



**Northeastern Association
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Forensic Scientists**

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Drug Chemistry Abstracts

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

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The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). Historically, SWGDRUG recommended minimum standards for the forensic examination of seized drugs and to seek their international acceptance. In light of the formation of the Organization of Scientific Area Committees (OSAC), SWGDRUG will continue to work as part of the international community to improve the quality of the forensic examination of seized drugs. In addition, the resources provided on the SWGDRUG website will continue to be updated and available.

This presentation will provide attendees with an update on SWGDRUG activities during the year 2017 and currently in 2018. Recent activities include revising Part IIIB of the SWGDRUG Recommendations relating to Methods of Analysis/Analytical Scheme for Identification of Drugs or Chemicals and the publication of a new supplemental document (SD-7) exemplifying the Construction of an Analytical Scheme. SWGDRUG is currently seeking public comment on both of these documents. A calculator is being validated to accompany SD-6 which details the extrapolation of net weight in conjunction with a hypergeometric sampling plan. Lastly, the SWGDRUG spectral libraries and monographs remain an extensively utilized resource within the forensic community and current status as well as future plans will be reviewed.

Using the QuEChERS System for Sample Preparation for Delta-9 THC Analysis in Food Samples

Wendy Alger, Vermont Forensic Laboratory

QuEChERS (Quick Easy Cheap Effective Rugged Safe) was originally developed by the United Chemical Technologies (UCT, Levittown, PA, USA) as a sample preparation technique for testing pesticides in food samples. It was further developed by the RESTEK Corporation (Bellefonte, PA, USA) for testing cannabinoids in food samples. QuEChERS includes an extraction step followed by two clean-up or wash steps that remove sugars, fats, organic acids, steroids, proteins, pigments, and excess water to provide a clean sample prior to analysis by Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry. The Vermont Forensic Laboratory purchased testing materials from RESTEK for the development of a cleaner, more efficient, and faster extraction method to test for delta-9 tetrahydrocannabinol (THC) in food samples submitted as criminal casework. In collaboration with the New Hampshire Forensic Laboratory and RESTEK, utilizing the RESTEK procedure "Extraction Method for



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Cannabinoid Analysis in Edibles: Too Much of a Good Thing" the developed method was successfully transferred to seized drug casework in Vermont. QuEChERS is a modified liquid-liquid extraction method using acetonitrile as the solvent. This solvent is miscible with water and is salted-out to form separate layers during the digestion step. The polar cannabinoids are soluble in the solvent and migrate to the top layer during the centrifugation step. QuEChERS also utilizes clean-up steps using dispersive solid phase extraction (dSPE) sorbents to remove the oils, fats, and sugars from the extraction supernatant before adding it to the final test vial while maintaining the drug of interest, THC. As a note, please be aware that these clean-up steps also remove cannabinol (CBN) and other acid cannabinoids. This presentation will illustrate the steps involved in using this product and the differences seen in the GC/MS results of these samples vs the traditional extraction method.

Investigation of Artifact Formation through GC/MS Analysis of Controlled Substances

Branden Brunner, MSFS, F-ABC and Caroline Mackay, MSFS, F-ABC, NMS Laboratories, Willow Grove, PA

As forensic chemists, identifying any illicit (or controlled) components in complex mixtures is a requirement for thorough testing. It is important for chemists to make the correct identification of controlled and non-controlled substances. Situations occur in which compounds are being “artificially” formed through sample preparation and analytical analysis techniques, specifically gas chromatography-mass spectrometry. Sample preparations and analytical techniques include extraction solvents, internal standard addition, pH adjustments, and gas chromatographic injection port temperatures. Those compounds that are observed in the analysis data, but are potentially formed during analytical manipulation, are referred to as artifacts. Artifacts differ from impurities in that impurities are byproducts formed during drug synthesis, making them inherently present prior to sample submission. This byproduct formation occurs during both illicit drug production as well as pharmaceutical drug synthesis. As chemists, we do not want to report compounds that are not truly present in case samples, which makes being aware of artifacts extremely important. Many times, these artifacts and impurities can be a higher Drug Enforcement Administration (DEA) or state schedule than the main parent compound. Specifically, this is seen with methamphetamine, cannabidiol, and fentanyl. This project investigates major artifacts being seen at NMS laboratories. The presence of artifacts is discussed in terms of various sample types, drug types (i.e. functional groups), and possible mechanisms for artifact formation. It is assumed that artifacts are common findings in seized drug samples in many testing laboratories.

Optimization of a Gas Chromatographic-Mass Spectrometric Method for the Analysis of Thirty Fentanyl Analogues

Delilah DeWilde, Thomas Brettell, Ph.D., D-ABC, Cedar Crest College, and Matthew Wood, Ph.D., Ocean County Sheriff's Department

Forensic drug chemists are responsible for reporting the composition of commonly seized drugs. The recent opioid epidemic has resulted in many commonly seized drugs such as heroin, cocaine, and marijuana being observed laced with fentanyl and fentanyl analogues. The DEA in a 2017 report about NPS drugs reported a 117% increase from 2016 in opioid drugs, of which fentanyl accounted for 66% of those drug identifications. The fentanyl epidemic includes fentanyl analogues that are being synthesized in clandestine laboratories, some of which may not be considered illegal



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due to lag time for legislation. The close structural similarity of these analogues makes the identification challenging. Drug chemists typically use gas chromatography-mass spectrometry (GC-MS) to identify these compounds in drug seizures.

This presentation will discuss a confirmatory method using GC-MS that has been developed for the analysis of 30 fentanyl analogues. The fentanyl standards included in optimizing this method were fentanyl, crotonyl fentanyl, acetyl fentanyl, butyryl fentanyl, para-fluorofentanyl, meta-fluorofentanyl, ortho-fluorofentanyl, cis-3-methyl fentanyl, trans-3-methyl fentanyl, para-fluorobutyryl fentanyl, meta-fluorobutyryl fentanyl, ortho-fluorobutyryl fentanyl, acryl fentanyl, valeryl fentanyl, isobutyryl fentanyl, carfentanil, ocfentanil, cyclopropyl fentanyl, alfentanil, sufentanil, remifentanil, W-15, 4-ANPP, para-methoxybutyryl fentanyl, thiofentanyl, -hydroxythiofentanyl, -methyl fentanyl, -methyl fentanyl, and furanyl fentanyl. This method utilized a Thermo Scientific Trace 1310 gas chromatograph outfitted with two FID detectors and coupled with a Thermo Scientific ISQ LT single quadrupole mass spectrometer to improve resolution between fentanyl standards utilizing a split injection. The optimized GC-MS conditions were developed by analyzing variables such as the split ratio, injection temperature, injection volume, and the oven program. This development also investigated the employment of complementary dual columns. The primary column employed was comprised of a 5% diphenyl: 95% dimethyl polysiloxane stationary phase (30 m x 0.25 mm x 0.25 μ m). The other two columns utilized for analysis were a 5% diphenyl: 95% dimethyl polysiloxane column optimized for amines (30 m x 0.25 mm x 0.5 μ m) and a trifluoropropylmethyl polysiloxane column (30 m x 0.25 mm x 0.25 μ m). The columns were compared using calculated linear retention indices. The column with a trifluoropropylmethyl stationary phase provided complementarity data for standards containing fluorine compounds that originally had elution times overlapping with non-fluorinated compounds using the primary column.

The development of a confirmatory method using GC-MS for the analysis of fentanyl analogues has provided contributory insight for the implementation of a split method, an intricate oven temperature ramp rate, and results from atypical columns for the use of drug analysis. The procedure can be utilized to accurately identify 30 different fentanyl analogues. The linear retention indices and retention times from the developed method results can be referenced for future research.

Analysis of Fentanyl by Gas Chromatography/Solid-State Infrared Spectroscopy

Emily Prisaznik, MSFS, Lindsay Welch, Ph.D, and Thomas Brettell, Ph.D, ABC-D, Cedar Crest College

An investigation of the analysis of fentanyl and 10 fentanyl analogues by gas chromatography/solid-state infrared spectroscopy has been performed. Gas chromatography was performed using an Agilent 6890N gas chromatograph. Infrared (IR) Spectroscopy was performed on a Spectra-Analysis DiscovIR-GC using an operation system with Thermo Galactic GRAMS 8.0. The cryofocused disk was cooled with liquid nitrogen to -40°C and the dewar was set to 20°C for all experiments. GC-solid-state IR spectroscopy has shown to be capable of identification of fentanyl and ten of its analogues. The method, using the conditions in this study, is capable of producing a reproducible quality solid-state IR spectrum from injection of 1 μL of a 1 mg/mL methanolic solution of sample onto a chromatographic column. Using the chromatographic conditions in the method developed most of the fentanyl analogues can be



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separated such that infrared spectra can be produced for identification of the compound. Most of the fentanyl analogues were separated with 99% confidence ($s = 3$ sd.) except for three pairs, isobutyryl fentanyl/fentanyl, fentanyl/acryl fentanyl and butyryl fentanyl/cyclopropyl fentanyl. Multiple injections of the fentanyl compounds resulted in reproducible retention times (standard deviation = 0.023 and a CV % of 0.012 %). The data also produced spectra containing absorption peaks with an average standard deviation = 0.272 and an average CV % = 0.022, showing good reproducibility for the IR spectra. The solid-state IR spectra of fentanyl and the ten fentanyl analogues analyzed in this study were quite similar but were easily discriminated by comparison of the fingerprint region of the spectrum. The amide region (1650 cm^{-1}) clearly showed different absorption peaks and pattern as well as the region for the C-H stretches of the aromatic and alkyl region ($2700\text{ cm}^{-1} - 3050\text{ cm}^{-1}$) and the spectral region between 1200 cm^{-1} and 1500 cm^{-1} . The linear retention indices for the fentanyl compounds were calculated using this retention index standard mixture. Overall GC-solid-state IR spectroscopy is a viable and reproducible method for the analysis and identification of fentanyl and fentanyl analogues that may be found in seized drug material.

Sky High: Sorbent-Facilitated Headspace Mass Spectral Analysis for the Detection and Identification of Plant-Based Legal Highs

Meghan G. Fogerty, M.S. and Rabi A. Musah, Ph.D., SUNY Albany

Due to both the increase in legislation against the use of opioids and prescription drugs, as well as user perceptions that there are products that can be safely used to wean themselves off of other more highly addictive scheduled substances, there has been an increase in the use of legal-high psychoactive plant materials such as Damiana (*Turnera diffusa*), Wild Dagga (*Leonotis leonurus*), and Marshmallow (*Althaea officinalis*). The United Nations Office on Drugs and Crime has identified twenty plant species as “plants of concern” because of their increased use as “legal highs”. It is difficult for law enforcement to identify these materials and to distinguish them from innocuous plant material (i.e. herbs, spices, and food). It would be valuable to develop a technique that would permit both crime scene analyst and field agents to detect and identify plant-based “legal highs” in an open space. This is because most of the psychoactive “legal high” plant material sold in the US is sourced internationally and arrives in large shipping containers at US ports of entry. It would be beneficial to be able to confirm the veracity of the labels listed on packages of these imported goods, especially if importation of these products were to be outlawed. This project seeks to address this issue by developing a technique that would allow detection of plant-based “legal highs” through the recognition of diagnostic small-molecules signatures in the headspace of the plant material. It is hypothesized that headspace volatiles of plant-based “legal highs” can be concentrated on sorbent material (e.g. SPME fibers) which can be subsequently analyzed by a mass spectrometric technique (e.g. direct analysis in real time-high resolution mass spectrometry, DART-HRMS) to identify and differentiate “legal high” plant material. The first step in this method was to concentrate the headspace volatiles of the plant material onto solid-phase microextraction (SPME) fibers, followed by analysis using DART-HRMS. The fibers were exposed to the plant material for 30 min and this sampling was performed in replicates of ten. Multivariate statistical analysis processing (Kernel Discriminant Analysis) of the DART-HRMS data showed that the headspace signatures could be used to accurately identify “legal high” plant material at the species level, with a leave one out cross validation of greater than 95% and an external validation of 100%. DART-HRMS permitted rapid analysis and produced diagnostic chemical fingerprints, but it was limited in enabling identification of the detected molecules. Therefore, complementary techniques are needed for further identification of the molecules in the headspace. Thus, the plant material was placed in empty thermal desorption tubes and analyzed using thermal desorption coupled with gas chromatography-mass spectrometry which enabled the identification of diagnostic



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biomarkers including p-cymene in Damiana, caryophyllene oxide in Wild Dagga, and dihydroactinidiolide in Marshmallow. Through the creation of a database of headspace mass spectra and using these techniques in tandem, law enforcement agencies can efficiently and safely survey crime scenes and shipping containers for the detection and identification of plant-based “legal highs”.

