evolved to providing advice to the Laboratory Director on a variety of other topics such as regulation changes, interpretation of statutes, assisting with "best practices" guide for evidence management to assist with post-conviction work, responding to court orders, public records requests and assisting a busy case management unit with responses to discovery motions and many other tasks. The authors will discuss the achievements of the National Association of Forensic Laboratory Counsel since its inception. Specific emphasis will be on the role of an attorney to assist laboratories with navigating various legal issues as well as the benefits of collaborating and sharing resources with other lawyers that represent forensic laboratories.

Scientific Support of HAZMAT Transportation Regulations and Incident Resolution: Can the Forensic Laboratory Have a Role?

Vincent Desiderio, United States Postal Inspection Service Security Group, VA

Background/Introduction

By definition, hazardous materials (HAZMAT) are any products, articles, or substances that are capable of posing a significant risk to health, safety, or property when transported by air, rail, ground, or sea. The transportation of HAZMAT is strictly regulated on a domestic level by the United States Department of Transportation via the Code of Federal Regulations, specifically 49CFR Parts 100-185. Additional levels of regulation exist at the international level as enforced by the International Civil Aviation Organization (ICAO) which sets regulations for air transportation, and the International Maritime Organization (IMO) which oversees oceanic transportation.

Although HAZMAT encompasses a broad range of materials, the large majority of materials of concern include organic and inorganic chemicals. The analysis of these materials for identification purposes and/or physical property determination is often a critical component of enforcement requirements. This is especially important with respect to undeclared HAZMAT shipments that represent an elevated threat. In addition to ensuring regulatory compliance, scientific support can be even more critical when it comes to the characterization of materials when an incident occurs (e.g., spill, leak, or fire).

As ecommerce continues to expand, the presence of undeclared HAZMAT in shipments at the consumer level has become a growing threat. Home business owners and online marketplace vendors rarely take the steps necessary to educate themselves on the proper packaging and handling of hazardous materials. As such, a growing volume of HAZMAT has been making its way into business to consumer transportation networks. With this growing volume, there has been a noticeable increase in the number of incidents involving product fires (e.g., lithium batteries and strike anywhere matches), undeclared firework shipments being exposed, and leaking powders and liquids that may be either flammable, toxic, or corrosive. Along with the increase in the frequency of incidents, there is now a concomitant increase in the need to identify these materials when they are encountered as unknowns.

Unfortunately, transportation agencies do not typically have their own laboratories, often relying on private contract laboratories for analyses when required. To this end, the forensic network, having the analytical capabilities and courtroom experience required for such determinations, may be able to play a critical role in this system.

Objective

This presentation will provide a brief discussion on hazardous materials transportation requirements with an emphasis on areas in which analytical support would typically be required. The overall objective will be to demonstrate an entirely

new area in which forensic laboratories may be able to provide much needed analytic support in the world of public safety. In order to further demonstrate the need and general utility of forensic laboratories in this role, several real-world examples will be discussed.

Conclusion
After attending this presentation, the need for collaboration between forensic laboratories and the transportation sector should be clear. As more incidents are encountered, the enforcement of transportation regulations will undoubtedly end up in court at a greater frequency. Who better to handle the analysis and courtroom presentation of this information than the forensic examiner that is well versed in the analysis of general unknowns and the presentation of findings in court?

The Visualization of Bruises Using Alternate Light Source

Wan Yu Tan, Boston University School of Medicine, MA, Karen Kelly, Brody School of MedicineEast Carolina University, NC, Ann Marie Mires, Anna Maria College, MA and Sabra Jones, Boston University School of Medicine, MA

With the global pandemic, there has been mandatory movement restrictions by countries around the world. There has also been an increase in domestic abuse; such violence often presents in many forms with physical abuse heading the list.1,2,3,4 This study was conducted to enable forensic officers to make use of existing crime scene equipment to enhance the visualization of bruises on victims of abuse.

When a case of abuse is reported, evidence of the abuse must be documented. Traditional methods of investigation involve questioning the victim or abuser, followed by documentation using photography and note-taking which may not accurately represent the injuries. In addition, the amount of force used, area of injury and the age of the injuries could affect the appearance of blunt force trauma including bruising. At times only redness is observed on the victim's skin making the injury difficult to document; such injuries would constantly be overlooked.5,6 Alternate Light Source (ALS) is a common, cheap, and effective piece of equipment used by forensic examiners at the crime scene to reveal objects missed by the naked eye. With the use of ALS, the documentation of existing bruises can be enhanced, while bruises that are missed by the naked eye can be revealed.

In this study, the effectiveness of visualization of blunt force injuries (contusions) to the skin at different ALS wavelengths was evaluated to determine the optimal wavelength for documentation of bruises.7,8,9,10 Bruises were inflicted on 57 participants with no known medical conditions following institutional approval. The participant was in a seated position while a cylindrical ball of ~465 grams was dropped at a height of 1.5 meters through a vertically positioned tube onto the ventral surface of the participant's forearm. The injury site was then observed and documented under white light, 415nm, 460nm and 550nm. Photographs of the forearm were taken under at all wavelengths prior to bruising, immediately after bruising, 3 hours after bruising, and at specific time points over a period of 21 days. The results showed better visualization of the injury observed at a wavelength of 415nm and 460nm.

A blind study was conducted using the same methodology to determine the validity of the experiment. A colleague was briefed and tasked to conduct a blind trial on 12 participants following institutional approval where the researcher has no knowledge on which participant the bruise was inflicted on. Photographic documentation and observations were recorded with the results only made known to the researcher at the end of the experiment. It showed that the

methodology is accurate at about 75%.

This study shows that the use of ALS provided an effective alternative with the visualization and documentation of blunt force traumatic injuries compared to traditional documentation methods without added cost and should be considered for use in future cases involving trauma and physical abuse. Additionally, since ALS is the standard crime scene equipment, the documentation of bruising by forensic examiners can be initiated in the field prior to transport of victim to either the hospital or morgue setting.

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Volatile Organic Compounds (VOCs) Produced by Bacteria Associated with Decomposition

Veronica Cappas, Megan Morris, Dan Sykes and Reena Roy, The Pennsylvania State University, PA

Microorganisms play an important role in decomposition and are known to produce volatile organic compounds (VOCs) that contribute to the odor of decomposition. Although microorganisms and VOCs have been studied independently regarding decomposition, few studies have linked the two subject matters. As the number of decomposition studies increases, a clearer picture of the temporal evolution of VOC profiles is emerging. However, identifying the origin(s) of specific VOCs remains elusive (i.e., microbial processes, general chemical decomposition, insects, environmental effects). The volatile organic compounds produced by specific species of bacteria associated with decomposition were characterized in order to determine origins of VOCs detected during decomposition. In the present study, the volatile organic compounds produced by specific species of bacteria associated with decomposition were characterized. It may provide the potential origin of VOCs observed throughout decomposition, giving further insight to impact of microorganisms on the process, and improving techniques for locating remains.

Microbial communities were sampled at various time points during decomposition of a swine placed in an indoor enclosure and sequenced using NextGen Illumina sequencing. Because the indoor enclosure inhibited insect activity and colonization by extrinsic microorganisms, the VOC profile likely reflects the intrinsic microbial community and autolysis.

Over 1,000 taxa at the genus level were identified and over 600 taxa at the species level. Prevalent phylum includes Bacteroidetes, Firmicutes. Tenericutes, and Spirochaetes, which are seen in other swine decomposition studies (1). Further analysis of the swine's microbiome succession is discussed with an emphasis on the potential correlation of the microbiome succession and decomposition as seen in other studies like Hyde et al. and Metcalf et al. (2,3).

Based on the relative abundance of sequenced reads, Alcaligenes faecalis, Lysinibacillus fusiformis, and Lactobacillus gasseri were selected. They were independently cultured in headspace vials on a modified chopped meat medium created from ground pork broth and sheep blood, to provide similar nutrients to the decomposing swine. Solid Phase Microextraction (SPME) fibers were used to sample the VOCs produced by the bacterial species. The VOC profiles produced by each bacteria species are compared with the overall VOC profile collected from the same decomposing swine to distinguish the VOCs associated with bacterial decomposition from those originating from the general breakdown process. A. faecalis produced VOCs categorized as sulfides and carboxylic acids. L. fusiformis generated sulfides and hydrocarbons, while L. gasseri was seen to produce just one VOC. However, this may be due to the nutrient source and growth conditions. To investigate how interactions between species could affect VOC production, A. faecalis and L. fusiformis were cultured together. Different VOCs versus in the independent cultures were produced, like nitrogen-containing compounds that have been seen in other decomposition studies (4). This may support that species interactions can have an extraordinary effect on VOC production.

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DART-High Resolution Mass Spectrometry (DART-HRMS) for Identification of the Feeding Resource of Necrophagous Insects

Samira Beyramysoltan, University at Albany-State University of New York, NY, Jennifer Rosati, John Jay College of Criminal Justice, NY, Amy Osborne and Rabi Musah, University at Albany-State University of New York, NY

Necrophagous insects that have colonized decomposing remains can play a critical role in forensic investigations, as their species identity can be used to estimate time since death (or postmortem interval—PMI). However, insect evidence has the potential to reveal much more about the circumstances associated with the death. For example, drugs in the system of the decedent are ingested by the insects feeding on the remains, and thus detection of these drugs in the insects provides evidence that may be relevant to the cause of death. In a similar vein, it would be useful to be able to determine the identity of the tissue (food resource) on which insects have fed (e.g., human or animal) from direct examination of the insects. Recently, the metabolomics data derived from DART mass spectrometry (MS) was used to accurately determine insect species from analysis of the various life stages. Here, we examined DART-MS-derived insect molecular profiles as a function of food resource consumption to ascertain whether the identity of the tissue which was ingested by the flies could be determined. Multiple eggs of three species (C. vicina, L. sericata, and P. regina) were reared on five resources: beef liver, pork chop, dog feces, chicken breast, and decaying tilapia. The multiple individuals of each species

were collected in larval and adult life stages and stored in 70% aqueous ethanol until analysis. DART mass spectra were acquired in positive ion mode in replicates of 5 from analysis of the aqueous ethanol suspensions of the individual samples under soft-ionization conditions. The resulting spectra were imported into MATLAB software (The MathWorks, Inc.) for further multivariate analysis. The MS data were binned and scaled, and the resulting matrix was explored by the multifactor method ANOVA simultaneous component analysis (ASCA) to reveal variations in the chemical profiles that were a function of resource type. A fusion of partial least square-discriminant analysis (PLS-DA) and principal component analysis-discriminant analysis (PCA-DA) was performed to create a discriminative model for the reliable identification of not only species and but also food resource using selected m/z values. The performance analysis of the method showed 91%, 80%, 95%, and 52% identification accuracy by five-fold venetian blind cross validation for determination of species identity for larvae and adults, and food resource identity for larvae and adults, respectively. Two sets of discriminative features for each life-stage were identified to be responsible for discrimination of species and the ability to identify resource type. The results showed that the chemical profiles of adult samples were more influenced by resource variations and external conditions, in comparison with larva samples, which affected identification accuracy. Therefore, analysis of the larval life stage, which is the most commonly encountered insect form in forensic investigations, can be used not only for species determination, but also for determination of resource substrate.

You Are What You Eat: Utilization of Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) for the Toxicological Examination of Insect Evidence in Death Investigations

Amy Osborne, University at Albany-State University of New York, NY, Jennifer Rosati John Jay College of Criminal Justice, NY, and Rabi Musah, University at Albany-State University of New York, NY

In death investigations, entomological evidence is most well-known for providing a means by which to estimate postmortem interval (i.e., time since death), particularly in cases where decomposition is at an advanced enough stage that conventional techniques for PMI estimation cannot be used. However, the evidentiary value of insects could be dramatically enhanced beyond the scope of PMI if the chemical information they potentially contain could be harnessed to reveal additional information of relevance to the cause of death. One such form of information is toxicological evidence that is lost or irretrievable when the level of decomposition has progressed to the point where matrices that are typically probed in this type of analysis (i.e., blood, urine, and internal organs) no longer remain. The solution to this problem? Entomotoxicology. As maggots feed on decomposing remains, they ingest the tissue along with any drugs or chemical toxins that are contained therein. To the extent that these chemical compounds and/or their metabolites remain within the flies, their presence can serve as a historical record of the factors that may have led to the cause of death. In other words, you are what you eat!