A LADI doesn't lift a finger: Laser ablation direct analysis in real time imaging-mass spectrometry (LADI-MS) of psychoactive small molecules in latent fingerprints

Kristen L. Fowble, B.S., Cameron Longo, B.S., and Rabi A. Musah, Ph.D., SUNY Albany

Traditional fingerprint analysis uses dusting powders and cyanoacrylate fuming, among other methods, to reveal the physical pattern of the fingerprint ridges. However, these techniques neglect vast amounts of chemical information that can also be contained within a deposited latent fingerprint, such as various materials with which an individual has come into contact. Some approaches have been developed to extract the chemical components of a deposited fingerprint, but most require the destruction of the print by swabbing its surface. Imaging mass spectrometry, a technique that maps the spatial distribution of detected analytes in the sample of interest, provides a means of retaining the fingerprint image while also providing chemical information. Most conventional imaging mass spectrometric techniques require sample preparation steps and the use of solvent, high vacuum, and/or the application of a matrix. These steps can be time-consuming and can contribute to casework backlogs as well as complicate the ability to detect certain analytes of interest, particularly small molecules.

It is demonstrated in this study that using laser ablation direct analysis in real time imaging-mass spectrometry (LADI-MS), small-molecule spatial distribution maps can be acquired for endogenous compounds in latent fingerprints while simultaneously detecting psychoactive materials within them. The ability of the technique to detect small molecules within latent fingerprints was demonstrated using prints that were deposited after exposure of hands to psychoactive “legal high” plant material (Piper methysticum), cocaine, and pseudoephedrine. LADI-MS revealed spatial distributions of endogenous cholesterol, squalene and fatty acid esters localized to fingerprint ridges, while providing confirmation of exposure to the psychoactive small molecules cocaine, kavain and other kavalactones (derived from P. methysticum), and pseudoephedrine. The spatial distribution mapping of cholesterol in a lifted print on the non-conductive adhesive side of tape was also accomplished. The ion images obtained of the endogenous compounds revealed the fingerprint ridge pattern necessary for potential identification of an individual. The newly developed LADI-MS technique can be applied to a number of forensic sample types including latent fingerprints, without the need for sample pretreatment.

Reproducible Analysis of Fentanyl and Its Analogs with a Fieldable Mass Spectrometer

Gwen Bone, 908 Devices, Inc.

Fentanyl and its many analogs represent an increasingly frightening threat to public safety. Due to their extreme potency, accidental overdoses have been skyrocketing, and first responders dealing with the aftermath are put at



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significant risk of accidental exposure. Synthetic opioids' potential for use as a weapon of mass destruction in terrorist incidents has also not escaped the notice of many in the

security and defense communities worldwide. A handheld ion trap mass spectrometer with an atmospheric pressure ionization source has been configured with in-source CID to detect this class of compounds with ng-level sensitivity and high specificity. Automated detection algorithms for identification as well as classification are evaluated against neat material, as well as dilute cuts consistent with street drugs.

Excellent selectivity across a set of 9 different fentanyl analogs evaluated were observed independent of salt form (hydrochloride, oxalate, citrate, or free-base) providing unique identification of the base form of all the analogs. Detection limits for all the measured fentanyl analogues were in the 25-100ng range. In-source CID was crucial for differentiating between isobaric analogs such as Butyryl fentanyl and cis-3-methylfentanyl. These isobars, which cannot be distinguished by parent molecular ion, can be clearly distinguished based on the multiple fragment masses. Simultaneous detection of multiple drug mixtures such as Fentanyl in the presence of 100X Heroin, and fentanyl analogues at 0.1% of a mixture are demonstrated. At doses 100X the LOD and above, the instrument recovers a true negative baseline after 1-2 thermal desorption cycles which can be automatically managed by instrument algorithm. Independent test data across five separate systems show very similar detection thresholds.

Synthetic opioids often differ by a single, relatively inert modification from a controlled substance. As synthetic opioids continue to evolve Fentanyl-analog classification based on molecular structures, neutral loss and dissociation pathway appears to be able to detect novel analogs that aren't directly in the target library. Handheld MS repeatably demonstrates highly sensitive and selective fentanyl analysis with CID, a promising approach for novel analog detection.

Rapid NMR Spectroscopic Identification of Opioids

Nicole Homburger and Ling Huang, Ph.D., Hofstra University; Megan Chambers, B.S., SUNY Albany

Due to the ongoing opioid pandemic, it is imperative to develop new tools for forensic analysts to rapidly identify traditional and designer opioids. Many designer opioids are highly potent and often used as adulterants to or substitutes of heroin (Breindhal et.al, 2016; Taffe and Olive, 2018). Sometimes fentanyl was the purported main ingredients and fentanyl “cousins,” or opioids which are structurally similar to fentanyl, are the substitutes. In nearly every street sample of heroin, fentanyl or designer opioids, caffeine or acetaminophen were used as cutting agents (Gufour et.al, 2015).

We propose using proton NMR as a screening agent for designer opioids. Previous studies in our lab have used NMR for rapid spectroscopic identification of cannabinoids (Fowler et. al, 2015, Marino 2015). A similar study by another lab employed DOSY and QNMR to assess the impurities and cutting agents of street heroin samples (Balayssac et.al, 2014). In our current study, standards of each opioid were dissolved directly in NMR solvent. Additionally, mock street samples of the opioids were prepared by combining one opioid standard with caffeine, acetaminophen, and glucose, which are common cutting agents of opioids.



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With H1 NMR and proton-proton correlation spectroscopy or COSY NMR, we found that each opioid produces NMR spectra unique to that specific compound, and an opioid spectrum could be isolated even from a solid mixture mimicking street drug composition. NMR is nondestructive, and all the sample used may be preserved for further investigation with conventional tools such as FT-IR, GC-MS or LC-DAD. Because the opioids are immediately dissolved in NMR standard, the health risk to the analyst associated with opioid aerosol exposure is minimized. No prior physical separation of the opioid from the mixture is required for NMR analysis. COSY has been proved to be a rapid method with minimal sample preparation. It has increased resolution because the signals are spread along the orthogonal direction on a 2-D map, making it easier to identify compounds within a mixture (Fowler 2015). We found that each opioid produced a unique COSY NMR spectrum, which could be used as a fingerprint for identification. Within an hour, close designer opioids can be effectively differentiated with the identification of a handful of signals on proton or COSY spectra generated from several milligrams of sample. Semiquantitative analysis may be performed with H1 NMR without use of an internal standard. NMR may also be used to predict or to identify future opioids, as many of the compounds have peaks within the same regions.

An overview of the Technical Working Group for Seized Drug Analysis (TWG DRUG) in New York

Eric Sorrentino, M.S., Suffolk County Crime Laboratory

The Technical Working Groups (TWGs) are the foundation of a robust infrastructure that makes up forensic science in New York. These groups are comprised of experts in each discipline from each forensic laboratory that is accredited by New York State.

The TWGs provide discipline specific expertise to the New York Crime Laboratory Advisory Committee (NYCLAC), the Commission on Forensic Science (CFS), and its DNA subcommittee in order to improve quality and effectiveness of forensic science in New York.

This presentation will provide and explain:

- In-depth overview of the infrastructure surrounding the TWGs
- Examples of expertise we may be asked to provide
- Information and data sharing through TWGs
- Projects that have been developed in the TWGs and implemented in the forensic laboratories of NY

Current ongoing projects that are being worked on at the moment:

1. Creation of large scale validation reports.
2. Draft a white paper for eliminating pure weight drug laws in New York.
3. Best practices for Statistical Sampling/weight estimation schemes.
4. Best practices for weighing of vegetative material.



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5. Newly controlled drugs and their analytical schemes.
6. Best practices for evidence inventory (for evidence storage locations).
7. Uncertainty of measurement (aggregate weight, pure weight, pill counts).
8. Annual compilation of drug identifications to mirror the annual report done by currently in TWG TOX.



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Forensic Biology Abstracts

The Detection of Male DNA Using Y-STRs in Post Coital Samples of Vasectomized Males

Sarah Lighthart, Janine Kishbaugh, MSFS, Cedar Crest College

In the United States, 1 in 6 women will be raped. The perpetrators in the crimes were male 94% of the time (1). In the case of a male perpetrator and female victim, there is the potential for deposition of semen in the vaginal cavity; however, not all cases with semen present will have sperm present. A study reported that approximately one-third of men that commit sexual crimes suffer from some form of sexual dysfunction, which can mean a decrease or lack of sperm (2). Lack of sperm vastly decreases the amount of DNA present for subsequent testing. This study aimed to establish how long after an assault a viable DNA profile might be obtained from samples without sperm. Using post-coital intervals of 1, 3, 5, and 7 days from five separate vasectomized couples, Y-STR analysis was performed. The resulting DNA profiles were analyzed to determine the total number of male-specific alleles present. After one day, the results from 5 couples contained 20.6 of 23 alleles (89.5%). The average number of alleles found after three days was 4.4 (19.1%). An average of 3.2 (13.9%) alleles were present after five days. After seven days, the average number of alleles identified was 0.8 (3.4%). Thus, based on the current results the time frame to detect nearly full DNA profiles from samples without the presence of sperm is approximately one day post-coitus. Future work utilizing low copy number (LCN) methods may increase the amount of alleles obtained from longer intervals. 1. NIBRS. Sex offenses reported via NIBRS in 2013. National Incident Based Reporting System.

What Can be Done with a Bag of Bone: Utilizing DNA and Osteological Analysis on Human Skeletal Remains from the Flevaeis Plot in Rhodes

McKenna Lohr, B.S., Lisa Ludvico, Ph.D., Forensic Science and Law Program, Duquesne University

When human skeletal remains are found at a crime scene or archaeological site, there are several forms of analysis that can be performed to determine identifying characteristics of the individual(s), including osteological and DNA analysis. In the case of the Flevaeis Plot in Rhodes, two graves were uncovered containing six to seven skeletons each. The dates of the objects buried with the remains range from 3200 BCE to 650 CE, indicating potential occupation of this site by several cultures. Osteological analysis was performed on the remains brought to Duquesne University to determine size/intactness of each bone, the type of bone, side of origin, and in some cases sex, age, and overall health. Based on this data, it is estimated there are twelve bones total from at least four individuals, two males and two females. To determine if this estimation is accurate, DNA analysis was performed in conjunction with osteological analysis. A decalcification and extraction protocol obtained from the Human Identification Center at the University of Northern Texas was utilized to isolate human DNA from the skeletal remains. A mitochondrial DNA (mtDNA) mini primer set created by the Armed Forces DNA Identification Lab (AFDIL) was used on the extracted DNA to amplify and sequence hypervariable region 1 (HV1). This data was used to determine haplogroups which can be used to differentiate between the skeletal samples as well as determine maternal lineage.



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Quantifying neuropeptide expression to estimate pain endured by an individual prior to death

Emily Neverett¹, Lawrence Quarino², Ph.D. and David San Pietro¹, Ph.D., ¹Forensic Science Program, University of New Haven, ²Department of Chemical and Physical Sciences, Cedar Crest College

In cases of wrongful death, the sentencing of an individual convicted of homicide can be based subjectively on how much pain the victim is estimated to have suffered. This study aims to develop a simple quantitative PCR assay that could estimate the possible pain endured by an individual prior to death by measuring gene expression levels of two neuropeptides, TAC1 (encodes the peptide Substance P) and the Substance P receptor. Substance P and the corresponding Substance P receptor are neuropeptides that assist in the transmission of the inflammatory response that is stimulated by tissue damage. Individuals suffering from more chronic pain, such as illness or alcoholism, are hypothesized to have greater expression of Substance P and receptors due to the prolonged inflammatory response. Individuals suffering from acute pain, such as a motor vehicle accident or homicide, are hypothesized to have less expression of Substance P and receptors.

Eleven whole blood samples were collected from deceased individuals by The Office of the Chief Medical Examiner in Connecticut. The manner of death was classified into two different cohorts: acute (homicide, suicide, or motor vehicle accident) and chronic (illness or drug toxicity). Total RNA from whole blood samples were extracted with the Qiagen® RNeasy Mini Kit. Total RNA was quantified using the Qubit 3 Fluorometer (ThermoFisher Scientific). The Invitrogen™ SuperScript III™ Reverse Transcriptase was used to convert mRNA transcripts to cDNA. Custom primers for TAC1 and Substance P Receptor were purchased from ThermoFisher and SYBR™ Green Master Mix was purchased from Applied Biosystems™. qPCR was performed using the Applied Biosystems™ 7500 qPCR instrumentation and supplied software. All acute and chronic samples were quantified in triplicate.

The results of this assay were consistent with the hypothesis that an individual suffering from chronic pain would have greater expression of TAC1 and Substance P Receptor. There is a statistically significant difference in expression levels of both TAC1 and Substance P Receptor between the chronic and acute cohorts. This qPCR assay could be used as a more objective method of evaluating pain endured by an individual prior to death. Further research is aimed at determining the rate of degradation of the transcripts as well as the sensitivity of the assay.

Forensic Application of microFLOQ® Direct Collection Device

Reena Roy, Ph.D., Shayna Gray, Sara Walton and Teresa Tiedge, Forensic Science Program, The Pennsylvania State University

Body fluids such as blood, saliva and nasal secretions are often collected as evidence at crime scenes. In these cases, specimens such as an insects or bullets, collected at the crime scene, can be useful in aiding the investigation. In forensic cases sometimes a female victim remains missing. DNA profiles obtained from bloodstains or other body fluids retrieved from the crime scene cannot be compared to her due to lack of reference samples. Papanicolaou test slides collected routinely from women are preserved in the laboratory. Since these slides are prepared from intimate source body fluid, and collected from within the individual's body, they are accepted as reference samples by the judicial system.



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Standard methods currently used for generation of DNA profiles require extraction, purification, and quantification of DNA before they are subjected to thermal cycling and DNA profiling. These are labor intensive, time consuming steps. In this study, direct amplification of various types of body fluid was performed using microFLOQ® Direct collection device from COPAN Italia, Brescia, Italy. The swabs were used to collect minute amounts of blood from mosquito midgut, saliva, nasal secretions from humans, cells from Papanicolaou stained slides, and, from touch evidence such as cell phone and bullets. Samples were amplified using PowerPlex® Fusion 6C System from Promega Corporation and the Investigator 24plex GO! Kit from Qiagen. The tips of the devices containing the body fluids were left in the amplification reagents during the amplification steps.

Complete and concordant STR profiles were successfully generated within a very short period of time. This study demonstrates that minute amounts of body fluids can be amplified directly using the microFLOQ® Direct collection device.

Effect of organic acid on false positive results using immunochromatographic assays

Catherine O. Brown, M.S.F.S., Megan M. Foley, M.S.F.S. and Heather E. McKiernan, M.S.F.S., Center for Forensic Science Research & Education; Phillip B. Danielson, PhD, University of Denver

With an increasing sensitivity of genetic testing methodologies targeting trace levels of genetic material, serological techniques have failed to demonstrate adequate detection limits, prompting a shift in current forensic biology workflows. However, serological screening and identification of body fluids can greatly aid an investigation. The current methodology most routinely applied to forensic casework for body fluid detection and identification are immunochromatographic assays. Manufacturers of these tests include Seratec®, Abacus Diagnostics®, and Independent Forensics, marketing assays for blood, semen, saliva, and urine detection. However, it is important to understand the limitations of such techniques to most effectively elucidate test results. Regardless of the manufacturer, immunochromatographic assays function in a similar manner and therefore suffer due to equal limitations. For example, cross-reactivity of target biomarkers present in lower concentration in non-target fluids in addition to non-specific binding events have been demonstrated. The latter category was the focus of this study.

Common immunoassay tests utilized in forensic casework including ABACard® p30 and ABACard® HemaTrace® by Abacus Diagnostics®; RSID™-Urine, RSID™-Semen, RSID™-Blood, and RSID™-Saliva by Independent Forensics; and PSA Semiquant, HemDirect, and Amylase Test by Seratec® were evaluated. Four studies evaluating the presence of organic acid on false positive rates were assessed. Manufacturers' guidelines were followed for each assay evaluated. First, a pH series of 300 mM citric acid ranging from 1.78 to 12 was tested. Similarly, a pH series of 300 mM lactic acid was also assessed. False positive results were observed for each assay evaluated for both organic acids and were seen between a pH of 2 to 12 on various tests. False positive repeatability was observed by running assays in triplicate at the extremes of the pH range.

Second, a molarity series of citric acid ranging from 150 mM to 9.3 mM at pH 4 was evaluated across all assays. It was determined that organic acid concentration did not affect the occurrence of false positive results, with false positives observed at 9.3 mM on various tests. A third study evaluated the presence of organic acid in manufacturer-specific buffer at a range of pH values. Samples were prepared using 300 mM citric acid in a 1:1 ratio with buffer. pH was recorded before and after the addition of the organic acid. All assays exhibited a false positive test result. And lastly, the pH of deionized water was manipulated to ensure the false positive results observed were due to the presence of organic acids and not due to acidic/alkaline pH. The acidic and alkaline extremes in addition to a midpoint pH were



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assessed on all assays. All deionized water solutions produced negative test results as expected, indicated the role of organic acids in generating non-specific binding events.

Additionally, common household products containing organic acids were evaluated on all assays for the presence of false positive results. It should be emphasized that based on the findings exhibited in this study, immunochromatographic tests display presumptive findings due to lack of specificity. A proposed mechanism causing the false positive results will be discussed.

Post- Conviction DNA testing: A Laboratory's Perspective and Participation in a Multi-Agency Collaboration for the Identification and Evaluation of Post-Conviction Cases

Lynn Schneeweis and Kerry Collins, Dorothea Sidney Collins and Kristen Sullivan, MA State Police Crime Laboratory

In 2012, Massachusetts passed Chapter 278A, a post-conviction DNA and forensic analysis law. As a result, in 2013, five criminal justice agencies within MA, the Committee for Public Counsel Services (CPCS), Middlesex County District Attorney's Office (MCDAO), Middlesex Superior Court Clerk's office, Suffolk County District Attorney's Office, and New England Innocence project (NEIP), formed the Massachusetts Working Group (WG) on Post-Conviction Testing Assistance. This working group received funding through a Post DNA Testing Assistance award for the purpose of identifying DNA based innocence claims in serious violent felonies, locating and testing evidence in said cases, and adopting best practices for inventory and storing evidence. In 2014, the working group recognized the potential benefits of expanding this partnership and invited the MA State Police Crime Laboratory (MSPCL), Boston Police Laboratory, and Suffolk County Superior Court Clerk's office to join. The newly expanded working group subsequently received funding under the 2014 DOJ Post Conviction Testing of DNA Evidence to Exonerate the Innocent. The Working Group's goal for these funds included conducting comprehensive evidence inventories in participating counties, creating a best practices guide for evidence management, and conducting a review of pre- 2000 cases at MSPCL where microscopic hair comparison was performed.

The MSPCL's initial contribution to this project focused on providing technical expertise on evidence handling and storage procedures. Laboratory personnel served as resources for police departments conducting evidence room inventories and provided guidance for inquiries as to improving the storage and packaging of pieces of evidence previously subjected to less than ideal conditions. Additionally, the MSPCL provided technical resources for the construction of a "best practices" guide for evidence management to assist the legal community.

The primary role MSPCL undertook in this project was to identify forensic cases within MA where microscopic hair comparisons had been performed and resulting associations between items of evidence and known samples were made. Criteria were determined by the WG to prioritize which categories of these cases would be identified for further review by CPCS and NEIP to assess for potential post- conviction DNA testing. A twenty-year time frame of cases for review was established and over 20,000 serology cases were administratively screened to determine those in which hair was examined and associations were made.

During this project, the Working Group convenes regularly to evaluate progress towards these goals. Through this collaboration, the WG expanded the scope of their work to include training on post-conviction cases for attorneys, developing a post-conviction template motion for DNA testing, drafting a discovery materials agreement between the MSPCL, MCDAO, and CPCS, and creating a DNA technology timeline for attorneys litigating post-conviction cases.

The authors will discuss the achievements of the Working Group's efforts over the past 5 years since its inception. Specific emphasis will be on the role of the MSPCL's collaboration with the legal community in the identification and evaluation of the suitability of cases for post-conviction DNA testing.



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Partial Match in NY State CODIS Database helps solve 20 year old Homicides

Robert Baumann, Suffolk County Crime Laboratory

In late 1993 and early 1994, three females were found murdered in Suffolk County on Long Island under similar circumstances. RFLP analysis confirmed that biological evidence collected from two of these women was from the same person. Subsequent testing with STRs was performed and the profile was entered into CODIS in 2000. Over the years, dozens of exemplars were submitted for comparison with no resolution to these cases. Finally, in 2013, we were notified that a partial match was made to a convicted offender in the New York State database. The investigation gained momentum and in 2014 an arrest was made. The presentation will highlight the biological evidence that helped the prosecution win a conviction in 2017.

Reduction of Sexual Assault Evidence Backlog by Implementation of a High Throughput Automated Differential Digestion Process

Amanita LeMon, Helena Wong, City of Oakland Police Department Criminalistics Division

Forensic laboratories are being faced with an increase in public and legislative demand for the timely examination of sexual assault evidence (SAE). Limited staffing and the time consuming and labor intensive work required for a differential digestion process (for the separation of sperm and non-sperm DNA) are common factors behind the number of requests for SAE analyses becoming a backlog. The demand for an increase in request processing, and a simultaneous decrease in expected turn-around time of SAE, led the Forensic DNA Unit at the Oakland Police Department (OPD) Crime Lab to transition to an automated and high throughput differential digestion process. While many areas of DNA analysis have adopted automation, the differential digestion process has remained a sample limited, time consuming, manual task for many laboratories.