Currently, forensic entomotoxicology research has focused mainly on applying traditional methods of toxicological analysis and drug detection to insects. These methods can involve long, complicated sample preparation that requires the complete destruction of the collected insect evidence. Here, we demonstrate Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) as a new means to extract toxicological information from insects. For this study, blowflies (L. sericata) were fed beef liver laced with fentanyl derivatives at physiologically relevant concentrations, and the life stages of the flies following emergence of the maggots from the eggs that were laid was followed through to the emergence of adult flies. Specimens representative of each life stage were collected over two weeks. Following standard insect evidence collection procedures and to minimize future sample preparation, all specimens were stored in 70% aqueous ethanol prior to further analysis. DART-HRMS was then utilized to generate

insect metabolome profiles through the direct interrogation of the individual insect specimens. These profiles were subjected to kernel discriminant analysis (KDA) in order to determine whether the profiles of the liver control (no drug) and the druglaced liver could be differentiated through pattern recognition techniques. While the results show marked differences in the metabolome profiles of drug versus control samples for every life stage (i.e., larvae, pupae, and adults), the most significant changes were observed in the puparial casings. The findings provide a new avenue by which to access toxicological information of potential relevance to a death investigation that circumvents the challenges often encountered when using conventional techniques and provides a means by which to retrieve important forensic information even under conditions of advanced decay, particularly since puparial casings can remain unchanged for many decades.

False Negative Results for Blood Tested in the Presence of Chemical Interferents

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Blood is one of the most widely tested biological matrices. The first step in forensic blood identification involves visual examination followed by presumptive testing. Once a positive presumptive result is observed, confirmatory tests may be performed to determine that a stain is human blood, thus reducing the time and resources spent on forensically irrelevant samples. When interfering agents are present, this general workflow is hindered as presumptive tests can render false negative results. General awareness of these interfering agents can help analysts to recognize forensically relevant evidence that may have otherwise been deemed immaterial.

The main objective of this study was to understand various interfering agents and their effects on presumptive blood tests such as Kastle Meyer (KM) and Orthotolidine (O-tol) reagents and confirmatory tests such as HemaTrace® and Rapid Stain Identification (RSIDTM)-Blood. In the first part of the study, bloodstains in varying concentrations were exposed to ten chemical interferents over a period of time to understand how blood dilution, age of the stain, and the chemical nature of the interferent affect presumptive blood test results. Antioxidants, active oxygen, and tannins are known to interrupt the mechanism of presumptive tests. Thus, ten interfering agents (ascorbic acid, chlorogenic acid, catechin, sodium percarbonate, hydrogen peroxide, oxalic acid, proanthocyanidins, quebracho extract, chestnut extract, and theaflavin) were selected based on these characteristics. Six blood dilutions (neat, 1:10, 1:50, 1:100, 1:500, and 1:1000) were exposed to the interferents, and presumptive tests for blood were conducted on six separate days (day 1, 8, 22, 43, 71, and 106). The second part of the study examined bloodstains deposited on real-world samples (wines, citrus fruit juices, teas, coffee, cleaning agents, and leather products) containing chemical interferents. In addition, confirmatory testing for human blood was conducted with HemaTrace® and RSIDTMBlood on day 106 using the 1:500 dilution.

Nearly all chemical interferents and household products tested had an inhibitory or altering effect on bloodstain identification. The results showed that as blood concentration reduced, more falsenegative results were observed when chemical interferents were present. Further, chemical interferents produced frequent atypical color changes in tests with KM and O-tol reagents that were not characteristics of a positive result, while only some atypical color changes were observed with the household products tested. Immunochromatographic assay results indicated both HemaTrace® and RSIDTM-Blood could detect the presence of blood when most interfering agents were present, although the positive result bands with RSIDTM-Blood were faint and sometimes difficult to visualize; however, nearly one third of the samples tested yielded a negative result. Overall, the data indicates that valuable blood evidence may be overlooked due to faint or false negative results when these chemical interferents are present. Future studies should focus on how these interferents may affect downstream DNA analysis.

RSID™-Semen and RSID™-Saliva Validation at the Boston Police Department Crime Laboratory
Kathryne Hall, Boston Police Crime Laboratory, MA

The objective of this validation was to evaluate the sensitivity and reproducibility of the Rapid Stain Identification (RSID™) tests for semenogelin and saliva manufactured by Independent Forensics. The goal was to determine whether these tests would be suitable replacements for the ABAcard® p30 test and the detection of amylase via radial diffusion that the laboratory was previously performing. An important consideration was to ensure that any new tests brought online would be as similar as possible to the current laboratory protocols. It was also important to evaluate if the RSID™ Universal Buffer would be a suitable replacement solution for the dH2O that was in the validated laboratory protocols for extraction and preparation of microscopic slides with the Christmas tree stain.

All samples were run in duplicate. Serial dilutions of pooled semen were applied to Puritan cotton swabs, Texwipes®, and 100% cotton pre-washed fabric. Serial dilutions of pooled saliva were applied to cotton swabs. Significant differences were observed when the RSID™-Semen results were compared to the ABAcard® p30 results from extracted cotton swabs. Invalid results were obtained for the RSID™-Semen test cards when samples were extracted from Texwipes® due to the fact that the negative control during these test runs yielded positive results. Similar results were observed between the RSID™-Semen test cards and ABAcard® p30 test cards from extracted samples on 100% cotton fabric. Overnight extractions for semen dilutions on cotton swabs and 100% cotton fabric were also evaluated. Similar results were obtained for extracted cotton swabs when comparing RSID™-Saliva and amylase detection via radial diffusion.

The use of the RSID™- Universal Buffer during the extraction and washing steps of the microscopic slide preparation procedure appeared to prevent the picroindigocarmine stain from being fully rinsed from the slide, leading to difficult visualization under the microscope. Several different changes were made to the procedure to adjust for the difficult visualization. Finally, it was determined that using the universal buffer during the extraction and dH2O in the wash step aided in the visualization of the microscopic slides.

Pollen Grain Assemblage for Geolocation in Mock Crime Scene
Jacqueline Goetz and Heather Coyle, University of New Haven, CT

Pollen analysis may be employed in a forensic investigation to provide evidence to link a suspect to a crime scene. Every geographic location has a pollen assemblage that consists of the combinations of plant species unique to that specific area. This distinction allows for pollen assemblages to be obtained and related to specific locations. Pollen grains can be utilized as trace material due to their microscopic size, abundance in the environment, and their resistance to chemical degradation. Grains can also provide characteristics of the environment from which they are from, and their morphology can be used to distinguish specific plant taxa. However, experimental research in utilizing pollen assemblages to link forensically relevant items to specific crime scenes has been limited. This study focuses on the collection of pollen grains from T-shirts and footwear that has been worn at a simulated crime scene through surface swabbing techniques. Utilizing traditional morphological comparisons, pollen quantification counts and an optimized staining technique to enhance grain structure, pollen assemblages from the T-shirts and footwear were compared to control samples taken at the mock crime scene. Results indicate that each scene had a unique assemblage that could be matched to the samples collected from the T-shirt and footwear that was worn at the scene, with most of the pollen assemblage coming from the upper front of the shoe. Limitations to this study include having a controlled, contained scene, and participants who wore clean footwear/clothing that were seized and processed immediately unlike a typical crime scene. However, this study

demonstrates the usefulness of the pollen analysis technique pollen analysis that could be utilized more prevalently in forensic investigations for associative evidence.

A Tree-Mendous Method for the Forensic Identification of Illegally Traded Timber by DART-HRMS and Multivariate Statistical Analysis

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One of the concerns of wildlife forensics is the identification of endangered species, the trade of which is illegal. The Convention on International Trade of Endangered Species (CITES) was created to address the conservation of imperiled wildlife by controlling their trade. Regulation status is defined by appendices: CITES Appendix I species are exceptionally endangered and trade of any kind is outlawed; CITES Appendix II species are threatened in the wild and international trade is managed; and CITES Appendix III species are regulated by a particular nation. While trade in fauna, including elephant parts (such as tusks), rhinoceros horns, and pangolin scales, are well-known examples of wildlife crimes, there are a host of flora that are heavily trafficked, including trees. Dalbergia species serve as a case in point. These species fall under the Leguminosae family, with most species being commonly known as rosewood. Illegal trade of these species is common because they are highly prized for making exclusive furniture, cabinetry, musical instruments, and artifacts. Depending on the species, trade is either totally or heavily restricted. However, when specimens are intercepted by law enforcement, it is extremely challenging to identify the evidence as either legal or illegal, because many of the species that are illegal to trade have an appearance that is similar to species that are not restricted. The traditional technique for species identification of timber is morphological examination based on diagnostic anatomical features. However, this requires high levels of training and may not be successful when attempting to distinguish between species with similar wood anatomy (e.g., D. nigra and D. spruceana). Therefore, a technique is urgently needed by law enforcement for the rapid and efficient identification of endangered timber in order to circumvent the aforementioned challenges. We demonstrate accomplishment of this by an integrated approach using solid phase microextraction (SPME), direct analysis in real time-high resolution mass spectrometry (DART-HRMS), and multivariate statistical analysis. Seventeen Dalbergia species, including D. baronii, D.cearensis, D. oliveri, D. occulta, D. madagascariensis, D. latifolia, D. melanoxylon, D. normandii, D. purprascens, D. retusa, D. nigra, D. decipularis, D. stevensonii, D. tucurensis, D. spruceana, D. maritima, and D. cochinchinensis were provided by the U.S. Fish & Wildlife Forensic Lab, all of which are listed as CITES Appendix II species, except for D. nigra, which is listed as a CITES Appendix I species. Three samples of each species were analyzed in multiple replicates. The headspace volatiles of the wood samples were concentrated on SPME fibers for thirty minutes, which were then analyzed by DART-HRMS. Multivariate statistical analysis processing of the DART-HRMS data revealed intraspecies similarities and interspecies differences that resulted in the ability to assign species attribution to the chemical signatures. The classification model that was developed could therefore be used for rapid forensic identification of species based on simple analysis of the headspace of the wood. The results show that this approach can be used as a technique for species identification of these illegally traded timber.

Reducing Required Ink Sample Size for Analysis Using Microvolume UV/Vis Spectroscopy

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Questioned document analysis often deals with the validation of documents that contain a particularly small sample of

handwriting. A sample can be as small as a set of initials, making some conventional chemical analysis unfavorable if it requires extraction of the entire sample. Because using the entirety of a sample in analysis prevents further visual, microscopic, or chemical analysis an analyst may elect not to use methods that necessitate extraction1.

Conventional UV/Vis spectroscopy is an established method for chemical ink analysis but faces issues of large sample size and destruction of the sample. Additionally, the suggested solvent for UV/Vis ink analysis, pyridine1 , produces a nauseating odor and is a systemic toxicant2 . Pyridine also has limitations when attempting to extract water-based gel ink or porous point pen ink1 . The use of UV/Vis spectroscopy is favorable because the spectral output is easy to visually compare to other samples without specialized training.

In our new analytical method, a 0.7mm steel mechanical pencil tip as a micro-punch to transfer tiny ink-on-paper samples from written characters, before the ink is extracted by a micro-volume surfactant solution and investigated spectroscopically on a micro-volume UV/Vis spectrometer. Recognizable and repeatable spectra are generated with this method and the spectra allow for visual comparison between types of inks. Testing began with black and blue ballpoint, gel, and porous point pens. Analysis was also expanded to thermochroic, or "erasable," gel pens and alcohol based porous point permanent markers. These samples were selected for their forensic relevance. QD analysis frequently looks at evidence material where handwriting is meant to have intense staying power, as in "permanent" inks, or is meant to be easily destroyed, as lay-people feel they achieve with pigment that deactivates. Additionally, these formulations are popular among consumers3.

It is still possible to see general physical and microscopic characteristics of the characters after micropunch samples have been taken. Samples can be taken with strategic placement in order to preserve the most relevant or distinctive aspect of any given character being examined. This method works on a sample as small as a set of initials and leaves them readable with some lines of ink still present for further analysis.

The results of these tests were found to be replicable when analysis was in keeping with the set procedure. It was found that ballpoint, gel, and porous point pen inks were differentiable from one another, and the spectra for ink formulations were clearly different from the spectra obtained by running paper blanks, indicating the ink spectra were notable due to the ink itself and not any paper interference. The method was additionally found to work on deactivated thermochroic pigment and alcohol based porous point pen inks, indicating this form of analysis is suitable for a wide variety of samples. The intention of this research is to develop an accessible, replicable methodology for minimally destructive chemical ink analysis, and to eventually establish a searchable database of reference UV/Vis spectra.

1. "Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography." ASTM International, E 2227 – 02: 2-5.
2. U.S. EPA. Health And Environmental Effects Profile for Pyridine. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/ X-86/168 (NTIS PB89123384), 1986.
3. "The Science Behind FriXion Erasable Pens," Na., accessed October 4, 2020, https://www.nippon.com/en/features/c00520/; "Sharpie-About Us," Na, accessed October 4, 2020, https://www.sharpie.com/about

Evaluation of the GelSight Mobile 3D Imaging System for Collection of Postmortem Fingerprints

Mason Carlson and Amy Brodeur, Boston University School of Medicine, MA

Current methods for collecting postmortem fingerprint exemplars in a forensic investigation most commonly include

using ink, black powder, or a two-dimensional camera/scanner. Disadvantages with these methods include smudging or distortion from inconsistent pressure and movement during collection as well as detail being lost from excessive ink/powder or finger deformation. These problems are exemplified when rigor mortis or decomposition is present.

The GelSight Mobile is a portable three-dimensional contact imaging device that is primarily used in the aerospace industry and has recently been utilized in the field of ballistics. The device uses an elastomeric sensor to measure the topography of any surface and is sensitive to the micron level regardless of lighting conditions. The handheld device contains six LED lights and individual images are simultaneously captured using each of the lighting conditions while the gel pad is conformed to the surface of interest. The six images are then automatically combined to create a 3D point cloud through a process called photometric stereo. The GelSight Mobile device is used in conjunction with companion software to give instant photographic feedback.

To evaluate the GelSight Mobile for forensic use, the fingerprints of decedents with varying postmortem intervals were evaluated at the Connecticut Office of the Chief Medical Examiner. Fingerprints were captured first with the GelSight Mobile, followed by the FingerScan Decedent ID twodimensional scanner, the standard method of fingerprint collection for that office, and lastly using ink and a postmortem fingerprint collection kit. The electronic images and inked prints from each individual were compared to one another to evaluate the level of detail visible. Further, the ease and efficiency of using the GelSight Mobile was compared to current methods.

Overall, the GelSight Mobile was efficient in collecting postmortem fingerprints and in many instances provided a higher quality fingerprint impression than other methods. In particular, friction ridge skin that had begun to slip or decompose contained more identifiable minutiae when viewing the 3D images as compared to the inked prints and 2D scanned images. The 3D models created can be measured, scaled, rotated, or exported for further processing. The device requires minimal training to use and images of all ten fingers can be captured in under three minutes. One disadvantage to the GelSight Mobile is the limited size of the field of view, which only allows an area measuring 17mm by 14 mm to be captured in a single image. Thus, using the current model, multiple scans are required if capturing the entire finger pad is desired.

Double Homicide in South Boston: "Call 111 … Gunman in house … serious"

Kathryne Hall, Boston Police Crime Laboratory, MA

On Friday May 5, 2017, at approximately 20:38 hours, Boston Police officers responded to Penthouse A at 141 Dorchester Ave. in South Boston for a 911 call reporting a male in the home with a gun. Front desk security at 141 Dorchester Ave. received a phone call from a friend of the resident explaining that he had received a text message from Richard requesting that police be notified that it was a serious situation and there was a gunman in the house. Upon officers' arrival, a male dressed in dark clothing and possibly armed with a firearm was observed in the home. The male made hand movements consistent with a person aiming a firearm at the officers, resulting in the officers discharging their firearms at the male suspect. The male was wounded in the left hand, abdomen, and leg. After a struggle, the suspect was taken into custody. It was later determined that the suspect had a BB/Pellet pistol and not a working firearm. At this time, the suspect told officers there was a sniper in the building who was going to shoot at officers if they went into the residence. Due to this statement made by the suspect, the Boston Police Entry Team was used to gain entry into the home. BPD SWAT officers then made a protective sweep of the residence during which the bodies of 38- year-old Dr. Lina Bolaños and 49-year-old Dr. Richard Field were discovered. Lina had been stabbed more than 20 times

along with multiple blunt force injuries. Richard sustained one stab wound to the neck along with several blunt force injuries.

The night of May 5, 2017, through to the night of May 6th, crime scene analysts processed a large, complex crime scene at 141 Dorchester Ave. Crime scene processing involved the bodies of Lina and Richard, several bottles of cleaning detergents and solutions, bloodstain patterns, shooting reconstruction, and over one hundred items of evidence that were collected for further analysis. The case was presented at trial in December of 2019 where Bampumin Texeira was found guilty of two counts of murder for the deaths of Dr. Lina Bolaños and Dr. Richard Field and sentenced to life in prison. This presentation will explore the processing of the crime scene, the forensic evidence, and will discuss the presentation of the evidence in the courtroom.

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Drug Chemistry Abstracts

Using LC/MS Q-TOF for the Analysis of Seized Drug Evidence
Anna Weaver, New Hampshire State Police Forensic Lab

The New Hampshire State Police Forensic Laboratory has been working with an Agilent 1260 Infinity II Liquid Chromatograph (LC) with a 6530C Quadrupole/Time-of-Flight Mass Spectrometer (Q-TOF) detector in the Drug Chemistry Unit for approximately four years. The intent of this instrument purchase was to assist in the analysis of thermally labile compounds like psilocybin, gabapentin and GHB. Additionally, the LC/MS Q-TOF was considered a likely candidate for the testing of other troublesome analytes, such as cannabinoids, which tend to stick around on the gas chromatograph-mass spectrometer (GC/MS) leading to carry-over issues. This presentation will cover the pros and cons of using this technology for drug chemistry casework, particularly the significant differences that set it apart from the current "gold standard" confirmation technique in drug chemistry, the GC/MS. This presentation will review the daily instrument preparation and quality control measures that were developed to ensure that the instrument is functioning as expected before samples are run, especially as they compare to that of the GC/MS. The fully validated psilocybin/psilocin/bufotenine method will be discussed, as will the method to qualitatively identify the presence of Δ9-THC in edibles. Finally, additional analytes that are in the method development process currently, as well as those that are in the pipeline, will also be covered. This presentation will be helpful to other forensic chemists who are considering the purchase of an LC of any kind and those who are currently or shortly will be in the process of method development or validation of a similar instrument.

Development of Novel Approaches for Efficient Cannabinoid Detection and Quantification in Edibles, Beverages, Personal-care Products and Plant Materials
Megan Chambers, University at Albany - SUNY; Rabi A. Musah, PhD., University at Albany – SUNY

The most recent National Institute of Justice Report to Congress: Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices (2019), identified several challenges that have emerged in forensic laboratories due to the "legalization and decriminalization of marijuana." As a result, new methods must be developed for the detection and quantification of the major psychoactive cannabinoid tetrahydrocannabinol (THC) in a variety of plant-based substances, edibles infused with cannabinoids, and extracts derived from Cannabis sativa plant material. Protocols currently implemented in forensic labs lack uniformity and require extensive sample preparation steps. This project focuses on utilizing the unique abilities of direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) for the detection and quantification of THC and cannabidiol (CBD) in cannabinoid-infused complex matrices, including edibles, beverages, personal-care products, and C. sativa plant materials.

Previous accomplishments under this project demonstrated the application of DART-HRMS to rapidly detect cannabinoids in C. sativa plant material (i.e., hemp and marijuana), edibles prepared inhouse and edible certified reference materials with no sample pretreatment. Therefore, to demonstrate the versatility of this method, additional complex matrices were investigated, including beverages and personal-care products. Aliquots of various beverages were spiked with either THC or CBD standards at concentrations representative of those in commercial products. When analyzed by DART-HRMS in positive-ion mode, a peak at m/z 315, which is consistent with the protonated mass [M+H]+ of THC and CBD, was readily detected in all spiked beverages. DART-HRMS analysis of several personal care-products (e.g., soaps, lotions, balms) derived from hemp extract/oil readily detected CBD in each product in their native form. Control samples (either blank beverage matrices or personal-care products not manufactured with hemp extract/oil) were also analyzed in this study. Despite the complexity of their matrices, none of the experimental controls exhibited peaks that overlapped with those that would be consistent with the presence of cannabinoids.

The successful detection of cannabinoids in complex matrices prompted the development of quantification protocols using DART-HRMS. Protocols have been developed for the quantification of CBD in traditionally challenging edible matrices (i.e., gummies, chocolates, marshmallows) prepared in-house. Extraction protocols and the DART-HRMS method for quantification are being optimized for integration into current forensic laboratory workflows.

In addition, the application of DART-HRMS for differentiation of hemp and marijuana varieties of C. sativa was investigated. Plant materials of both varieties were obtained from multiple geographical locations and sources.

Preliminary statistical analysis revealed the potential for differentiating hemp and marijuana by DART-HRMS. These results prompted application of advanced statistical processing to the DART-HRMS data, which revealed m/z values important for differentiating these two C. sativa varieties with a high level of certainty. The identities of several m/z values have been confirmed, while the identification of the remaining masses is currently underway.

Results of this work will substantially impact forensic science and criminal justice practice in the U.S. by providing crime labs with novel, validated methods for the rapid detection, differentiation, and quantification of complex Cannabis-derived evidence that circumvent some of the most frequently reported problems associated with traditional methods.

Development and Evaluation of a New DART-MS Data Interpretation Tool

Edward Sisco, National Institute of Standards and Technology; Arun S. Moorthy, National Institute of Standards and Technology, Stephen Tennyson, University of Maryland, College Park

As seized drug chemists continue to face a number of analytical challenges due to the presence of emerging drugs and novel psychoactive substances, many laboratories are adopting new technology. One technology that is being increasingly implemented is direct analysis in real time mass spectrometry (DART-MS). While DART-MS provides a rapid, information rich analysis with minimal sample preparation, data interpretation is not straightforward. This is especially true when the sample contains multiple components. To address this gap, researchers at NIST have been developing analysis software referred to as the DART-MS Data Interpretation Tool (DIT). The DIT has several features, such as report generation and library viewing, that have been developed with input from the forensics community. The DIT works with a wide range of input files and is now freely available. A key function implemented in the DIT is the Inverted Library Search Algorithm (ILSA). The ILSA is a new search method specifically designed for DART-MS data, leveraging multiple in-source collision induced dissociation (is-CID) spectra to produce numeric similarity scores for potential components in an unknown sample. This presentation will discuss the process of creating the DIT as well as the evaluation and ongoing optimization of the ILSA. Initial evaluation efforts, using spectra from actual casework, have shown excellent agreement between results obtained with the DIT and the gas chromatography mass spectrometry (GC-MS) results. Better performance than peak-list searching approaches which are commonly used with DART-MS data has been demonstrated largely due to the ability to rule out one or more constitutional isomers since multiple is-CID spectra are leveraged.

Detection of Δ9-Tetrahydrocannabinol and Metabolites in the Meibomian Lipids of Tear Samples Through LC-MS/MS

Allen Mello, Boston University – Biomedical Forensic Sciences; Sabra Botch Jones, Boston University – Biomedical Forensic Sciences, Denise Valenti, OD, IMMAD: Impairment Measurement Marijuana and Driving, Jamie Foss Perkin Elmer, Inc.

There exist limitations with current methods of detection of Δ9-Tetrahydrocannabinol (THC) drug analyte in Driving Under the Influence of Drugs (DUID) cases. This research explores the use of meibomian tear fluid as a novel matrix to detect THC and its accompanying analytes.

This research focused on the detection and quantitation of THC, 11-Hydroxy- THC (11-OH THC), and 11-nor-carboxy-THC (THCOOH) as these analytes are produced in the metabolism of Δ9-THC. Meibomian fluid maintains a high lipid concentration and Fatty Acid Binding Protein 5 (FAPB5), a protein known to bind to cannabinoids. Due to the lipophilic nature of THC, tear fluid could be used as a less-invasive biological matrix to test for the presence of THC and its metabolites.

This project optimized a collection of tear fluid, to create a method suitable for direct injection. Collection was completed by BVI Weck-Cel® Sterile Cellulose strips, measuring approximately 2 x 20 mm, and placed in Thompson eXtreme PVDF 0.2 ∝m, pre-slit, red cap, filter vials containing Quantisal buffer solution. All analysis and calibrations were completed with fortified matrix standards with concentrations ranging from 0.25 - 250 ng/mL. Method validation was consistent with Academy Standards Board (ASB) Standards of Forensic Toxicology Standard 036, First

Edition 2018.

Tear samples were collected from participants according to Institutional Review Board (IRB) standards before and after administration of Marijuana. Samples were collected approximately 30 minutes post. Samples and calibration standards were analyzed using Liquid Chromatography Tandem Mass Spectrometry (LC/MS-MS) with the QSight® 220 CR LC/MS/MS and using a Halo® C18 3.0x50 mm (2.7 μm) column. Limit of Detection (LOD) and Quantitation (LOQ) for THC was calculated at 0.25 ng/mL. The LOD of THCOOH was detected at 0.25 ng/mL and LOQ was calculated at 1 ng/mL. The LOD of 11-OH-THC was detected at 2 ng/mL and was not quantitated. Upon analysis of participant samples, it was determined that THC and metabolites could be detected and quantitated in tear fluid. However, it is noted that insufficient sample volume in collection is an issue that leads to poor quantitation and can readily be optimized in future research.