An automated differential digestion protocol using a selective degradation step was developed by the OPD Crime Lab for the VERSA (Aurora BioMed) 1100 liquid handler. The selective degradation technique replaces labor intensive wash and centrifugation steps by using the enzyme DNase1 to digest residual non-sperm DNA from the sperm fraction prior to lysis of the sperm cells. The automated protocol utilizes 96-well plates for high throughput of samples and incorporates the preparation of microscope slides for the observation of sperm. By using this automated differential digestion process, the SAE kit backlog at OPD Crime Lab was eliminated in less than a year, without compromise to the quantity and quality of the DNA obtained or to downstream processes.

Construction of an Allelic Ladder for an *Odocoileus* STR Multiplex

Jolene Strand, David San Pietro, Ph.D. and R. Christopher O'Brien, Ph.D., University of New Haven; Brian Hamlin and Mary Burnham Curtis, Ph.D., USFWS; Erin Meredith, California Department of Fish and Wildlife



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Short Tandem Repeat (STR) analysis is a useful tool in deer poaching cases. Like with human cases, it can provide linkages between evidence items. However, there is no consistent STR multiplex used by all wildlife forensic crime labs. Currently, a STR multiplex for the genus *Odocoileus* (white-tailed deer, black-tailed deer and mule deer) is being developed through a collaboration of federal, state and private labs for this purpose. There is also a plan to develop a database where DNA profiles from cases can be uploaded in hopes of discovering linkages between cases from different labs that is currently not possible. However, for the creation of a shared database for case samples, a standardized reference allelic ladder is also necessary to ensure concordance between labs. The objective of this research is to develop this allelic ladder.

Allele frequencies will vary between species of deer as well as between their different geographic populations across the United States. Using the *Odocoileus* STR multiplex, over 1500 samples from the various deer populations were run on an ABI 3500 genetic analyzer with POP7 polymer with a 50 cm capillary array to capture the most common alleles and collect their frequency data. All DNA and primers were provided by US Fish & Wildlife Service National Forensic Laboratory. The QIAGEN Multiplex PCR kit was used for PCR set up of 10uL reactions and the PCR protocol provided along with the *Odocoileus* multiplex was used for amplification. This consisted of a 15-minute initial denaturation at 95°C followed by 28 cycles of 95°C for 30s, 56°C for 90s and 72°C for 60s with a final 10 minute extension at 72°C. Based on this data, samples have been chosen for incorporation into the ladder. First, a ladder for each individual locus is being constructed, with individual samples being amplified following the same PCR amplification protocol in 10uL singleplex reactions. Next, 1uL from each sample was combined, diluted to 10mL with sterile water and amplified in a second reaction, resulting in an allelic ladder for that particular locus. The *Odocoileus* STR multiplex consists of 12 loci, including X and Y sex markers. The ladder of locus OHET256 V has been successfully completed with the next loci to be looked at are Ohe C186 J, OheC10 B and OheC143 F.

The STR DECoDE Multiplex for MPS: A Novel DNA Mixture Deconvolution Tool

Nicole Novroski, Ph.D., University of Toronto & Center for Human Identification, University of North Texas Health Science Center; August E. Woerner, Frank R. Wendt, Magdalena M. Bus, Michael D. Coble and Bruce Budowle, Center for Human Identification, University of North Texas Health Science

De-convolution of complex mixtures can be challenging. Various improvements in polymerase chain reaction coupled with capillary electrophoresis (PCR-CE) chemistry and downstream statistical analyses have been developed and implemented to attempt to better resolve two or more person DNA mixtures. However, CE electropherograms describe STR variation solely based on allele size and do not exploit the full genetic information contained within target markers to distinguish between or among component contributors.

Massively parallel sequencing (MPS) for typing forensically-relevant STR loci has dramatically impacted our abilities to identify allele diversity due to sequence variation within STR repeat and flanking regions. Studies have described STR sequence variation in large population groups and demonstrated that there are enormous amounts of diversity and complexity within the currently utilized STR markers for forensic genetic analysis. However, recent studies have demonstrated that some of the current core CODIS loci lack repeat or flanking region sequence diversity, minimizing the relative information gain via MPS for these STRs. Thus, novel STRs with increased sequence variation should be sought to facilitate mixture deconvolution.



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This presentation will highlight the *in silico* and empirical testing of 73 novel STRs from individuals comprising Caucasian, African American and Hispanic US populations. Sequence variation within these markers was assessed using MPS, STRait Razor 3.0, PANDAseq, and in-house analysis workbooks to evaluate their suitability for distinguishing component contributors and stutter from true alleles in simulated simple and complex DNA mixtures generated from empirical population sequence data. Additional population genetic analyses were performed to characterize these novel STR markers, which were selected based on their high heterozygosity, reduced allele length spread, and increased sequence diversity. The incorporation of additional forensically-useful markers into a novel STR panel in conjunction with current core CODIS markers will allow the forensic scientist to more effectively address the challenges of interpreting some complex mixture samples, increase the number of resolved profiles being compared to reference and suspect profiles, and expand the DNA database by increasing the number of forensic samples that may be uploaded.

Post-Conviction Testing: The Continuing Search for Answers

Jonathan S. Kui and Kendra Hardy, Department of Forensic Biology, Office of Chief Medical Examiner

Post-conviction DNA testing fills a vital role in science serving justice. The search for answers using modern DNA techniques must often continue beyond the answers that were achievable at the time, as questions persist and exoneration may remain a possibility. To do so is our responsibility as scientists and members of the communities in which we live and work.

The New York City Office of Chief Medical Examiner's Department of Forensic Biology is an accredited laboratory with approximately 150 analysts who process over 13,000 cases per year. In addition to homicides, assaults, robberies, firearms and property crimes under active investigation, and cold cases and missing persons identifications, the laboratory also tests evidence requested in post-conviction contexts, where testing may yield results that would support exoneration. Our laboratory's history of post-conviction testing began two decades ago, but its scope expanded dramatically as part of the efforts of the New York City Joint Working Group on Post-Conviction DNA Testing. This initiative was originally funded by a 2015 grant submitted by NYPD and the Innocence Project, and has since transitioned into the course of our standard workflow.

In the past three years, our laboratory has tested a wide variety of evidence types in post-conviction inquiries, as newly developed technologies have continued to increase the sensitivity and efficacy of our testing methods. Given the challenges of processing samples that are, in some cases, decades old and highly degraded, the use of modern techniques may represent one of the last remaining options in uncovering elusive answers in these enduring cases.

This presentation will provide an overview of our laboratory's post-conviction testing process, and will showcase some of the challenges and results from several recently tested post-conviction cases. We will also provide a glimpse into the future of post-conviction testing at OCME.

Genetic Genealogy as a New Tool for Forensic Investigation

Colleen Fitzpatrick, Ph.D., Identifinders International



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Genetic genealogy autosomal SNP testing has recently emerged as a new tool for forensic investigation, with the capability of solving cold cases sometimes decades old. Although genetic genealogy itself has developed rapidly over the last twenty years in response to the ever-increasing demand by consumers for personal genomic information, the use of DTC services by the forensic community has been prohibited by testing companies due to Fourth Amendment search and seizure issues. However, due to the decrease in cost of large scale SNP typing, it has become possible to circumvent DTC service providers by using independent laboratories to replicate DTC data. The coincident growth of GEDmatch, a third-party genetic genealogy database, has provided a means of using this replicated data to find DNA matches that can be turned into investigative leads for forensic cases. Perhaps the most notable instance where the methodology was successfully used to solve a cold case was the identification of the Golden State Killer in April 2018. The advantages of using genetic genealogical data for forensic casework are numerous, as are the related controversies. The success of genealogical analysis depends primarily on the composition and size of the database; unlike CODIS, it is not necessary for a suspect's DNA, nor the DNA of an immediate family member, to have been entered into the system at all. Yet it is just this dependence of the genealogical identification process on even distant family members that has created privacy concerns. While most US states prohibit familial matching using CODIS, there is no similar legal restriction on using genetic genealogy data that has been voluntarily uploaded to GEDmatch. This has prompted much discussion on privacy issues and informed consent among both the genealogical and forensic communities.



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Toxicology Abstracts

Validation of 15 Synthetic Cannabinoid Metabolites in Urine by LC-MS/MS,

Erika Phung, Boston University School of Medicine, Nichole Bynum, RTI International, Raleigh, NC, Megan Grabenauer, RTI International, Raleigh, NC, Sabra Botch-Jones, MS, MA. D-ABFT, Boston University School of Medicine, Boston, MA, Katherine Moore, RTI International, Raleigh, NC.

Introduction/Objective:

Synthetic cannabinoids (SCs) have emerged as a global health and societal issue. Their use has resulted in many adverse and life-threatening effects leading to poison control center calls and emergency department visits. Despite efforts to control synthetic cannabinoids, clandestine manufacturers continue to modify their structures to avoid legal consequences, creating an ever-changing analytical target for forensic laboratories. The objective of this project was to identify and quantitate 15 SC metabolites in urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analytes validated include AB-CHMINACA M4, FUB-PB-22 3-carboxyindole, AB-PINACA pentanoic acid, AB-CHMINACA M2, 5F-AKB48 4-hydroxypentyl, UR-144 5-hydroxypentyl, XLR11 4-hydroxypentyl, 5F-ADB M7, AB-FUBINACA M3, 5F-AMB M5, AB-FUBINACA M2A, and 5F-AB-PINACA 4-hydroxypentyl.

Methods

Samples (300 μ L) were extracted by supported liquid extraction (SLE) with ethyl acetate as the elution. Data were acquired on an Agilent 1290 LC interfaced to an Agilent 6490 MS/MS. All data were acquired using dynamic multiple reaction monitoring (DMRM). Samples were injected (4 μ L) onto an Agilent Poroshell120 SB C18 column (2.1 x 100 mm, 2.7 μ m) and held at 55 $^{\circ}$ C. A gradient elution with a flow rate of 0.6 mL/min was used with mobile phase A consisting of 5mM ammonium formate in water with 0.1% formic acid and mobile phase B consisting of methanol with 0.1% formic acid. Analytes were validated using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation. Parameters evaluated were bias, precision, carryover, interference, ionization suppression/enhancement, limit of detection (LOD), limit of quantitation (LOQ), and stability.

Results

A six-point calibration curve was extracted and run over five separate runs with low, medium, and high quality control (QCs) samples extracted in triplicates. QCs were used to evaluate within-run and between-run precision and accuracy. The overall average within-run and between-run %CV was within $\pm 20\%$ for all analytes. The accuracy was within $\pm 20\%$. All analytes were linear and weighted 1/x except 5F-AKB48 4-hydroxypentyl and UR144 5-hydroxypentyl were quadric. The calibration curve ranged from 0.3 ng/mL to 40 ng/mL for all analytes except the LOQ for AB-CHMINACA M4 was 0.5 ng/mL and FUB-PB-22 3-carboxyindole and AB-PINACA pentanoic acid were 5 ng/mL. The average R^2 was > 0.99 for all except AB-PINACA pentanoic acid. No carryover was observed after the highest calibrator. All analytes stable for 96 hr in autosampler at 4 $^{\circ}$ C for processed stability.



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Conclusion

A quantitative method was validated for the 15 synthetic cannabinoids in human urine providing the forensic community with a reliable method for SC analysis.

Examining Validity, Reproducibility and Sensitivity of the Quantification of 11-Nor-9-Carboxy- Δ^9 -Tetrahydrocannabinol in Urinary Samples via GC-MS with Various Extraction Methods,

Pinaz Mehta, Syracuse University, Sarah Baquero, Syracuse University, Syracuse, NY

Marijuana is one of the most widely abused drugs throughout the world, thus being able to detect and quantify it is now more relevant than ever with its legalization status in several states of the US, in Canada, and several other countries around the world. There is also a growing support for legalization across the United States, although federally marijuana and all cannabis products are still listed as a Schedule I drug. The main psychoactive constituent of cannabis is tetrahydrocannabinol (THC), which can be detected and quantified in blood, urine, hair, oral fluid, or sweat samples. In forensic and clinical applications, urinary samples are preferential in identifying recent as well as chronic marijuana consumption. This is because THC is quickly metabolized in the human body to its oxidation products, 11-hydroxy- Δ^9 -Tetrahydrocannabinol (hydroxy-THC) and 11-Nor-9-Carboxy- Δ^9 -Tetrahydrocannabinol (carboxy-THC), causing the blood levels to drop. In comparison, there is an abundance of carboxy metabolite in renal fluids and analysis of renal fluids involves a non-invasive process. Therefore, carboxy metabolite in urinary samples is commonly used to detect recent as well as continued and chronic marijuana consumption.

The purpose of this study was twofold: In the first step we have examined the validity, reproducibility and sensitivity of a previously established method by Mohammed, English, and Salem (2017) for quantification of carboxy-THC in urine samples using a mixed mode ion exchange solid phase extraction, based on OASIS MAX from Waters, followed by GC-MS. Specifically, the goal was to achieve high sensitivity, reliability and reproducibility for the lower detection limit of carboxy-THC in the range of 5 ng/mL to 100 ng/mL. In the second step, we compared the results of the SPE method above with solid phase extraction methods based on disposable pipets from DPX Technologies, LLC, the DPX Reverse Phase (RP) and DPX WAX-S tips. The results with respect to linearity, detection limits and limit of quantitation will be presented, and some of the interferences in the analysis that we encountered will be discussed. The acquired data from solid phase extractions in both steps will be compared to show the efficiency of DPX Reverse Phase and WAX-S tips.

Detection and Quantitation Of 10 Synthetic Cannabinoid Metabolites In Human Urine,

Cassandra Swart, B.S., Boston University School of Medicine, Daniel Lee, M.S. Boston University School of Medicine, Mikayla Caldwell, B.S., Boston University School of Medicine, Nichole Bynum, M.S., Center for Forensic Sciences, RTI International, Raleigh, NC, Moore, Katherine, M.S., Center for Forensic Sciences, RTI International, Raleigh,



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NC, Megan Grabenauer, PhD. Center for Forensic Sciences, RTI International, Raleigh, NC, Botch-Jones, Sabra, MS, MA. D-ABFT, Boston University School of Medicine, Boston, MA

Background/Introduction:

Synthetic cannabinoids remain in the top 25 drug-encountered analytes in seized drug evidence based on the U.S. Drug Enforcement Administration's National Forensic Laboratory Information System 2017 mid-year report. Continued illegal manufacturing of structural analogs of synthetic cannabinoids causes forensic laboratories to continually update their analytical methods. In addition, due to structural modifications of these synthetic cannabinoids, many of these compounds may bind to endogenous CB1 and CB2 receptors with greater affinity causing severe adverse and life-threatening effects.

Objective:

With a focus on synthetic cannabinoids of different core structures such as naphthoylindole, adamantoylindole, quinolinyl, and carboxamide, the purpose of this research was to develop and validate a robust and reliable method to accurately identify and quantify 10 synthetic cannabinoid metabolites in human urine including UR-144 degradant N-pentanoic acid, UR-144 N-(5-hydroxypentyl), PB-22 N-(5-hydroxypentyl), MDMB-FUBICA metabolite 3, JWH 250 N-pentanoic acid, ADB-PINACA pentanoic acid, ADB-PINACA N-(4-hydroxypentyl), ABFUBINACA metabolite 3, 5-fluoro PB-22 3-carboxyindole, and 5-fluoro MDMB-PICA metabolite 7 (Cayman Chemical, Ann Arbor, MI, USA).

Method:

A six-point calibration curve and three quality control (QC) samples each in triplicate were extracted using supported liquid extraction with ISOLUTE cartridges (SLE, Biotage, Uppsala, Sweden). Samples were analyzed on an ultra-performance liquid chromatography (UPLC, Shimadzu, Kyoto, Japan) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS, SCIEX, Waltham, MA, USA) in positive ionization mode. The method was validated in accordance to SWGTOX guidelines for quantitative analysis by evaluating calibration model, precision, bias, limit of detection (LOD), limit of quantitation (LOQ), carryover, interferences, and ion suppression and enhancement.

Results:

With this developed method, total analysis time was 8 minutes. Elution time ranged between 3.26 and 4.47 minutes. Calibration curves for each analyte had acceptable R² values >0.99. The calibration model was linear using a weighting factor of 1/x. A linear dynamic range of 5 – 40 ng/mL was used for all analytes. Percent recovery and ion suppression/enhancement was assessed at a low (10 ng/mL) and high (30 ng/mL) concentrations. Percent recovery and ion suppression/enhancement ranged 65.4 to 93.0% and 67.6 to 102.2%, respectively across both concentrations. Bias and precision were assessed at 15, 25, and 30 ng/mL for all analytes and analyzed in triplicate over 5 runs. Samples had acceptable calculated percent bias and percent coefficient of variation within ±20%. Dilution integrity was assessed at



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1:50 and 1:10 dilutions. Samples had acceptable dilution integrity accuracy within $\pm 20\%$. No carryover was observed following 40ng/mL. No matrix interference or interference from other commonly encountered drugs at 2,000 ng/mL was observed.

Conclusion/Discussion:

The overall development and validation of this method demonstrates a reliable way to positively identify 10 different synthetic cannabinoid metabolites in human urine in rapid time. Extraction of analytes using SLE cartridge improved sample preparation time by more than half, compared to traditional solid phase extraction (SPE) methods.

Key Words: Synthetic Cannabinoids, Supported Liquid Extraction, Metabolites

Conversion of Combined Drugs LC-MS/MS Method Into a Multi-Method Approach by 2D LC-MS/MS Technology,

Paul Iarussi, M.S., Boston University School of Medicine, Claude R. Mallet, PhD, Waters Corporation 2, Milford, MA, Sabra R. Botch-Jones MS, MA. D-ABFT, Boston University School of Medicine, Boston, MA

Introduction:

Forensic, clinical, and sports-doping toxicological analysis all utilize some form of combined quantitative analysis. When dealing with trace analysis in a complex matrix, this type of work will require a time and labor consuming sample preparation protocol. Thus, creating single target analytical protocols to cover a wide range of compounds is not cost effective for laboratories (e.g., time, labor and consumables). Therefore, when dealing with the chemical and physical properties for multiple and wide ranges of compounds, the challenge is to identify which analytes behave with a similar chromatographic response to a given set of separation conditions, such as elution strength, pH, buffer additives and retention. The separation becomes difficult to optimize when the number of target analytes increases and keeping the total run time as low as possible. This trend tends to lead in most cases to poor performances. This work will show the conversion of a multi-residue target analysis using traditional Liquid Chromatography (LC)-Mass Spectrometry (MS)/MS method from a forensic laboratory into a multi-method 2 Dimensional (2D) LC-MS/MS workflow.

Methods:

All target analytes in this study were first separated in three groups based on their chemical structure (25 total). Once in solution, target analytes were individually infused to acquire their respective precursor and product ions. The chromatography conditions were optimized for each class using a 6x6 automated methods development protocol. Each target analyte was subjected to a total of 144 LC-MS/MS methods which were carried out within 72 hours. The



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multi-dimensional LC was configured for "Trap & Elute" with At-Column Dilution. The total run time was 10 minutes. The mass spectrometer was a XEVO TQD operated in positive ionization mode.

Results:

The main objective of this work was to improve the overall chromatography performance and apply the upgraded method toward biological fluids. In this instance, human urine was selected as target matrix. The single LC-MS/MS method utilized a short 5 minutes gradient with the bulk of the analytes eluting at a single retention time at 2.5 minutes. This observation suggests that all target analytes are behaving as later eluting analytes on a typical reversed-phase analytical column. The fact that all analytes are chromatography co-eluting is a strong indication that the method was crafted for the mass spectrometer to take the bulk of the work, both separation and quantification. Ultimately, the 2D LC/MS/MS approach yielded two 10 minute methods with a single urine extract from a 30 minutes mixed mode extraction protocol.

Conclusions:

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

1-Mallet, C.R, Botch-Jones, S.R., Ilicit Drug Analysis Using Two-Dimension Liquid Chromatography/Tandem Mass Spectrometry, *J. Anal. Tox.*, 2016:1-11

Key Words: Forensic Toxicology, Biological Matrices, Single dimensional chromatography, Multi-Dimensional Chromatography, LC-MS/MS

Analysis of Microcystins LR, YR, and RR in Biological Fluids by 2D-LC Technology,

Beatriz Garcia-Barboza, M.S., Boston University School of Medicine, Claude R. Mallet PhD, Waters Corporation, Milford, MA, Sabra R. Botch-Jones MS, MA. D-ABFT, Boston University School of Medicine, Boston, MA

Introduction:



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Algae “super blooms” are a commonly encountered environmental issue in fresh water that occurs due to the buildup of cyanobacteria. Many of the commonly encountered cyanobacteria produce potent cyanotoxins (microcystins) that pose serious health threats and even death to local wild life and humans. Microcystin contaminated fresh-water that empties into the ocean has been shown to lethally affect marine life in the area of contamination. Human consumption of tainted sea life can lead to microcystin exposure and even death. Thus, a method was developed for forensic postmortem analysis of microcystins RR, LR and YR by 2-Dimensional (2D) Liquid Chromatography (LC)-Mass Spectrometry (MS)/MS.

Methods:

A final 2D LC-MS/MS method was selected from 6x6 automated method development experiments. Each microcystin was subjected to a total of 144 methods which were completed over a 72 hour period. The extraction process was performed using a reversed-phase sorbent with a 3 cc SPE barrel using a sequential elution. From an acetonitrile stock solution, 15 μ L of Nodularin was added to the final extract. The concept of sequential micro extraction was designed to capture the retention behaviour of a target analyte in response to various extraction parameters (sorbent strength, elution polarity, solubility ... etc). Therefore, optimized conditions can be selected to excise a region of interest during extraction. In this application, the elution solvent chosen was acetonitrile with the incremental set at 10 % increments. Since microcystins exhibit a zwitterionic structure, two set of elution solutions were created to evaluate the elution profile (pH 3 and pH 10).

Results:

When the elution profile for low pH and high pH are compared, microcystin RR was eluted in a single fraction (20% acetonitrile) with low pH conditions, but can be seen into the 20% and 30% fractions (50/50) under high pH conditions. This elution behaviour suggests that the acidic moieties of the structure show a stronger retention for the stationary phase. For microcystin LR and YR, they were eluted 40 % acetonitrile under acidic conditions. The urine sample gave recovery values for all three microcystins in the 80% range, as to be expected with type of complexity associated with biological sample.

Conclusions:

The sequential extraction protocol produced a clean extract after 30 minutes workflow using a single and optimized 2D LC-MS/MS method. The total analytical run time was set at 10 minutes.