Integrating the Thermo Scientific™ TruNarc™ Analyzer into a Seized Drug Laboratory Workflow – A Compilation of Curious Circumstances in Casework

Stephanie Minero, M.S., ABC-DA Nassau County Office of the Medical Examiner, Division of Forensic Services

Classified as a SWGDRUG and ASTM Category A technique due its increased level of selectivity, the Thermo Scientific™ TruNarc™ Analyzer has the ability to rapidly identify drugs of abuse, precursors, and common diluents with little or no sample preparation required. An accompanying Type H test kit allows for the analysis of many fluorescent compounds and some low concentration analytes. Over the last several years, the Nassau County Office of the Medical Examiner, Division of Forensic Services purchased several units to implement into its seized drug laboratory workflow as a screening technique. The laboratory has previously presented that integration of this technique resulted in a more efficient workflow for routine cases and decreased case turnaround time in both the analytical and technical review phases.

Almost two years after its qualification for use in casework, several circumstances have been encountered that may pique the curiosity of the forensic community and fellow seized drug analysts. These include the performance of a proper self-check, scanning of colored packaging and the dangers it presents, the analysis of colored solid material with the Type H Kit, fentanyl analogues and possible "false positives" in complex mixtures.

This presentation will discuss modifications to the procedure manual and scheme of analysis to continue to mitigate false positives/negatives, differences between field and laboratory applications and their potential impact, potential root causes, and beneficial information received from Thermo Scientific technical support requests.

Novel Presumptive Tests for Drugs of Abuse Using Chemiluminescence

Giulia Romano, Cedar Crest College; Lindsey A Welch, PhD, Cedar Crest College

Benzodiazepines are often used for treatment of insomnia, convulsions, and many psychiatric disorders. The widespread use of this class of drugs has raised concern about recreational benzodiazepine abuse. This highlights the importance of chemical detection and concentration determination of the benzodiazepine drugs within a human system. Although there are many different approaches to detection, this proposal outlines a novel presumptive testing method using chemiluminescence. A comparative study was designed to analyze three azepine drugs, carbamazepine, clonazepam, and diazepam, in a range of concentrations. The chemiluminescent reagents to be discussed are tris(2,2'-bipyridyl) ruthenium (II) chloride hexahydrate Ru(bipy)3Cl2•6 H2O with cerium (IV) sulfate, known as method A and n-bromosuccinimide, known as method B. Chemiluminescence was detected using a Berthold Lumat3 tube luminometer. Method A was more selective toward carbamazepine and clonazepam. While method B was more selective toward diazepam. These drugs were detected in concentration ranges of 100 mM to 0.1 mM. The results from this work suggest novel presumptive tests for these drugs of concern. These methods require minimal sample preparation, offer a rapid screening process, and facilitate the detection of these compounds in biologically relevant concentrations.

Tetrahydrocannabinol (THC) Identification and Semi Quantitation by GC/MS

Alexandra Kocaj, Nassau County Office of the Medical Examiner, Division of Forensic Services; Nicholas Ciccone, Nassau County Office of the Medical Examiner, Division of Forensic Services

On March 8th, 2020, New York State first integrated the definition of hemp into the New York State Agriculture and Markets Law, Title 505. This amendment defined hemp as "Cannabis Sativa L. containing less than 0.3% (w/w) delta 9-tetrahydrocannabinol" and thus currently requires an analytical scheme that allows the differentiation of cannabis from hemp. The Nassau County Office of the Medical Examiner, Division of Forensic Services has developed a semi-quantitative analysis method using Gas Chromatography-Mass Spectrometry in order to meet this new requirement. By comparing an unknown sample to a decision limit of 1% (w/w) delta 9-tetrahydrocannabinol (THC), the laboratory can determine if submitted evidentiary material in vegetative form can be confirmed as cannabis.

An overview of the advantages of developing, validating, and implementing a semi-quantitative method will be discussed, in addition to the challenges encountered and their resolutions. Specific studies conducted during the validation including selectivity, linearity, stability, accuracy, and intermediate precision will be included. Noted challenges include the selection of an internal standard, preparation of the positive control, potential interfering compounds, and integration parameters. Recommendations for training and competency testing as well as successful participation in the National Institute of Standards and Technology (NIST) Cannabis Quality Assurance Program will be included. Completion of the program and the successful training of the analysts showcase the robustness of the method and prove it reliable for confirming the presence of cannabis.

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Forensic Biology/DNA Abstracts

Determining Human Identity from Leeches Using Copan microFLOQ® Direct Swabs

Veronica Cappas, Elizabeth Knapp and Reena Roy, Ph.D., The Pennsylvania State University

Leeches are worms commonly found in water and can suck blood from a human host. These worms carry an anticoagulant, known as hirudin, in their salivary gland. These annelids have suckers, they conceal their cutting plates which hook onto human flesh, and after making a 2mm incision, they can then ingest from 5 mL to 15 mL blood from one human. If a perpetrator disposes a victim's body in a river, stream or pond, the leeches in the pool of water can hook onto the suspect's body. Once they are gorged with blood within about 30 minutes, they can fall off in the nearby area. In addition, they can help with identification of a victim or determine if a body had been in a certain area of the water. Therefore, they can become a valuable source of evidence for the forensic scientists.

Our research group has previously determined human identity from anopheles stephensi mosquito blood meals using Copan microFLOQ® Direct swabs and PowerPlex® Fusion 6C System to identify short tandem repeat (STR) DNA profiles. Massively parallel sequencing was also performed on the blood from the midgut of the same mosquitoes. In this current study, a similar approach was taken to identify human STR profiles from blood meal ingested by leeches. North American medicinal leeches were obtained from commercial source, and blood from male and female donors were fed to individual leeches. After a certain period of time, each leech was frozen quickly and then dissected. Once blood was identified in the midgut with presumptive test, presence of human blood was confirmed with various biological tests. After that, the tip of one swab was used to collect a minute amount of blood from the midgut area of each worm. These swabs, containing the human blood on their tips, were then used for direct amplification and STR profiles were determined using PowerPlex® Fusion 6C PCR amplification reagents. Known blood samples (reference samples) were also amplified using the Copan microFLOQ® Direct swabs and analyzed similarly. The Applied BiosystemsTM 3130xl Genetic Analyzer was used for capillary electrophoresis. GeneMarker® HID V2.9.5 software (SoftGenetics LLC, State College, PA, USA) was used for analyzing the STR profiles.

Complete and concordant profiles, consistent with the reference profiles of the donors of blood were obtained from the midgut of the leeches using the above method. The results of this study indicate that Copan microFLOQ® Direct swab is an excellent way to amplify blood directly, even when the blood has been ingested by leeches. Complete STR profiles can be generated within a very short period using these swabs and the PowerPlex® Fusion 6C PCR amplification kit. Since only a minute quantity of blood is required, this method of collection and amplification is an excellent procedure for obtaining human identity from minute amounts of blood ingested by leeches and similar organisms. Future research will include sequencing the blood from the leech midguts in order to obtain additional information about the human donors.

Determining Y-STR Profiles from Leeches Using Copan microFLOQ® Direct Swabs and the Yfiler® Plus Amplification Kit

Elizabeth Knapp, Veronica Cappas, and Reena Roy, Ph.D., The Pennsylvania State University

Leeches are annelids which have been used for many centuries in the field of medicine. These worms carry an anticoagulant, known as hirudin, in their salivary glands. Once they hook onto human flesh, they make a small incision, and can ingest from 5 mL to 15 mL blood from one human.

Projects in our research group has included identifying human donors from blood meal ingested by mosquitoes using autosomal loci and massively parallel sequencing. Loci found on the Ychromosome have been successfully used in many cases including sexual assault cases, particularly when there is more than one perpetrator or if the level of male DNA is low and amount of female DNA is very high. In this current project we used Copan microFLOQ® Direct swabs and Yfiler® Plus Amplification Kit to directly amplify Y-STR markers from blood ingested by leeches. This amplification kit is a 27-plex Y-STR system which include seven rapidly mutating Y-STR loci which allow for discrimination among related individuals. North American medicinal leeches were obtained from commercial source, fed blood meal and frozen at certain times. Blood from the midgut of the leeches were collected on the tip of the swabs and Y-STR loci

amplified using the amplification kit. Known blood samples (reference samples) were also amplified using the Copan microFLOQ® Direct swabs and analyzed similarly.

Y-STR profiles were generated successfully from blood ingested by the leeches, and they were consistent with the reference profiles of the donors. The results of this study indicate that Copan microFLOQ® Direct swab is an excellent way to amplify Y-STR loci directly, even when the blood has been ingested by leeches. The direct amplification bypasses the time-consuming, labor-intensive extraction and quantitation steps. Complete Y-STR profiles were generated within a very short period using these swabs and the Yfiler® Plus Amplification Kit. Only a minute amount of blood is used for amplification, saving the evidence for other types of analysis such as autosomal STR and massively parallel sequencing.

Determining the Efficiency of Nylon-flocked Swabs Versus Cotton Swabs for Fellatio Samples

Brianna Gregory and Janine Kishbaugh, M.S., Cedar Crest College

The presented research hopes to establish a way to increase the efficiency of sexual assault collection kits by determining optimal swab types for sample collection within 24 hours after oral sex. A lesser type of sexual assault, fellatio, was looked at to determine if male DNA can be prevalent enough in a female's mouth to create a full or partial profile. Choosing the optimal swab type is essential for picking up foreign material, such as sperm cells or male epithelial cells, from the mouth of the sexual assault victim. This study examined cotton and nylon flocked swabs to determine which of these may yield better profiles for collection of samples after fellatio. After a DNA extraction containing DTT was performed, the collected cells were amplified via PCR with Y-STR primers. The amplicons were then analyzed on a 3130xl genetic analyzer to see a Y specific STR profile of the male participant. Each swab type was also swabbed in three specific locations in the mouth to determine what location Northeastern Association of Forensic Scientists 2021 Annual Meeting – Program Chair, Adam B. Hall Newport, RI 84 was the most efficient for retaining male material. In addition, this research investigated different time intervals within 24 hours to determine the length of time a usable male DNA profile can be detected in the oral cavity after fellatio was performed. Participants in this study were asked to record activities that included eating, drinking, and oral hygiene to help gauge the effects that those activities have on the amount of DNA profile obtained. Resulting DNA profiles from analysis of samples at all time intervals have produced male DNA profiles. Swabs of the lips yielded the best results with cotton and nylon. An increase in observed alleles was obtained by increasing the electrophoresis injection to 10 seconds. The identification of successful sample collection methods including swab type and sampling areas could improve sexual assault investigation.

DNA Quantitation Levels of Common Saliva Coated Forensic Samples

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In forensic biology there is an ever present need to process evidentiary samples in a timely manner, without completely consuming the samples. By skipping quantitation, samples can be conserved and have less chance for contamination. This project is examining whether predictable amounts of DNA are present on oral-source DNA samples on common types of physical evidence, namely cigarettes, chewing gum, flaps of sealed envelopes and used drink containers. Predictable quantities of DNA would negate the need for DNA quantitation. In addition, the project is also statistically assessing the effects of outdoor environmental factors on predictable levels of DNA, if in fact predictable levels exist on control samples. Test samples were extracted using silica-based technology followed by quantitation using a SYBR® Green Alu-based qPCR method. Control samples used for comparison were kept under controlled conditions. Environmentally exposed samples were placed in an outdoor environment and left unattended (but protected) until sampling. In addition, some smoked cigarettes were collected from public areas. Statistical analysis will be performed to

assess the effect of environmental conditions on DNA concentration. After collection and analyzation of over two hundred and fifty samples, preliminary results have shown that cigarettes are the most likely evidentiary type to have predictable quantities of DNA. It is hoped that the results of this study can aid forensic scientists in casework analytical schemes for these common evidence types.

Exploring Bodily Fluid Stain Identification Using Raman and Microchemical Tests

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The methods for body fluid stain identification used in current crime laboratories lack efficiency in time and cost as well as preservation of limited samples. Some bodily fluids including menstrual blood, saliva, urine, and feces still require an identification test. Recent literature describes the use of Raman microspectroscopy to identify body fluid stains; however, substrate interference and spectral intensity appear problematic. This research aims to capitalize on the use of Raman microspectroscopy for body fluid stain identification while eliminating the interference and sensitivity issues. To achieve this, extraction and isolation steps are employed to separate abundant and specific components within the four main body fluid types (blood, saliva, urine, semen). These isolated components, either in the form of microcrystals or precipitates, allow for signature spectra with Raman microspectroscopy, with greater spectral intensity and specificity. Currently, a method for urine stain identification has been developed by utilizing the enzymatic breakdown of urea and complexing the resulting ammonium with iodic acid to form characteristic crystals which give a characteristic Raman spectrum. A blood stain identification test involving hemoglobin precipitation followed by the addition of iodic acid appears to produce a Raman signature spectrum; however, the chemistry at this time is unknown. Concordance results with commercially purchased hemoglobin however have been obtained. In order to be effective with time and cost, the most limiting time frames and amount of reagents are being investigated. As of now, the urine test needs approximately thirty minutes for the enzymatic reaction to occur while the blood test can be reduced to ten minutes. Additionally, saliva and semen stain identification methods are being developed in a similar approach using microcrystal and precipitate formation.