1-Mallet, C.R, Botch-Jones, S.R., Illicit Drug Analysis Using Two-Dimension Liquid Chromatography/Tandem Mass Spectrometry, *J. Anal. Tox.*, 2016:1-11

Key Words: Postmortem Forensic Toxicology, Biological Fluids, Multidimensional Chromatography



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Opioids: Choosing the Right Solution for Your Laboratory,

Jillian Neifeld, Lynn Jordan, Biotage, Charlotte, NC

Much of the United States is experiencing an opioid epidemic due to addiction to prescription pain killers and newly emerging designer opioids. In 2016 alone, it is estimated that over 42,000 people died from an overdose of opioids. It is becoming increasingly important for toxicology laboratories to be able to quickly and accurately analyze for these drugs of abuse, preferably all in one panel. Common matrices for analysis include urine, plasma, and whole blood, as well as tissue in the case of post mortem samples. There are several extraction techniques that can be used to analyze for these opioid compounds, including solid phase extraction (SPE) and supported liquid extraction (SLE). Both extraction techniques have their advantages and it is important to be able to pick the best one based on compounds in the panel and laboratory capabilities. There are also multiple ways to perform these extractions. They can be manually performed using a vacuum manifold or a positive pressure manifold and it is also possible to automate these extractions. Analysis for these methods can be done using a GC/MS or an LC/MS/MS. With these various methods, recoveries for the opioid compounds are usually higher than 75%. These techniques show that it is possible to analyze for a large panel of opioids without the need to develop multiple extraction methods.

Correlation of Ethanol Concentrations in Human Blood and Oral Fluid Samples

Emily Parchuke Cedar Crest College and Rutgers University, Matthew Wood, Ph.D., D-ABC Forensic Science Laboratory, Ocean County Sheriff Department, Toms River, NJ, , Marianne Staretz, Ph.D, Forensic Science Program, Cedar Crest College, Allentown, PA and Thomas A. Brettell, Ph.D., D-ABC Forensic Science Program, Cedar Crest College, Allentown, PA

The use of oral fluid as a forensic specimen for driving while impaired cases has the potential to provide an accurate estimation of blood alcohol concentration (BAC) at the time of the incident. The ability to collect oral fluid at the scene can potentially reduce the lag time between traffic stop and sample collection. This sample type is also markedly less invasive to collect from individuals in the field. In order for oral fluid to be utilized in casework for this purpose, a correlation between ethanol levels in both blood and oral fluid must be determined. A headspace gas chromatographic method was utilized for the analysis of ethanol in blood and oral fluid from human-dosing study subjects.

A controlled human dosing study was performed utilizing female subjects who consumed a pre-determined amount of wine (11.5%) in order to reach a target BAC of 0.05 g/dL for each individual. Blood, breath, and oral fluid samples were collected from subjects prior to the consumption of alcohol. Blood samples were collected every 15 minutes over a 3-hour period; oral fluid and breath samples were collected every five minutes for the first 30 minutes post-consumption and every 15 minutes following for 3 hours.



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Blood and oral fluid samples were prepared using 3 mL of internal standard (0.016% n-propanol), 300 μ L of sample, and $\frac{1}{4}$ teaspoon of NaF/NaCl salt mix. Breath samples were measured with a portable breath-testing device. In this study, a Perkin Elmer HS-Clarus 580 headspace gas chromatograph with two flame ionization detectors and a TurboMatrix™ 40 autosampler was utilized. A single headspace injection was split between two columns, Elite-BAC1 (30 m x 0.32 mm x 1.8 μ m) and Elite-BAC2 columns (30 m x 0.32 mm x 1.2 μ m). Helium carrier gas at a flow rate of 12.30 mL/min was utilized; the column temperatures were set to 70°C.

Results showed the ethanol concentration profiles correlated well between blood and oral fluid. Pearson correlation values between samples of oral fluid and blood averaged 0.95 with all blood and oral fluid samples collected 15 minutes post-consumption. Oral fluid to blood ratios averaged 1.18, approximating previously published results. Results from these studies and subsequent data analyses indicate that at least a 15-minute waiting period is necessary for the dissipation of residual mouth alcohol.

Contamination Issues Utilizing Preliminary Breath Testing (PBT) Instruments,

John W. Drawec, JD, Western New England University, Springfield, MA

This presentation will focus on research and data collected on the preparation and motion to dismiss by the defense in a recent Massachusetts criminal case. In the case, an individual was charged with possession of a firearm while intoxicated after a Preliminary Breath Testing (PBT) instrument registered a significant result when administered to the defendant. At the time of the alleged offense, the defendant was cleaning firearms with a commercial solvent solution.

Research showed that the presence of the solvent, which contained a high percentage of ethyl alcohol, would produce false readings on the PBT instrument. This presentation will focus on the testing of the cleaning solution through GC/MS, the suggested proper administration of a PBT screening, and the effects of contaminants on PBT results.



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Trace, Arson & Explosives Abstracts

The Effects of Exposure to Various Environmental Conditions on the Analytical Data of Manufactured Fibers.

Alexis Weber and Virginia Maxwell, University of New Haven

The forensic analysis of fiber evidence involves the ability to link a questioned fiber back to its known source. Before the collection and analysis of fiber evidence, fibers are potentially exposed to various environmental conditions for an extended period. There has been significant research done on how natural fibers are affected by environmental conditions showing how natural fibers degrade overtime. However, there has been little research on the impact of environmental conditions on manufactured fibers. To avoid the potential of erroneous exclusions of fiber evidence it is necessary to determine if the analytical data from manufactured fibers is measurably altered when the fabrics are exposed to environmental conditions for an extended period.

Colorless manufactured fabrics nylon, polyester, acrylic, and rayon were exposed to multiple environmental conditions for a nine-month period. These conditions ranged from solid mediums including soil, cow manure, chicken manure, mixtures of soil and manure, and sand to liquid mediums including calcium chloride road pretreatment, calcium chloride road pretreatment and water, salt road pretreatment, and oil. Squares of each fabric type were placed in glass containers, each containing a different environmental condition and stored in a controlled environment. Fibers from each fabric square were removed from the environments every two weeks and analyzed microscopically and instrumentally.

Microscopic examination of the fiber followed the standard operating procedures established by SWGMAT. The instruments used to examine the fibers after exposure were a Raman spectrometer, Fourier Transform Infrared Spectrometer (FTIR), and Ultraviolet/Visible Microspectrophotometer (UV/Vis MSP) in fluorescence mode. Comparisons of the exposed fibers were made back to the control fibers to determine if the fibers were measurably altered over time to a point where they are inconsistent with their known source. Throughout the first eight weeks of exposure, rayon completely broke down leaving no remnants behind. As rayon is a semisynthetic fiber composed of cellulose, cotton was added to the containers to compare the decomposition rate of rayon to cotton.

Microscopic analysis was used to determine if there are any significant differences in the fiber's physical appearance over time. Within the first two months of microscopic analysis there was no significant change in the microscopic properties of the manufactured fibers. Instrumental data was analyzed using statistics to determine if there were significant differences between the instrumental results over the course of the time study. Apart from rayon, the increased strength and resilience of manufactured fibers should allow for there to be no significant changes to the analytical data from the fibers despite the exposure to various environmental conditions.



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Glass Population Study and Discrimination of Glass Samples using Glass Refractive Index Measurement III and Scanning Electron Microscope and Energy Dispersive Spectroscopy.

Meghan Smoker, Amy Reynolds, Elizabeth Ziolkowski, and Sabra Botch-Jones, Boston University School of Medicine, Boston Police Department Crime Laboratory, and United State Postal Service Forensic Laboratory Services

Glass is a hard, amorphous, and transparent or translucent substance, and it is examined in forensic science to place a person or object at a scene or with a victim when a crime is committed. Due to its brittle nature when combined with some force, glass is often broken, and is then submitted as a type of trace evidence to a crime laboratory in cases such as hit and runs, breaking and enterings, and homicides. Broken glass is most often obtained from bottles, windows, doors, and automobiles, and can easily be found on the street. Previous published research has examined known samples of glass and compared these samples with their known categories or types of glass. In this current research, a population study was conducted based on the collection and analysis of broken glass with unknown origins in Boston, MA. Glass samples (n=100) were collected from the streets and sidewalks around Boston neighborhoods, and an analytical scheme, constructed by the Boston Police Department Crime Laboratory, was utilized for every sample. This analytical scheme included physical characteristics, such as color, transparency, thickness, curvature and the observance of UV fluorescence. Further instrumental analysis was performed using the Glass Refractive Index Measurement III (GRIM III) for the measurement of refractive index and the Scanning Electron Microscope and Energy Dispersive Spectroscopy (SEM/EDS) for elemental composition of each sample. Refractive index varies with glass depending on the manufacturing process and its added components and is defined as the ratio of the speed of light in a vacuum to the speed of light in the substance. Using a SEM/EDS it was possible to qualitatively determine the elemental components in each unknown glass sample. Using this analytical scheme, it may be possible to distinguish every unknown sample of glass from each other using differences in physical, optical, and elemental characteristics. This study showed the differences observed in a population of glass within the city of Boston, which ultimately could help with better statistics for testimony when asked about the significance of determining an inclusion or exclusion with casework samples.

Plastic Garbage Bags: The Effects of Exposure to Various Conditions.

Jamie LiCausi and Ted Schwartz, Westchester County Forensic Lab

Plastic bags are present in many criminal cases. They are frequently used by perpetrators of crimes to dispose of evidence and/or bodies or body parts. In forensic cases, laboratories typically receive bags that are simply the original packaging for various types of evidence. What examiners sometimes overlook is that, in some cases, the most important aspect of the evidence might be the bag itself.

The purpose of this project was to determine if class characteristics of plastic bags are altered when subjected to various conditions. More specifically, to determine if the dimensions of plastic bags change after the addition of weight to the inside. Also, to see if the dimensions and/or chemical composition of the bags were altered after exposure to different environmental conditions.



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Five different brands of trash bags were used in this study. They ranged from very thin (0.5 mil) to relatively thick (3 mil). Initial measurements were taken. Then objects totaling twenty pounds in weight were added to the bags, and the bags were carried around for a short distance. The dimensions of the bags were re-measured.

The thinnest bags (“Good Sense”) and the thickest bags (“Tough Guy 3mil”) were chosen for the environmental studies. Some of the bags were placed outside for up to four months, while others were buried in soil. The dimensions were measured at various time intervals. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) analysis and Scanning Electron Microscopy - Energy Dispersive Spectroscopy (SEM-EDS) analysis was conducted on the bags prior to exposure and again after four months.

In this paper, the results of the experiments will be presented. It was noted that, in some instances, the dimensions of the bags changed. Instrumental testing showed that the bags acquired some surface materials during the environmental studies. The chemical composition of the bags themselves, however, did not change.

Bringing the Laboratory to the Field: The Evolution of Field Identification Technologies.

David Godin, 908 Devices

The miniaturization of technology has increasingly placed chemical detection and identification instruments normally associated with laboratory analysis into the hands of first responders and law enforcement professionals. From colorimetric techniques, to IR, Raman, and even MS and GC/MS, laboratory analytical techniques are being ruggedized, miniaturized, and moved from the lab and into the field. When utilized effectively by trained and validated operators, field technology can greatly increase effective evidence collection, reduce confirmatory laboratory backlog, and prioritize investigative resources with real time analysis and intelligence. This presentation outlines the evolution of field technology from its initial role providing air monitoring for confined spaces, through its expansion into the public security sector after 9/11, to its current role in the opioid epidemic. The presentation will additionally provide an introduction to the instrumentation in use by responders, specifically where they overlap with ASTM E2329-17 and SWGDRUG guidelines. The overall goal is to provide familiarity with equipment already being utilized in many jurisdictions, and to encourage interagency communication, coordination, training, and sharing of best practices.

The Center for Advanced Research in Forensic Science (CARFS): An NSF and NIJ supported Industry/University Cooperative Research Center.