American Forensic Practitioners' Opinions on Activity Level DNA Reporting

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The technical advancements made in DNA detection and profiling have allowed very low amounts of DNA to be analyzed; therefore, the argument often made in criminal courts is not who the DNA belongs to but rather how the DNA was deposited1-3 . Activity level propositions have been considered to address and answer this question1-3 . However, there are many factors that should be included when formulating propositions at activity level, such as transfer, persistence, prevalence, and recovery (TPPR). Despite the complexity, European laboratories have used evaluative reports of activity level propositions, and the European Network of Forensic Science Institutes (ENFSI) has issued specific guidelines on DNA reporting. The overall views on activity level DNA reporting in the U.S, however, are not well known. Therefore, this study aimed at obtaining an overview on the opinions of using activity level reporting held by forensic DNA practitioners in the U.S.

A seventeen-question survey was distributed through QualtricsTM to members of the American Society of Crime Laboratory Directors (ASCLD) via the weekly Crime Minute and the International Society for Forensic Genetics (US members only) via email. The survey included multiple choice and open response questions and reached around 650 people.

Overall, there were 54 respondents to the survey in which 44 of them had over ten years of experience in DNA reporting at activity level. Of those 44 with over 10 years of experience, 72% agreed that despite having some concerns activity level reporting would be very (27%) or moderately (45%) useful. Only 31% believed that the current studies on DNA transfer were moderately adequate to provide empirical information on a case. A total of 41% of participants agreed that one year of training on activity level DNA reporting would be sufficient for a scientist to testify as an expert witness. There were six major concerns for implementing activity level DNA reporting in the U.S.: 1) the number of variables to be considered in activity level reporting such as shedder status, amount of starting DNA, or differing transfer rates based on surfaces; 2) educating practitioners and the legal system; 3) lack of controlled studies with realistic scenarios; 4) issues in court with admissibility. With this regard, some participants expressed concern that activity level propositions will have a difficult time passing a Daubert hearing because the approach is not as concrete or statistically sound as a DNA profile match. Moreover, 5) need for a standardized approach or guidelines in the U.S.; 6) convincing the forensic community and reaching consensus.

These concerns expressed by U.S. forensic practitioners revealed a range of varying opinions on activity level reporting. Future research will involve expanding the participants to a global level.

A Perfect Match: Identifying the Victim of a Cold Case Homicide Using DNA, NamUs, and CODIS

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In criminal investigations, a case with an unidentified decedent deprives family members of answers and may present a major obstacle to resolving the case. There have been significant scientific developments in the past twenty years that greatly improve the chances of success in cases with an unidentified victim. Today it is standard practice at forensic laboratories to develop DNA profiles of all unknown decedents and upload to the Unidentified Human Remains Index of the Combined DNA Index System (CODIS). Having the profile in the national database makes it available for comparison to antemortem profiles from the missing, profiles from relatives, and profiles from convicted offenders. Since 2007, the National Missing and Unidentified Persons System (NamUs) has been a government-sponsored nationwide clearinghouse to help expedite case associations. Investigators can upload case information about unidentified victims, which is then compared to information uploaded about missing persons. Possible matches are vetted and often confirmed with modern-day DNA technology.

In 1991, a male victim was fatally shot at close range in Queens, NY. The deceased man was not identified, and his body was interred at the Hart Island Cemetery. No biological evidence was submitted for forensic examination because DNA testing was not available. An absence of leads stalled investigative progress and the case grew cold. Then, in 2019, a woman submitted information about her missing father to NamUs. She also submitted a family reference sample, from which a DNA profile was developed and uploaded to the Relatives of Missing Persons Index of CODIS. Based on the information provided to NamUs, the NYC Office of Chief Medical Examiner (OCME) was notified of similarities in the details about the woman's missing father and the 1991 homicide victim. Agency identification and anthropology experts began by evaluating the cases. Based on the contextual information, a match could not be excluded, and the case was referred to the Department of Forensic Biology. Due to the absence of a postmortem specimen, a disinterment was initiated, and a bone specimen was provided for DNA testing. Specialized bone protocols were employed to develop a nuclear STR profile, which was uploaded to CODIS where it hit to the profile of the woman seeking her father. Kinship statistics were calculated and supported the familial relationship of father and daughter. Based on the DNA results, the unidentified male was conclusively identified, and an amended death certificate was issued in his name. The case remains an active homicide investigation, which may now advance with the knowledge of the victim's identification.

The outcome of this case demonstrates the results that are possible when resources at the investigator's disposal are used to their maximum potential. Strong emphasis is placed on the need for relatives seeking the missing to be made aware of options such as NamUs and CODIS. Forensic laboratories must also recognize the benefits of retroactively creating DNA profiles for cases stemming from the pre-DNA era. Overall, this example illustrates how cases with an

unidentified decedent can be resolved when three major tools coalesce – DNA technology, NamUs, and CODIS. None of these advancements were available until the recent past, and each makes a unique contribution to modern forensic investigations.

Forensic Genetic Genealogy: Myth or Fact?
Melissa Kotkin, Verogen

Forensic genetic genealogy (FGG) is a powerful tool for investigative lead generation. To date, it has been applied to more than 500 cold cases including violent crimes like sexual assaults, as well as the identification of unidentified human remains. As forensic laboratories consider how to implement this technique as part of their own workflow, multiple questions, concerns, and myths around FGG still exist. This presentation will address common questions associated with FGG and shed light on evaluation and implementation considerations for those determining how they might move forward with FGG.

Common Misconceptions and the Need for Best Practices in Forensic Genetic Genealogy
Claire Glynn, Ph.D., Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Forensic Genetic Genealogy (FGG) is a rapidly evolving professional field and sub-discipline of genomics, genealogical science, and forensic genetics. While the roles and impact of FGG experts, including scientists and other professionals in the field, have rapidly expanded, the best practices in the forensic use of genetic genealogy have yet to be established, nor are there clear standards for the pedagogy and competencies that must be mastered through expert training and continuing education. Most importantly, the field demands constant, fundamental, and critical analysis of the societal, privacy, and ethical impacts of the use of genetic genealogical data in forensic settings. Much of the privacy and ethical impacts that have come under scrutiny, both within the forensic science industry and the public view, are derived from a lack of understanding, or indeed perceived misconceptions about the FGG process, the tools and databases used, and the handling of individuals genetic data. This presentation will address several of the common misconceptions surrounding this new investigative tool and will discuss the critical need for establishing a collaborative framework bringing together academics, forensic practitioners, legal and ethical professionals, and law enforcement agencies, to establish comprehensive best practices for practitioners in this field, both nationally and internationally.

Investigating the Use of Forensic Genetic Genealogy (FGG) on Degraded DNA Samples
Julia Dollen, and Claire Glynn, Ph.D., Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Forensic Genetic Genealogy (FGG) has become an increasingly popular topic within the forensic science community, but there has yet to be any robust research regarding its use with biological samples that may be degraded. When a crime scene DNA sample does not generate a "match" in the Combined DNA Index System (CODIS), or is too degraded to make an identification, forensic genetic genealogy is an alternative method of identification that could be employed. This study investigated the manual "degradation" of raw DNA data derived from Direct-To-Consumer (DTC) DNA testing companies (e.g., Ancestry DNA, 23andMe etc.). Following written informed consent, volunteers who had previously taken a DTC DNA test downloaded their own raw DNA datafile from their account and submitted the file to the PI/Researcher. Using Microsoft Excel, the data from the Single Nucleotide Polymorphisms (SNPs) analyzed was manually, and randomly, deleted in increasing percentages (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 40%, and 50%) to mimic real degradation. Each of the new "degraded" datafiles were then uploaded to GEDMatch as "Research" uploads. GEDMatch generates match lists of other individuals who have uploaded their raw DNA datafiles to the database. The top 10 matches for each file upload were recorded to assess if those matches changed in their rank, or were lost, as the percentage of data deletion (i.e., "degradation") increased. Three volunteer's raw DNA datafiles were used in this study

to represent individuals who have high centimorgan (cM) top matches (~880cM), and low centimorgan (cM) top matches (~56cM). The aim of this is to investigate at which point of degradation will a familial relationship to a match be lost or altered. The results of this study revealed that as the degree of "degradation" increased, the order in which matches were ranked was altered, and in some cases lost completely. This information can greatly impact an FGG investigation as it is typically those top 10 matches that lead FGG investigators to the identity of their unknown subject. Therefore, if a crime scene sample is known to be heavily degraded, it is crucial to strategize appropriate methods of analysis to generate as much data as possible from the DNA sample, e.g., perform Whole Genome Sequencing (WGS), or indeed DNA repair, rather than the more standard approach of SNP microarrays. The topic of degraded biological samples has not been greatly investigated using this new investigatory tool, and this research will contribute to the growing body of knowledge about Forensic Genetic Genealogy and its use in forensic investigations.

Quantifying UV-Induced Primer Binding Site Damage in DNA

Sabrina Martins, and David San Pietro, Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

DNA analysis developments have significantly changed how evidence is processed. There are various conditions that exist where DNA can be damaged, intentionally, or not. One common condition that induces DNA damage is UV-light exposure. UV-light primarily causes dimer formation or abasic sites in DNA leading to gaps in the helix. Depending on the extent and location of the damage, DNA profiles may be incomplete due to partial or complete allele drop out. Alleles in DNA profiles are determined by the short tandem repeat (STR) region that vary among individuals. Prior to profiling, samples are amplified through the polymerase chain reaction (PCR) in which primers bind to the primer binding site (PBS) in DNA. Although the STR region is variable among individuals, the primer-binding site is a clearly defined known sequence present at the loci analyzed. Damage in these regions may inhibit sample amplification and the generation of a DNA profile. However, due the primer binding site's consistent size and nature, it may be possible that repair of that specific region may improve DNA profiles. Alternatively, it is possible that damage to the specific PBS regions may not have an appreciable effect on the ability to generate a STR profile. Although previous studies focused on the repair of damaged samples, there is a lack of research on the specific mechanism(s) of DNA damage to more specifically defined areas (i.e., primer binding sites). By gaining a better understanding of the mechanisms of DNA damage, more effective repair methods can be created and utilized without risking STR region alteration. Primers were exposed to UV-light at various time periods, ranging from 5 seconds to 300 seconds, and the TPOX locus was profiled through capillary electrophoresis. Peak height ratios were calculated over this range to measure the possible damage and its effect on the recovered DNA profile from a single individual. Observed trends in peak height ratios may indicate when damage starts to affect recovered DNA profiles that may lead to better repair methods with future studies.

DNA Recovery and Transfer on Non-Porous Surfaces Submerged in Spring Water

Morgan Korzik, and David San Pietro, Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Submerged items are commonly thought to lack evidentiary value. For instance, some investigators believe that all DNA could be lost once an item is exposed to a flowing current or tossed into a body of water. However, previous studies have shown the ability to recover DNA from submerged porous items for upwards of six weeks. The crevices or interweaving fibers in porous items are thought to protect DNA from being washed away. Smooth non-porous surfaces inherently lack the traits that might aid in DNA retention. Previous studies have shown that alleles from stains on non-porous surfaces can still be detected up to three days submersion, but allele dropout can occur as early as twelve hours into the submersion period. As far as the authors are aware, studies have reported the percentage of alleles but not the quantity of DNA recovered from submerged non-porous items. We have hypothesized that, because non-porous surfaces do not have traits that might aid in DNA retention, then DNA quantities and the number of alleles recovered will decrease over longer submersion periods. Additionally, we have hypothesized that DNA quantity and the number

of alleles will decrease at a slower rate in stagnant water versus in a flowing current. Neat saliva of known DNA quantity will be applied to glass slides and exposed to stagnant and flowing spring water to observe the effects on both DNA quantity and STR amplification. Four experimental phases will be run: (1) blank and sample slides from one donor exposed to stagnant water, (2) blank and sample slides from one donor exposed to flowing water, (3) blank and sample slides from two donors exposed to stagnant water, and (4) blank and sample slides from two donors exposed to flowing water. Results from the first two phases support that DNA quantities decrease, and allele dropout occurs when samples are submerged for longer times, especially when samples are exposed to flowing water. Additionally, preliminary results have suggested that transfer and allele drop-in may occur from sample to blank slides submerged in the same water vessel. The amount of transfer from sample to blank appears expedited when exposed to flowing water. Research is continuing in the last two phases to determine if mixed profiles can result from DNA transfer through the surrounding water. Observed results could indicate the possibility that DNA recovered from submerged non-porous evidence is a result of transfer.

Evaluating the Discriminating Power of Hair Amino Acid Ratios on Distinguishing Individuals Using GCMS

Timothy Yaroshuk and Alyssa Marsico, Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven

Currently, the conventional methods of analysing hair include microscopic hair comparison (MHC) and DNA analysis (nuclear and mitochondrial), with nuclear DNA analysis being the most individualizing. However, MHC is subjective and nuclear DNA analysis is not always possible if not enough adequate cells are present. Non-synonymous amino acid changes in the hair protein sequence – resulting from single nucleotide polymorphism profiles that differ between each individual – can be exploited to offer alternative routes for hair analysis. Currently, proteomics has successfully exploited genetically variant peptides (GVP) content in hair to differentiate at least non-related individuals. However, proteomics is complicated and requires the GVPs to remain intact. Analysing amino acid content is an alternative method that may simplify the analysis. It has been demonstrated that analysing amino acid quantities has abilities to differentiate people based on general class characteristics of sex, age group, and geographical origin. A study by Macri et al., analysing amino acid ratios of two individuals with morphologically similar hair, discovered 15 amino acid ratios that differed. Expanding on this study, this research will evaluate the discriminating power of using hair amino acid ratios to differentiate individuals with a focus on increasing the sample size and diversity. The purpose of this study is to develop a method that can supplement MHC to reduce subjectivity of hair analysis in case DNA analysis cannot be conducted.

Hair samples were obtained from 9 consenting individuals and were anonymized. Plucked hairs were obtained except for 1 individual where hair cut samples were obtained. 2 separate hair samples were collected from the same individual where one set was dyed, and another was natural. Hairs were thoroughly washed with deionized water and methanol to remove surface contaminants. They were then prepared in triplicates where hairs were cut into smaller pieces and hydrolyzed with hydrochloric acid for protein digestion. Subsequently, the sample was filtered to remove unhydrolyzed hair pieces and an aliquot was dried under a gentle stream of nitrogen. L-norvaline was then added as an internal standard. After reconstituting in ethyl acetate, N,O-Bis(trimethylsilyl)trifluoroacetamide was added for amino acid derivatization. GCMS was used for analysis. A set of 11 standard derivatized amino acids, including L-norvaline, in ethyl acetate was also analysed using GCMS for quantitation purposes.

Eight derivatized amino acids were detected from the hair samples in addition to a glutamic acid derivative. Identification was conducted by comparing sample retention times to standards as well as mass spectra library comparison. Quantitation relative to the internal standard was completed and 36 amino acid ratios were constructed from these quantities. Outliers for each ratio were determined by the Grubbs test for samples with more than 3 data points and outliers were discarded. Between the 10 hair samples analyzed, one-way ANOVA for all 36 ratios had $p<0.05$. Most individuals were differentiable using the post-hoc Tukey test for all ratios. For the samples that could not be differentiated with this method, 3D-PCA plot showed distinct clustering of individuals therefore allowing differentiation. Ratios between dyed hair samples were differentiable from undyed hair.

Paving the Way for New Technology in a Post-Pandemic Criminal Justice System

Rachel Oefelein, DNA Labs International

The COVID-19 pandemic rocked forensic laboratories across the United States with laboratories seemingly overnight having to resort to closures, shift work, social distancing, and massive supply shortages. As the light at the end of the tunnel begins to become clearer, new technology is emerging. Advances in probabilistic genotyping, Rapid DNA, SpentShell™ testing, new screening testings for urine and menstrual blood, pyrosequencing, hair shaft testing, phenotyping, Next Generation Sequencing (NGS), and genealogy testing tailor-made for forensics will all be ushered into casework processing in 2021. How will this new technology be implemented? How will it be presented in court with many courts still shut down or limited to virtual hearings and trials? Finally, this presentation will discuss the lessons learned as a result of 2020 and what that means for the technology utilized in the laboratory.

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Poster Session Abstracts

Analysis of Benzodiazepines in Urine by UHPLC-MS/MS: Meeting the Requirements of ASB Standard 121
Abby Houliston, Boston University School of Medicine; Jamison Whitten (co-presenter), Boston University School of Medicine; Simone Rumph (co-presenter), Boston University School of Medicine; Nadine Koen, Boston University School of Medicine; Halia Haynes, Boston University School of Medicine; Reshma Gheevarghese, Boston University School of Medicine; Sabra Botch-Jones, Boston University School of Medicine; Jamie Foss, PerkinElmer, Inc.

Benzodiazepines are a widely prescribed class of drugs used to treat seizures, anxiety, and sleep disorders. They act as central nervous system depressants with rapid onset of action and although they are often administered voluntarily, their abuse rate in drug facilitated crimes (DFC) has become increasingly prevalent in today's society. A DFC is a crime which occurs when a person is victimized while mentally or physically incapacitated due to the effects of drug, and DFCs often involve the use of benzodiazepines due to their sedative and amnesic effects. Analytical detection of benzodiazepines in DFC cases requires high sensitivity because the drug analytes could be present in low concentrations due to their high potency. In accordance with ASB Standard 121 - Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Drug-Facilitated Crime Investigations, urine is the typical biological specimen for analytical testing in DFCs because it can provide an extended window of detection for analytes after the alleged incident. The main objective of this project was to develop a LC-MS/MS method for the separation and detection of six benzodiazepines in accordance with the ASB Standard 121 - Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Drug-Facilitated Crime Investigations.

The Center for Advanced Research in Forensic Science (CARFS): An Industry/University Collaborative Research Center
Adam B. Hall, Boston University School of Medicine; Jose Almirall, Florida International University; Michael Chambers, University of South Alabama; Aaron Tarone, Texas A&M University; Sarah Kerrigan, Sam Houston State University

The goals of the Industry/University Cooperative Research Center (I/UCRC) Center for Advanced Research in Forensic Science (CARFS) are to bring together industrial and governmental partners, including the end-user community, with academic forensic science researchers with an aim to develop, implement and commercialize tools that benefit the national forensic science research enterprise. CARFS tackles emerging forensic science problems. Our two main sites are located at Florida International University and University of South Alabama with affiliate sites at Texas A&M University, Boston University and Sam Houston State University. Our Industrial Advisory Board (IAB) provides guidance on the direction of projects at the forefront of forensic science. Faculty affiliated with the center are distributed across key disciplines in science, social science, engineering, and statistics, with interests that cover a wide array of forensic disciplines. The CARFS research program is designed to address key issues in forensic science identified by the 2009 NAS report, and to develop innovative technologies and investigative approaches for forensic practice.

NSF-I/UCRC enables industrially relevant, pre-competitive research via multimember, sustained partnerships across industry, academe, and government. NSF supports the development and evolution of I/UCRCs, by providing a financial and procedural framework for membership and operations. It also promotes best practices learned over decades of fostering public/private partnerships that produce significant value to the nation, industry and university faculty and students. Participating researchers perform cutting-edge, pre-competitive fundamental research in technology areas of interest to industry and government partners which can drive innovation and the U.S. economy. Members guide the direction of Center research through active involvement and mentoring. I/UCRCs offer a platform for significant leveraging of financial investment by members to accelerate the knowledge base in emerging technologies and manufacturing sectors while developing an industrially savvy workforce to benefit US economy.

Estimating Muzzle to Target Distance from the Physical Characteristics of a Bullet Hole in Different Wood Substrates

Alan Lee, John Jay College of Criminal Justice; Peter Diaczuk, PhD, John Jay College of Criminal Justice

Muzzle to target distance is an integral part of crime scene reconstruction, but methods of determining this distance can be limited depending on the condition the crime scene is in. In this study, our goal was to explore how the physical damage characteristics of bullet holes may lead to clues in determining muzzle to target distance. Test fires were conducted with a .22 caliber rifle over a range of muzzle to target distances and different bullet velocities. The goal of the study was to simulate an indoor shooting on plywood and Medium Density Fiberboard (MDF) panels. The results of our study show that as muzzle to target distance increases, bullet hole depth decreases. In addition, specific damage patterns were observed on the back of the substrates relating to shooter distance and bullet velocity. A predictions model was developed using this data that allowed shooter distance to be estimated based on bullet hole depth. Conclusions were made that with some finetuning, this method may aid forensic scientists in casework concerning ballistics.

Bullet Behavior After Perforation of Intermediate Substrate into Ballistic Gelatin

Alisia Tseytina, John Jay College of Criminal Justice; Peter Diaczuk, PhD, John Jay College of Criminal Justice

In wound ballistics, how a bullet will behave after perforating or penetrating an object is difficult to predict. Upon impact, there is loss of momentum and possible trajectory change as well as orientation changes. The aim of this experiment was to see, under controlled conditions, how a bullet behaves after it has perforated a substrate, then entered a tissue simulant at close range. The experimental design was set up to model a home invasion scenario; common household construction materials (wood, MDF, sheet metal, glass etc.) were used to simulate items that could be used for protection/cover; ballistic gelatin used as a simulant for a human body; and a Ruger 10/22 with .22 LR ammunition used as the firearm. Variables focused on throughout the experiment were depth of penetration (DoP), orientation, damage done (cavity formations inside the gelatin) and change in velocity of the bullet. Data taken from straight shots into the gelatin, with no intermediate object, was used as a baseline for each variable. The results of this experiment showed that thickness of the substrate affected the velocity of the bullet as well as its DoP, but the material of the substrate affected the damage done. Substrates that can shatter or splinter, like glass, wood, and sheet metals, were observed to deal the most damage from secondary shrapnel. By understanding how bullets can behave under controlled conditions, these observations and insights can someday be used in and applied to real life crime scene cases.

The Differentiation of Dark Colored Automotive Carpet Fibers using Plane-Polarized Light Ultraviolet-Visible Microspectrophotometry

Andra Lewis, Sam Houston State University; Dr. Patrick Buzzini, PhD, Sam Houston State University

Automotive carpet fibers found in vehicles are made from recycled polyester derived from plastic bottles and blends of fibers including polyamides, polypropylene (PP), polyester (PET), and polyolefins. The analysis of these fibers is challenging due to their peculiar and blended compositions. Most fiber examinations start with light microscopy for both identification and comparison purposes followed by visible microspectrophotometry and/or thin layer chromatography (TLC).

The inclusion of the ultraviolet spectral range may provide further discriminatory capabilities and studies have shown the discriminating potential of dichroism (plane polarized microscopy or PPL) in conjunction with visible MSP. This research aims to investigate the combined discriminating power of light microscopy in combination with different

capabilities of UV-vis MSP-PPL in determining objective criteria to develop a protocol for the capture, processing, and interpretation of spectral patterns for fiber specimens encountered in casework.

In this project, forty (40) macroscopically similar black automotive carpet fibers were analyzed using microscopical examinations to include color and fluorescence followed by different applications of UVvis microspectrophotometry. The microspectrophotometer used in this study was not only equipped with full polarizing capabilities (i.e., polarizing filters and a rotating stage), but the analyzer and the polarizer transmitted UV radiation down to 240nm, making pairing of the ultraviolet with plane polarized light a reality. The results of this study showed ~70% of the samples provided further discriminatory information through the combination of both ultraviolet radiation and plane polarized light to the characterization and differentiation of the fibers.

How Did the Case Cross the Fence?

Andrew Winter, Middlesex County Prosecutor's Office (NJ); Janell Chuddley, Centenary University; Peter Diaczuk, PhD, John Jay College of Criminal Justice

Law enforcement regularly encounters two primary types of handguns at shooting scenes – the revolver and the semi-automatic pistol. Both of these very common platforms of firearms are designed to discharge a projectile. However, 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 be present at a shooting scene and become quite significant to investigators and crime scene personnel in their effort to determine where the shooting occurred, how far the shooter was from his/her intended or unintended target, and the location of the shooter in relation to the spent cases with an obstacle (fence) in the scene. Various factors including ejection port location, materials utilized in different types of pistol ammunition, the height and way the firearm is held, and the caliber variations among pistols will affect the flight of the spent case. This project focuses on the height and distance that spent cases can travel from the ejection port of a semi-automatic pistol and travel over a fence commonly seen in crime scenes.

Evaluating the 12 gauge Less-lethal Baton Shotshell

Britania Walters, John Jay College of Criminal Justice; Peter Diaczuk, PhD, John Jay College of Criminal Justice

Less-lethal ammunition and other less-lethal weapons have been used to take control of certain situations that do not require lethal approaches. Less-lethal ammunition and other less lethal tactics are used all over the world. The use of less-lethal ammunition has gained popularity in the United States due to the recent protests of the Black Lives Matter Movement. Less-lethal ammunition, previously known as "nonlethal ammunition," was developed and used for crowd control during riots and protests or simply to incapacitate a suspect without causing any major harm to the individual. These projectiles were designed to minimize fatalities, permanent injuries, damage to properties and the environment. Soon after this ammunition was used in public, it was discovered that it could still be lethal to individuals. Less-lethal ammunition has shown to have caused fatalities during the recent protests. Since less-lethal ammunition is being used more by law enforcement and the military, many injuries have been reported. This project will have a critical review of scientific literature highlighting the history of less-lethal ammunition, past studies carried out highlighting some of the effects of less lethal ammunition, and statistical data of injuries to civilians caused by less-lethal ammunition during the Black Lives Matter Protests across the U.S. Several experiments will be performed to measure the effects and impact of lethal ammunition. The aim of this study is to determine the different components of the less-lethal projectile(s) used, to observe the possible damage that the tested less-lethal ammunition can do to simulated body tissue and to propose the design of an even "lesser-lethal" bullet.