Adam B. Hall, Northeastern University

NSF Industry/University Cooperative Research Centers (NSF-I/UCRC) Program:

NSF-I/UCRC enables industrially relevant, pre-competitive research via multi-member, sustained partnerships among industry, academe, and government. NSF supports the development and evolution of I/UCRCs, providing a financial and procedural framework for membership and operations in addition to best practices learned over decades of



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fostering public/private partnerships that provide significant value to the nation, industry and university faculty and students.

The Center for Advanced Research in Forensic Science (CARFS), now in its second year, aims to tackle emerging forensic science problems. With its two center sites at Florida International University and University of South Alabama, and affiliate sites at Northeastern University and Texas A&M University, it brings together forensic science leaders in state-of-the-art laboratories in a collaborative manner with industrial members, with the inclusion of an Industrial Advisory Board (IAB) that support our research and give guidance to directions of projects that are 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 an 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.

Projects will address the human, analytical, and statistical foundations of forensic evaluations in the following areas:

Chemical Analysis of Drugs, Toxicology, and Trace Materials

Quantifiable Approaches to Pattern and Impression Analysis

Human Perception and Decision Making

Cyber Forensic Analysis

Statistical Modeling and Prediction of Dynamics, Mechanistic Probabilities

Industrial members in the private sector, not-for-profit organizations, or [federal, state, or local] governments are integral to research directions and the funding of research projects done at the university level. Here, industrial members form an Industry Advisory Board (IAB) that governs research for general or specific needs. The practice of “one-center-one-mission” provides an open-platform where noncompetitiveness is practiced for the advancement of forensic science knowledge, tools, and techniques.

The Possibility of Identification of Turpentine in Fire Debris.

Eugene Zegocki, Monroe County Crime Laboratory

Turpentine is an ignitable liquid that is readily available and may be used as a fire accelerant. Distinguishing it from softwood background may be a difficult task. Twenty different commercial turpentines were examined and the major



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components were identified. The saturation of activated carbon strips (ACS) with various quantities of turpentine was also studied. Several test burns using turpentine as a fire accelerant were conducted at the NYS Fire Academy. Settings included simulated living rooms/bedrooms with typical fire load, including softwood flooring, rugs, furniture and electronics. Approximately 250 ml of liquid was used in each test burn. Fires were started and suppressed approximately 30 seconds after flashover. Samples of softwood flooring, and samples from locations above the softwood flooring (couch, bed, chair) with and without using turpentine as an accelerant were collected soon after suppression and analyzed following current Monroe County Crime Laboratory standard operating procedures for identification of ignitable liquids using gas chromatography/mass spectrometry. Experiments regarding possible cross transfer of terpenes during burning, as well as comparisons of some turpentines and softwood patterns after burning

were conducted. The results presented in this study. All experiments were conducted twice and displayed similar results. Typical fire scene scenarios involving turpentine/terpenes were discussed. Despite similarities between turpentine and terpenes in softwood, it may be possible to distinguish the source in certain cases.

Using liquid turpentine as a comparison sample would significantly help in the interpretation of the results. If turpentine is suspected as a fire accelerant and samples contain softwood flooring, detailed information about the fire scene and several flooring comparison samples may be required for interpretation of the gas chromatography/mass spectrometry results.

Automation Possibilities for Fire Debris Analysis using ChemStation Macros and PDF forms.

Eugene Zegocki, Monroe County Crime Laboratory

ChemStation macro language provides multiple possibilities for automation tasks. Using macros, it is possible to automate screening, identification and printing chromatography/mass spectrometry instrument results. The author designed and implemented a set of ChemStation macros used for the Agilent 7890/5975C instrument combined with smart PDF form for Fire Debris analysis. These macros automate entire identification and documentation process starting from notes to final report. The core is a set of macros, which screen GC/MS data for multiple samples and identify ignitable liquids (results must be checked by a qualified analyst). If a certain ignitable liquid is identified, the program selects appropriate reference. Results are printed in real time after the last sample of the case and blank after it are completed. The printout includes the ASTM Test Mix, blank sample, the total ion chromatogram and the library search for each item. If an ignitable liquid is identified additional data is printed, including extracted ion chromatograms for selected ions, peak comparisons for one-component ignitable liquids, and the corresponding data for references. There is a predefined directory, which contains a set of references covering all classes, and the most commonly encountered sub-classes of ignitable liquids except the miscellaneous class. All that data is combined in one PDF file (or printed on paper). Combining that with the notes and final report PDF file creates one PDF file which is easy to use in a paperless workflow process. Notes are automated with prompts for completing all required fields. Field heights self-adjust according to content. A final completely edited and formatted report may be produced automatically from the notes PDF form. If the examiner does not agree with the automated identification results, it is possible to make all required changes manually. Similar macros/forms may be used for any repetitive analysis, such as Controlled Substances analysis etc.



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Criminalistics/Crime Scene Abstracts

A Casework Review for Determining Time Since Intercourse in Boston, MA

Cassandra Swart, Boston University Caitlin Rogers, MS, Colorado Bureau of Investigation, William Cavedon, MS, Boston University, Amy Brodeur, MFS, Boston University, Kathryne Hall, MS, Boston Police Department Crime Laboratory

The objective of this study is to develop a reliable framework for estimating time since intercourse (TSI) in living victims based on casework received by Boston Police Department Crime Laboratory, in Boston, MA. Additionally, this study aims to determine if any statistical significance exists between the victim's reported post coital activities and the presence of intact sperm cells.

The estimation of TSI, or Post Coital Interval (PCI) can be crucial information for particular cases in which the time between offense and the collection of a Sexual Assault Evidence Collection Kit (SAECK) is in question. Developing a method to estimate TSI based on a more extensive review of forensic casework would provide investigators with a fundamental tool for estimating a general timeline in which the offense occurred. This information may play an important role in supporting or refuting a narrative, or weighing the significance of the evidence at hand.

The need to expand research on estimating TSI for sexual assault victims using actual forensic casework is crucial to provide a more reliable method for TSI estimation compared to previous studies, which have generally been based on fertility studies. Between the years of 2009 and 2017, over 1,800 reported Sexual Assault Evidence Collection Kits were submitted to the Boston Police Department for evidence processing. More than 250 cases met the qualifications for this study, which included: a living victim, smear slides prepared by a medical professional, and microscopic identification of sperm cells made from intimate swabs. In order to estimate TSI, the smear slides from these cases were microscopically examined for the presence of intact sperm cells with the aid of Kernechtrot Picroindigocarmin (KPIC) stain.

Preliminary results indicate that the observation of intact sperm on vaginal smear slides rarely surpasses a TSI of 25 hours, with an average of 12 hours. Preliminary results indicate that the observation of intact sperm on anal smear slides rarely surpasses a TSI of 15 hours, with an average of 6 hours. Preliminary results indicate that the observation of intact sperm on oral smear slides rarely surpasses a TSI of 3.75 hours, with an average of 3.75 hours. This study provides reliable evidence based on actual casework samples for more accurately estimating the time since intercourse in living victims of sexual assault crimes.



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Shallow Water Blackout

Hannah Xavier, Syracuse University

Shallow water blackout is a phenomenon in which persons employ hyperventilation before immersion to increase underwater time. Hyperventilation is implemented under the misconception of it increasing their blood oxygen saturation, thus increasing underwater time. However, oxygen saturation is relatively high at the resting respiratory rate, thus hyperventilation instead depletes carbon dioxide to an abnormally low level. This hypocapnic state is what makes shallow water blackout so dangerous, as swimmers lose consciousness underwater while never feeling the urgency to breathe.

Autonomic respiratory drive is dependent on a chemoreflex response concerned with oxygen, carbon dioxide, and hydrogen ion levels within the body. These chemical agents regulate the breathing feedback mechanism, which is intuitive due to their involvement in cellular respiration. Most importantly regarding shallow water blackout, cellular respiration produces the byproduct which most strongly dictates autonomic respiratory drive: carbon dioxide. Chemoreceptors measure levels of carbon dioxide, and when levels raise to an arbitrary threshold the respiratory center increases breath rate and depth. This is when the diver feels the urgency to breathe, signaling them to surface to inhale oxygen.

When a diver immerses themselves underwater breath holding ensues, thus oxygen is no longer inhaled for cellular respiration. Thus, cellular respiration byproduct carbon dioxide accumulates, driving autonomic breathing urgency. In a typical dive, carbon dioxide levels remain at normal baseline, thus hypercapnia triggers breathing urgency before hypoxemia takes effect. During a hypercapnic dive however, hyperventilation causes lower baseline carbon dioxide levels, therefore breathing urgency does not occur until after consciousness is lost and drowning has taken effect.

The largest contributing factor in shallow water blackout is hyperventilation. The term 'shallow' only speaks for the lack of partial pressure effects associated with deep water diving. However, when reviewing literature I saw a pattern of careless interchangeability between characteristics of shallow water blackout and these other diving phenomenon. I suggest shallow water blackout adopt a name more relevant to the mechanism at hand, as to better differentiate it from these conditions. I propose adoption of 'Isobaric Hypocapnic Blackout', a name which instead focuses on the true matter at hand: hyperventilation induced hypocapnia that is independent of partial pressure aggravation due to hydrostatic pressure.

Some literature reports that decedents found at the bottom of a body of water as a hallmark of shallow water blackout. While aspiration of water makes sinking more likely, it is not a definite rule nor is it unique to shallow water blackout. Therefore, I find using this scene marker for shallow water blackout an oversimplification. Glorification of apneic ability coupled with ignorance of its dangers encourages laypersons to test their own limits. I feel preventative measures should be taken to thwart this contributor to drowning frequencies across the nation. Improving signage



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around public pools and campaigns at schools before summer months are some of the first steps in halting the normalcy surrounding breath-holding competitions.

A Sticky Situation: The Chemometric Identification of Condom-Derived Residues by Direct Analysis in Real-Time High-Resolution Mass Spectrometry

Allix M. Coon, State University of New York at Albany; Samira Beyramysoltan, PhD, State University of New York, Rabi A. Musah, PhD, State University of New York

In order to avoid leaving behind incriminating DNA evidence that could link them to the crime, perpetrators of sexual assault are increasingly using condoms. In such cases, condom residue trace evidence takes on heightened importance in the corroboration of a victim's account or in exonerating the falsely accused. Thus, the ability to identify trace evidence as being condom-derived is essential. Furthermore, attributing brand information to condom residue would further enhance its evidentiary value. Previous reports have found that a variety of techniques can be used to confirm that a given trace residue is condom-derived, based on the detection of spermicides, slip agents, and/or other common additives. However, there

has been limited success in differentiating between brands from among a large range of condoms. In this study, direct analysis in real time-high resolution mass spectrometry (DART-HRMS) was combined with chemometrics, for the rapid and accurate ascription of brands to various condom residues. A database of condom residue spectra comprised of 110 different condom types representing 16 brands was generated, with the spectra serving as representative fingerprints for each brand. These condoms included both U.S. and international brands. The spectral fingerprints were subjected to pre-processing prior to the application of Partial Least Square-Discriminant Analysis (PLS-DA), which was used to generate a classifier that permitted identification of condom brands with an accuracy $\geq 97.4\%$. Using the Student's t-test, the m/z values representative of small-molecule markers that were the most important for defining brand classes could be determined. This further revealed a subset of 14 m/z values that were observed in all 110 condoms representing the 16 brands, some of which may serve as potential universal small-molecule condom markers. Overall, the results show that the DART-HRMS database of condom residue spectra can be used to identify residues based on differences in chemical components that are unique to each brand. The database can be continuously expanded to include a broader range of condom types.