Improving Current Methodology used in the Forensic Analysis of Bisulfite Modified DNA Samples
Deborah Silva, Hofstra University; Haley Ecker, Hofstra University; Johnisa Walcott, Hofstra University

In forensic science, DNA serves as a vital tool that can tie a victim, suspect, or witness to a crime. More recently, forensic scientists have started to explore epigenetics and its application in forensic analysis. The epigenome is the "layer of information" on top of our existing genetic code, and it participates in differences between individuals, complete human populations, and contributes to physical and behavior differences. Studying DNA methylation patterns is advantageous for the forensic field as it can be used to identify the type of tissue or fluid at a crime scene, determine the sex of the sample donor, estimate the age of the sample donor, and distinguish between monozygotic twins. Since PCR amplification does not give any information about a DNA strand's methylation status (methylation is not preserved in this process), bisulfite modification is often used in order to get this information. In this process, DNA is treated with sodium bisulfite to convert unmethylated cytosines into uracil but does not alter the methylated cytosines. This is a harsh process that changes the chemical structure of the DNA and can damage the strands, making it not ideal for forensic samples as the environment may limit the amount of DNA, we are able to work with. The main goal of this research was to modify the current methodology used in bisulfite modification and subsequent PCR to repair damaged DNA and to improve data generation and also the downstream analysis of results. To achieve this goal, we first tested two different kits to bisulfite modify DNA samples. Then we performed a modified PCR that involved adding repair enzymes to the reaction mix and an extra step to thermocycling conditions in order to repair the damaged DNA before the start of the amplification process. Using NanodropOne, we were able to analyze the results of the modified protocol, confirm the quality and quantity of methylated DNA samples and evaluate which protocol yielded the most PCR products (with or without repair enzymes). The protocol using the EZ DNA methylation-lightning kit coupled with the added repair enzymes to the PCR process presented the best performance and higher yield of PCR products. The results obtained in this initial study are important to show that it is possible to improve the quality of DNA samples after going through a harsh chemical modification, which will then provide better results in DNA amplification and other downstream methods and analysis.

Autolytic Generation of Ethanol in Decomposing Mammalian Liver
Gabrielle Messe, University of New Haven; Natalia Gabrys, University of New Haven; Robert H. Powers, University of New Haven

A postmortem MV crash case was recently presented to this group in which the detection of significant levels of ethanol in a postmortem liver sample had been utilized as evidence of antemortem intoxication of the decedent, with significant legal ramifications. Because of the traumatic nature of the death, blood samples had not been retained for analysis. However, a liver sample was recovered at autopsy and retained for toxicologic analysis. While the potential for improperly preserved or stored blood samples to generate alcohol is well recognized in the forensic community, the potential for similar generation in liver samples has not been similarly documented in the forensic literature.

During both the autolytic and putrefactive stages of decomposition, volatile organic compounds (VOCs) are generated by the biochemical reactions of tissue decay. Autolysis encompasses the first stage of decomposition and is characterized by endogenous enzyme activity that degrades cell membranes. Decomposition is then characterized by extensive microbial activity and a pattern of volatile compound generation distinct from the autolytic period. In working with volatile generation in hepatic homogenates of both rat and pig, consistent appearance of ethanol during the autolytic period has been demonstrated in a time/temperature dependent fashion. Hepatic homogenates were incubated at different temperatures for a several daytime span, with sample aliquots removed every twelve hours in order to evaluate the temporal relationships of VOC generation. Headspace-GCMS was utilized to identify volatile materials in triplicate at each time point. Ethanol generation was observed in rat homogenates as early as 12 hours at 28°C incubation temperature and 24 hours at room temperature. Similarly, ethanol generation was observed in pig

liver homogenate at 36 hours at room temperature.

Based on these findings, particularly with reference to the conditions under which the liver sample in the case had been stored prior to analysis, significant autolytic generation of ethanol appeared to be a reasonable underlying explanation for its presence. Subsequent testing of a properly preserved vitreous sample that had been reserved in the case (but had not previously been analyzed) was negative for the presence of alcohol, thus consistent with the suggestion of autolytic generation of ethanol in the liver.

The potential for generation of ethanol in the autolytic stage of decomposition may be important for evaluation of future postmortem casework.

High Resolution Mass Spectrometry Screening in Impaired Driving Investigations
Jessica Ayala, Sam Houston State University; Dr. Sarah Kerrigan, Sam Houston State University

Impaired driving investigations have become increasingly more challenging with the influx of new psychoactive substances (NPS) into the drug market. NPS become more prevalent as drug users pursue "legal highs." However, as these compounds gradually become controlled substances, new structural analogues emerge. As a result, traditional immunoassay-based drug screening is unable to keep pace with new and emerging drug trends. Immunoassays are not available for all drugs or drug classes, and due to their reliance on antibody-based reagents, they are expensive and time consuming to develop. When used alone, they have insufficient scope and sensitivity. As a result, forensic toxicology laboratories are exploring high resolution mass spectrometry (HRMS)-based technologies for toxicological drug screening. The purpose of this study was to re-analyze adjudicated blood specimens and compare HRMS-based drug screening to reported immunoassay results.

Evaluation of Cannabidiol and Δ9-Tetrahydrocannabinol Use via a Qualtrix Survey
Kourtney Albert, Arcadia University, Karen S. Scott, PhD, Arcadia University

Cannabidiol (CBD) has shown exponential growth over the past decade as an off-label alternative medicine mainly for the management of pain. Δ9-Tetrahydrocannabinol (THC) continues to be one of the most commonly used drugs of abuse worldwide, however increasing numbers of the population now own legal medical marijuana cards, adding a potential new subset of the individuals to the cannabis using population. Both CBD and THC are sold in many forms, including but not limited to pills, flower buds, tinctures, beauty products, and candies.

For this research, a survey was sent out to members of the extended Arcadia University community via Qualtrix. The survey asked questions regarding biological sex, age, CBD and THC usage, and forms of products used. The total number of respondents to the survey was 84. The most common age range of participants was 18-29, but a maximum age of 70+ was seen. 63 (75%) of respondents were female. 62 (73.8%) of the respondents reported using CBD products. For those who used CBD products, the most common product forms were oils, gummies, and lotions. The main three reasons for use were stress, pain/arthritis, and anxiety. 60 (71.4%) of the respondents reported using THC products. For those who used THC products, the three most used products were marijuana leaf, edibles, and dab pens. The main reasons for use were recreation, medical card allowance, and anxiety. 53 (63.1%) of respondents used both CBD and THC products. This survey has served as a steppingstone in understanding CBD and THC usage within the extended Arcadia community. In addition to the survey, participants who used only CBD products were asked if they would be willing to provide samples of product and biological samples, allowing for future research to be conducted on product integrity as well as CBD presence in urine and saliva by LC-MS/MS.

Analysis of Polymer Coated Bullets Using Spectroscopic Methods

Liana Albano, John Jay College of Criminal Justice; Peter Diaczuk, PhD, John Jay College of Criminal Justice

Polymer coated bullets have gained popularity in recent years. To determine the composition of two polymer coated bullets (American Eagle Syntech (red polymer) and Syntech Defense 9 mm Luger (blue polymer)), the solubility, melting point and molecular vibrations of the polymers were examined. Our results indicate that the blue and red polymers studied had very different solubilities, melting points and molecular vibrations. Infrared spectroscopy revealed that the blue polymer had similar functional groups to dimethyl iso phthalate while the red polymer had similar functional groups to poly(ethylene glycol terephthalate). These results confirm that both polymers have different compositions as evident by the vast differences in solubility, melting point, and their infrared signatures. The next step would be to study various targets shot with polymer coated bullets for the presence of polymer residue. This can be helpful to link evidence from a crime scene to known polymer coated bullets.

The Visualization of Bruises Using Alternate Light Source

Wan Yu Tan, Boston University School of Medicine; Karen Kelly, Brody School of Medicine, East Carolina University; Ann Marie Mires, Anna Maria College; Sabra Botch-Jones, Boston University School of Medicine

With the global pandemic, there has been mandatory movement restrictions by countries around the world. There has also been an increase in domestic abuse; such violence often presents in many forms with physical abuse heading the list.This study was conducted to enable forensic officers to make use of existing crime scene equipment to enhance the visualization of bruises on victims of abuse. When a case of abuse is reported, evidence of the abuse must be documented. Traditional methods of investigation involve questioning the victim or abuser, followed by documentation using photography and note-taking which may not accurately represent the injuries. In addition, the amount of force used, area of injury and the age of the injuries could affect the appearance of blunt force trauma including bruising. At times only redness is observed on the victim's skin making the injury difficult to document; such injuries would constantly be overlooked.5,6 Alternate Light Source (ALS) is a common, cheap, and effective piece of equipment used by forensic examiners at the crime scene to reveal objects missed by the naked eye. With the use of ALS, the documentation of existing bruises can be enhanced, while bruises that are missed by the naked eye can be revealed.

In this study, the effectiveness of visualization of blunt force injuries (contusions) to the skin at different ALS wavelengths was evaluated to determine the optimal wavelength for documentation of bruises.7,8,9,10 Bruises were inflicted on 57 participants with no known medical conditions following institutional approval. The participant was in a seated position while a cylindrical ball of ~465 grams was dropped at a height of 1.5 meters through a vertically positioned tube onto the ventral surface of the participant's forearm. The injury site was then observed and documented under white light, 415nm, 460nm and 550nm. Photographs of the forearm were taken under at all wavelengths prior to bruising, immediately after bruising, 3 hours after bruising, and at specific time points over a period of 21 days. The results showed better visualization of the injury observed at a wavelength of 415nm and 460nm.

A blind study was conducted using the same methodology to determine the validity of the experiment. A colleague was briefed and tasked to conduct a blind trial on 12 participants following institutional approval where the researcher has no knowledge on which participant the bruise was inflicted on. Photographic documentation and observations were recorded with the results only made known to the researcher at the end of the experiment. It showed that the methodology is accurate at about 75%. This study shows that the use of ALS provided an effective alternative with the visualization and documentation of blunt force traumatic injuries compared to traditional documentation methods without added cost and should be considered for use in future cases involving trauma and physical abuse. Additionally, since ALS is the standard crime scene equipment, the documentation of bruising by forensic examiners can be initiated

in the field prior to transport of victim to either the hospital or morgue setting.

DNA Recovery and Transfer on Non-Porous Surfaces Submerged in Spring Water
Morgan Korzik, University of New Haven; David San Pietro, PhD, University of New Haven

Submerged items are commonly thought to lack evidentiary value. For instance, some investigators believe that all DNA could be lost once an item is exposed to a flowing current or tossed into a body of water. However, previous studies have shown the ability to recover DNA from submerged porous items for upwards of six weeks. The crevices or interweaving fibers in porous items are thought to protect DNA from being washed away. Smooth non-porous surfaces inherently lack the traits that might aid in DNA retention. Previous studies have shown that alleles from stains on non-porous surfaces can still be detected up to three days submersion, but allele dropout can occur as early as twelve hours into the submersion period. As far as the authors are aware, studies have reported the percentage of alleles but not the quantity of DNA recovered from submerged non-porous items. We have hypothesized that, because non-porous surfaces do not have traits that might aid in DNA retention, then DNA quantities and the number of alleles recovered will decrease over longer submersion periods. Additionally, we have hypothesized that DNA quantity and the number of alleles will decrease at a slower rate in stagnant water versus in a flowing current. Neat saliva of known DNA quantity will be applied to glass slides and exposed to stagnant and flowing spring water to observe the effects on both DNA quantity and STR amplification. Four experimental phases will be run: (1) blank and sample slides from one donor exposed to stagnant water, (2) blank and sample slides from one donor exposed to flowing water, (3) blank and sample slides from two donors exposed to stagnant water, and (4) blank and sample slides from two donors exposed to flowing water. Results from the first two phases support that DNA quantities decrease, and allele dropout occurs when samples are submerged for longer times, especially when samples are exposed to flowing water. Additionally, preliminary results have suggested that transfer and allele drop-in may occur from sample to blank slides submerged in the same water vessel. The amount of transfer from sample to blank appears expedited when exposed to flowing water. Research is continuing in the last two phases to determine if mixed profiles can result from DNA transfer through the surrounding water. Observed results could indicate the possibility that DNA recovered from submerged non-porous evidence is a result of transfer.

Analysis of Benzodiazepines in Urine by UHPLC-MS/MS: Meeting the Requirements of ASB Standard 120
Nadine Koen, Boston University School of Medicine; Halia Haynes (co-presenter), Boston University School of Medicine; Simone Rumph (co-presenter), Boston University School of Medicine; Abby Houliston, Boston University School of Medicine; Jamison Whitten, Boston University School of Medicine; Reshma Gheevarghese, Boston University School of Medicine; Sabra Botch-Jones, Boston University School of Medicine; Jamie Foss, PerkinElmer, Inc.

Driving under the influence of drugs (DUID) cases have involved the use of impairing substances such as alcohol and many other psychoactive drugs, specifically sedative-hypnotic drugs. Benzodiazepines are classified as central nervous system depressants that have become more widely prescribed for the treatment of anxiety, PTSD, and sleep disorders. A study in Finland showed a trend of increasing DUID cases from 1977-2007 with the number of cases increasing 18-fold. The researchers found the most common class of drugs detected were benzodiazepines (75.7%). This upward trend of abuse seen in this class of drugs calls for the continued development of increasingly sensitive analytical methods. The main objective of this project was to develop a UHPLC-MS/MS method for the separation, detection, and quantitation of nine benzodiazepines in accordance with the ASB Standard 120 - Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing in Impaired Driving Investigations.

Quantifying UV-induced Primer Binding Site Damage in DNA

Sabrina Martins, University of New Haven; David San Pietro, PhD, University of New Haven

DNA analysis developments have significantly changed how evidence is processed. There are various conditions that exist where DNA can be damaged, intentionally, or not. One common condition that induces DNA damage is UV-light exposure. UV-light primarily causes dimer formation or abasic sites in DNA leading to gaps in the helix. Depending on the extent and location of the damage, DNA profiles may be incomplete due to partial or complete allele drop out. Alleles in DNA profiles are determined by the short tandem repeat (STR) region that vary among individuals. Prior to profiling, samples are amplified through the polymerase chain reaction (PCR) in which primers bind to the primer binding site (PBS) in DNA. Although the STR region is variable among individuals, the primer-binding site is a clearly defined known sequence present at the loci analyzed. Damage in these regions may inhibit sample amplification and the generation of a DNA profile. However, due the primer binding site's consistent size and nature, it may be possible that repair of that specific region may improve DNA profiles. Alternatively, it is possible that damage to the specific PBS regions may not have an appreciable effect on the ability to generate a STR profile. Although previous studies focused on the repair of damaged samples, there is a lack of research on the specific mechanism(s) of DNA damage to more specifically defined areas (i.e., primer binding sites). By gaining a better understanding of the mechanisms of DNA damage, more effective repair methods can be created and utilized without risking STR region alteration. Primers were exposed to UV-light at various time periods, ranging from 5 seconds to 300 seconds, and the TPOX locus was profiled through capillary electrophoresis. Peak height ratios were calculated over this range to measure the possible damage and its effect on the recovered DNA profile from a single individual. Observed trends in peak height ratios may indicate when damage starts to affect recovered DNA profiles that may lead to better repair methods with future studies.

Evaluation of a Facile Synthesis of Lefetamine Analogs

Savannah Brown, University of New Haven; Koby Kizzire, PhD, University of New Haven

An ongoing challenge forensic drug laboratories and law enforcement face is the development of new psychoactive substances (NPS), also commonly known as designer drugs. These NPSs are often structural analogs of controlled substances which are created to mimic their effects. These compounds can be purchased from online chemical research companies, or they can be produced in clandestine laboratories. As drugs are controlled based on their unique chemical structure, analogs can skirt legalities associated with certain controlled substances. These compounds also create analytical difficulties for forensic drug laboratories as new structures emerge to stay ahead of legal regulations and may not be available in databases or as reference standards for purchase.

Lefetamine is a Schedule IV drug in the United States, and its analogs are not explicitly controlled by current legislation. User reports have shown interest in lefetamine analogs with ketamine-like effects, such as ephenidine and diphenidine, which have already been regulated internationally. A publicly available lefetamine synthesis was identified as having abuse potential in a clandestine setting, and due to the simplicity of the structures of these compounds, it could easily be modified to pursue analog production. This method was investigated by both direct and modified reproduction of the synthesis to produce non-controlled analogs. While direct reproduction was found to have limited utility, minor modifications expected of operators with limited knowledge were found to easily produce the compounds of interest in higher purities. The analysis of products and impurities in this work has the potential to aid forensic identification of this synthetic strategy and provide literature resources for comparison.

Soil Mineral Analysis by Particle Correlated Raman Spectroscopy (PCRS): Optimized Dispersion and Double-Pass Raman Analysis

Savannah Brown, University of New Haven; Hannah Garvin, University of New Haven; Nicholas Gogola, University of New Haven; Gabrielle A. Messe, University of New Haven; Chase Notari, University of New Haven; Virginia Maxwell, PhD, University of New Haven; John A. Reffner, PhD, John Jay College of Criminal Justice; Peter R. De Forest, D.Crim, John Jay College of Criminal Justice; Christopher Palenik, PhD, Microtrace LLC; Peter de B. Harrington, PhD, Ohio University; Deborah Huck-Jones, PhD, Malvern Panalytical Ltd; Bridget O'Donnell, PhD, HORIBA Scientific; Andrew Whitley, PhD, HORIBA Scientific; Brooke W. Kammrath, PhD, University of New Haven

The analysis of soils has become a neglected field in forensic science due to the perception of current practices as being overly time-consuming, subjective, and ineffective. The potential value of soil evidence is incontrovertible, and thus several analytical approaches are being researched which can revitalize the use of soil as forensic evidence. Particle Correlated Raman Spectroscopy (PCRS) is a recently developed technique that has demonstrated value for forensic soil analysis, as it combines particle size distribution and morphological measurements with chemical identification by Raman spectroscopy. This technique is non-destructive, rapid, and can be automated; thus, PCRS may provide more detailed information than traditional methods about a soil sample.

Previous research identified optimized parameters for the identification of soil minerals via PCRS. Such parameters included the laser wavelength and power, magnification, exposure time, and grating. These parameters, which were initially optimized using 10 diverse minerals, have been applied to the 60 most commonly encountered minerals. Traditional figures of merit and response surface modeling of a multi-level experimental design was used to confirm the optimized Raman collection parameters. It was concluded that a double-pass Raman analysis, using two laser wavelengths, is needed to identify the majority of the soil minerals. Further, total Raman acquisition times of 1 second per particle were achieved which would enable a large number of mineral grains to be identified in a reasonable time period, thus making it possible to have robust datasets for subsequent statistical analysis. Prior research also began the process of parameter optimization for the vacuum dispersion of soil samples. Proper dispersion must be attained to minimize overlapping or clustered particles so that data can be accurately obtained for individual particles. Soil samples were prepared for analysis by washing alone, washing and sieving, or analyzed with no further preparation. Each sample was then dispersed onto glass analytical plates with various combinations of vacuum dispersion parameters (sample volume, vacuum pressure, and time for dispersal and settling). The dispersion was assessed microscopically via PCRS for reproducibility, uniformity, dispersion density, and the maintenance of particle morphological characteristics throughout the processing. PCRS was also used to analyze four morphological characteristics of these dispersed samples – area, diameter, ellipse ratio, and circularity – at three points on the analytical plate – center, 2 cm from center, and 3.5 cm from center.

The results from this research, optimized PCRS analysis parameters, will next be applied to a large collection of soil samples to create a robust database combined with statistical analysis to support the evidentiary significance of soil. Optimization of sample preparation, dispersal, and Raman analysis parameters are necessary to take full advantage of the discriminating power of PCRS for soil analysis.

Evaluating the Discriminating Power of Hair Amino Acid Ratios on Distinguishing Individuals using GCMS

Timothy Yaroshuk, University of New Haven; Alyssa Marsico, University of New Haven

Currently, the conventional methods of analyzing hair include microscopic hair comparison (MHC) and DNA analysis (nuclear and mitochondrial), with nuclear DNA analysis being the most individualizing. However, MHC is subjective

and nuclear DNA analysis is not always possible if not enough adequate cells are present. Non-synonymous amino acid changes in the hair protein sequence – resulting from single nucleotide polymorphism profiles that differ between each individual – can be exploited to offer alternative routes for hair analysis. Currently, proteomics has successfully exploited genetically variant peptides (GVP) content in hair to differentiate at least non-related individuals. However, proteomics is complicated and requires the GVPs to remain intact. Analyzing amino acid content is an alternative method that may simplify the analysis. It has been demonstrated that analyzing amino acid quantities has abilities to differentiate people based on general class characteristics of sex, age group, and geographical origin. A study by Macri et al., analyzing amino acid ratios of two individuals with morphologically similar hair, discovered 15 amino acid ratios that differed. Expanding on this study, this research will evaluate the discriminating power of using hair amino acid ratios to differentiate individuals with a focus on increasing the sample size and diversity. The purpose of this study is to develop a method that can supplement MHC to reduce subjectivity of hair analysis in case DNA analysis cannot be conducted.

Hair samples were obtained from 9 consenting individuals and were anonymized. Plucked hairs were obtained except for 1 individual where hair cut samples were obtained. 2 separate hair samples were collected from the same individual where one set was dyed, and another was natural. Hairs were thoroughly washed with deionized water and methanol to remove surface contaminants. They were then prepared in triplicates where hairs were cut into smaller pieces and hydrolyzed with hydrochloric acid for protein digestion. Subsequently, the sample was filtered to remove unhydrolyzed hair pieces and an aliquot was dried under a gentle stream of nitrogen. L-norvaline was then added as an internal standard. After reconstituting in ethyl acetate, N,OBis(trimethylsilyl)trifluoroacetamide was added for amino acid derivatization. GCMS was used for analysis. A set of 11 standard derivatized amino acids, including L-norvaline, in ethyl acetate was also analyzed using GCMS for quantitation purposes.

Eight derivatized amino acids were detected from the hair samples in addition to a glutamic acid derivative. Identification was conducted by comparing sample retention times to standards as well as mass spectra library comparison. Quantitation relative to the internal standard was completed and 36 amino acid ratios were constructed from these quantities. Outliers for each ratio were determined by the Grubbs test for samples with more than 3 data points and outliers were discarded. Between the 10 hair samples analyzed, one-way ANOVA for all 36 ratios had $p<0.05$. Most individuals were differentiable using the post-hoc Tukey test for all ratios. For the samples that could not be differentiated with this method, 3D-PCA plot showed distinct clustering of individuals therefore allowing differentiation. Ratios between dyed hair samples were differentiable from undyed hair.

Detectors for GC Analysis of Ethanol in Blood

Tom Mancuso, PerkinElmer, Inc; Alan Gallaspy, PerkinElmer, Inc

Accuracy of compound ID/quantitation is of paramount importance in forensic analysis of Blood Alcohol. Bad data = Not Guilty. Dual column confirmation is the historical gold standard for compound ID in Blood Alcohol analysis by FID and is known for its high accuracy/robustness to produce high quality forensic data. Mass spec detection is the gold standard in many other industries such as environmental as the mass spectrum produced is a fantastic tool for compound identification especially of unknowns. Concept here is to combine both technologies to obtain the power of the mass spec for compound ID but utilize the FID for its robustness/ability to produce highly accurate and precise results.

Effects of Improper Ammunition Storage

Victoria Andre, John Jay College of Criminal Justice; Peter Diaczuk, PhD, John Jay College of Criminal Justice; Patrick McLaughlin, MS, John Jay College of Criminal Justice

Firearms are commonly used by law enforcement, hunters, and civilians either for protection, sport, or criminal activity. Ammunition is used in traditional firearms such as rifles and handguns, and consists of a cartridge case, propellant, a bullet (projectile), and primer. The two types of priming systems within the cartridge that are commonly used today are rimfire and centerfire cartridges. For research purposes, cartridges can be fired to observe and record their performance. To obtain velocity measurements, optical chronographs, or a Doppler radar unit such as the LabRadar are commonly used. As with all ammunition, there are malfunctions that can occur. Improper ammunition storage is one major factor that plays a role in ammunition failure. Work stress and environmental factors can cause ammunition failure to occur as well. Another form of improper ammunition storage is where the storage environment being used abruptly changes due to an external liquid or solid material submerging the ammunition. As a result, ammunition failure and damage to the firearm may occur if used. While this situation can commonly happen, little research is seen if and how ammunition would function when used in a compromised condition. This study observed how ammunition function is affected when it is improperly stored in the following materials: water, soil, Hoppes 9 Solvent (a gun bore cleaner that contains kerosene, ethyl alcohol, and ammonium hydroxide), and WD-40, (a water-displacing spray that contains aliphatic petroleum distillates and petroleum base oil). This research showed that although there were distressed cartridges that fired successfully with an expected average velocity measurement obtained, the majority of the cartridges exhibited changes such as ammunition failure, low velocity and anomaly velocity measurements. All of the distressing materials used had an adverse effect on the cartridges, with Hoppes 9 Solvent being the most detrimental on all cartridge sets. These findings encourage future work to be done to observe any additional detrimental effects of improper ammunition storage in an external liquid or solid.

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