Detection of Prostate Specific Antigen and Salivary Amylase in Vaginal Swabs using Seratec® Immunochromatographic Assays

Sarah Lighthart, Cedar Crest College; Jillian Conte, PhD, Keystone College, Lawrence Quarino, PhD, Cedar Crest College, Shanan Tobe, PhD, Arcadia University, Amy Flynn, Keystone College

Presumptive tests for the presence of biological fluids are often one of the first steps in the processing of biological evidence. A common method of performing such tests is to use immunochromatographic assays.



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Immunochromatographic assays contain monoclonal antibodies specific for a marker found in the fluid of interest. A positive test would be indicated by the presence of a line in the test area of the cassette. The Seratec® PSA semi-quant immunochromatographic assay detects the presence of prostate-specific antigen (PSA), a component of seminal fluid; however, PSA can be endogenously found in other areas of the body, such as urine and the gastrointestinal tract (1). A similar assay is the Seratec® Amylase Test, which tests for alpha-amylase in human saliva. As with PSA, alpha-amylase is not specific to saliva. Various genetic forms of the enzyme exist and some form of alpha-amylase can be found in nearly every body fluid albeit at a lower concentration than saliva (2). Due to this non-specificity of fluids, false positive results become a concern. This large-scale study was performed to determine the rate of a positive result for human PSA and alpha-amylase in vaginal fluid. Clean, self-collected vaginal swabs were obtained from volunteers that had abstained from male ejaculation in the vaginal cavity for 10-14 days, oral-vaginal contact for 7 days, and female orgasm for 2 days. The volunteers also provided information on contraceptives and spermicides used. The swabs were extracted following the SERATEC protocol and subsequently tested. Results were obtained within 10 minutes, photographed, and documented. The resulting data indicates little to no cross-reactivity with vaginal fluid. This indicates high reliability when using the Seratec® PSA semi-quant and amylase immunochromatographic assays in conjunction with processing sexual assault kits.

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Adding an Objective Approach to Questioned Document Examination using Principal Component Analysis and Mahalanobis Distance

Loren Williams, Cedar Crest College; Lawrence Quarino, PhD, Cedar Crest College, Morgan Mills, Lab Corp.

Studies have shown that document examiners have a skill that lay people do not.¹⁻² This skill is based on experience and expertise, and is essential in questioned document examination. However, after the release of the National Academy of Science's report on strengthening forensic science in 2009, it was made clear that objective measures of comparison are needed to augment and strengthen conclusions that are essentially subjective in nature.³ One possible approach is coupling the skill of questioned document examiners with statistics creating a more objective methodology. This study incorporated the use of principal component analysis and Mahalanobis distance to classify groups of known writing, compare an unknown with exemplars, and explore traced and disguised handwriting sample analyses.

To test this, 20 handwriting samples were collected from 50 individuals. Various known features, such as height of capital and lower-case letters, distances between words, and ratios of letter heights were measured using Adobe Photoshop® 2017. Measurements were used to perform principal component analysis (PCA) in order to attempt discrimination of handwriting between individuals. Of the 50 individuals, 32 were able to be distinguished visually using a 3-dimensional plot. Unknown writing samples taken from 10 participants in the original 50 were collected and compared to the dataset. Principal component analysis indicated that each unknown could be visually placed into the correct data set. In addition, Mahalanobis distances were also calculated. Results from this statistical test showed that 8 of the 10 unknowns were able to be correctly classified into their respective data sets. An additional 10 unknowns were



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collected from individuals not in the original group of 50 and were compared to the dataset. The visual analysis using principal component analysis indicated 9 of the 10 unknowns were not classified into any of the original 50 databases, although the Mahalanobis distances placed several of the unknowns into one of the original 50 datasets based on criteria used in the study. Traced and disguised handwriting samples were collected from four participants of the original 50. The writing they attempted to imitate was also an individual from the original 50. Principal component analysis indicated that the traced and disguised samples were not classified with the writer's handwriting or the person's handwriting imitated. Mahalanobis distance data indicated 3 of the 4 traced samples were correctly classified based on the criteria used. Disguised data had no classifications based on the criteria, however one sample did correlate to the writer's own handwriting.

These results indicate that principal component analysis offers the potential of objectively confirming conclusions reached in traditional questioned document examination. Going forward, results reached in this study should be confirmed by the subjective examination of a trained document examiner. If results in this manner are consistent with the statistical analysis, it will lend credence to the use of statistical analysis to be used in tandem with traditional handwriting examination. This could help alleviate the criticisms often levied on handwriting comparisons as being too reliant on subjective analysis. Finally, PCA could be a tremendous tool in showing the variability in handwriting exemplars taken from individuals. Objective determination of this variability can be helpful in assessing the validity of particular exemplars used in writing comparisons.

Enhancement of Textile Impressions in Vehicle Surfaces

Jessica Hovingh, Penn State Forensic Science Program; Abigail Bender, BS, Penn State Forensic Science Program, Ralph R. Ristenbatt III, MS, Penn State Forensic Science Program

Numerous types of physical and material traces can be generated during a pedestrian-vehicle collision. Many traces recovered from the scene are often categorized as so-called "trace evidence," and originate from the vehicle. These traces may include fragments of paint, glass, metal, plastic, and other synthetic materials. Pattern evidence from the vehicle's tires or other structural components may also be present at the scene, on the victim, or on the victim's clothing. Traces originating from the victim can be transferred to the vehicle; if the vehicle is recovered, these traces may be used to associate the vehicle to the victim. Such traces include biological substances (blood, saliva, hair, skin, or other tissues), pieces of clothing fabric, and fibers. Three-dimensional (3D) patterns impressed in the vehicle's surface coating may result from the victim's clothing fabrics upon impact. These patterns provide several challenges to criminalists: they are frequently difficult to detect, visualize, and record, especially when the indented substrate is white or light in color. Due to these difficulties, they are often overlooked or deemed unsuitable for further analysis. This study aims to investigate familiar and novel methods of visualizing and recording 3D textile impressions in vehicle coatings, and provide criminalists with a recommended approach when fabric impressions in vehicle coatings are encountered during accident investigations.



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A large pendulum impact device was used to generate 3D fabric impressions similar to those seen in pedestrian-vehicle collisions. To simulate a clothed human knee, a domed metal weld cap covered in 1/2-inch thick foam was affixed to the end of the pendulum's 6-foot arm. A square piece of washed Levi's 550™ denim jeans was then fastened over the foam. The simulated knee was allowed to impact a previously cut section of vehicle, generating a 3D impression in the surface coating. All variables (pendulum drop height, simulated knee, fabric, and vehicle sections) were held constant to produce consistent impressions, allowing ideal comparison of different enhancement methods.

In this study, several methods were investigated and compared to provide a recommended approach for enhancing and recording 3D impressions. Familiar techniques, such as use of optical filters and application of fingerprint dusting powders and small particle reagent, showed potential for enhancement. Removal of excess dusting powder may provide improved contrast between the impression and substrate. Following enhancement, the pattern may be lifted using a gelatin lifter or Mikrosil™ casting. Specialized instrumentation provides a supplemental recording method. This study investigated use of the Zygo® Nexview™ 3D Optical Surface Profilometer. The profilometer scans the substrate, recording fine detail of the impression. The instrument also measures the depth of the impressions, providing additional useful information to analysts.

BODIPY-Modified Duquenois-Levine Test

Jesse Caron, Western New England University; Sean P. McClintock, Anne F. Poirot

The recent legalization of marijuana (MJ) for recreational and medicinal use has been associated with an increased incidence of DUI (driving under the influence) of MJ.^{1,2} Roadside testing methods that can detect THC at the relatively low concentrations (< 0.1-50 ng/mL)³ expected in biological samples (saliva, breath, blood) of active MJ users could facilitate law enforcement efforts to deter DUI by MJ.² Effective roadside testing methods should be simple to conduct, sensitive, and reliable. The traditional Duquenois-Levine (DL) test for MJ is based on the acid catalyzed condensation of acetaldehyde and vanillin to form DL-aldehyde, which under acidic conditions, reacts with the activated aromatic ring present in THC. Rapid dehydration of the initial product yields the highly conjugated DL chromophore⁴ resulting in the characteristic violet color (present in the organic layer) associated with a positive DL test result.

Previously, we successfully prepared modified DL reagents⁴ exchanging both the electrophilic (vanillin) and nucleophilic carbonyl (acetaldehyde) compounds required resulting in new chromophores providing for a more selective DL test. The current research involves the preparation of BODIPY (boron dipyrromethene) modified DL reagents to react with THC to yield a novel fluorescent chromophore (fluorophore). BODIPY derivatives are small highly fluorescent molecular compounds that exhibit high quantum yields (approaching 100%)⁵ and have good solubility in many organic solvents. Structural changes to the substituents attached on the BODIPY multi-cyclic core affects the fluorescent properties of the compounds providing derivatives that have distinctive absorbance maxima and emission spectra. BODIPY compounds typically are relatively insensitive to variations in environmental conditions, such as pH and solvent changes, making them especially suitable for use as fluorescent sensors. The relative stability of



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BODIPY derivatives to changes in pH, their high solubility in organic solvents as well as the distinct spectral changes which occur upon structural modification should contribute to the success of the BODIPY-modified DL test.

This presentation centers on the synthesis of a suitable BODIPY aromatic aldehyde used to prepare a novel modified DL reagent. The results of THC testing using this BODIPY-DL reagent will be discussed.

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The Identification of PCP and Designer PCP Analogues using Microcrystalline Tests followed by Raman Microspectroscopy

Matthew Quinn, BS, Cedar Crest College; Lawrence Quarino, PhD, Cedar Crest College, Monica Joshi, PhD, West Chester University, Thomas Brettell, PhD, Cedar Crest College

The development of designer drugs is changing the landscape of today's drug market. The circulation of a wide variety of compounds has created enormous case backlogs in crime laboratories across the world (1-3). Traditional analytical techniques are not universal, and they require time and resources for method development and validation to meet accreditation standards. To increase a laboratory's effectiveness in quickly identifying designer drugs and other unknown compounds, new analysis techniques should be explored. This study details a fast and confirmatory protocol for the identification of unknown compounds using microcrystalline tests followed by Raman microspectroscopy.

Here, phencyclidine (PCP) and twelve designer PCP analogs (tenocyclidine, rolicyclidine, benocyclidine, phencyclamine, eticyclidine, 3-methoxy phencyclidine, 4-methoxy phencyclidine, PCEEA, PCMPA, 3-methoxy eticyclidine, methoxetamine, diphenidne) were subjected to microcrystalline tests followed by Raman microspectroscopy on the successfully grown microcrystals. According to the recommendations by the Scientific Working Group for the Analysis of Seized Drugs, these two analytical techniques used in conjunction are sufficient for a confirmatory identification of



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an unknown compound (4). For compounds that successfully grew microcrystals, a test method was developed using exact concentrations and volumes to eliminate the ambiguity of previous microcrystalline testing. Microcrystal properties such as shape, habit, time of growth, color, retardation colors, type/angle of extinction, and sign of elongation (when applicable) were observed and documented. Using an optimized Raman method and a developed library containing pure drug spectra for PCP and the twelve designer PCP analogs, the spectra obtained from drug microcrystals were able to be used to identify the original compound. Further comparisons indicated peak shifts along with the addition or subtraction of specific peaks for each microcrystal. In this presentation, the effectiveness of microcrystalline tests followed by Raman microspectroscopy for the identification of PCP and designer PCP analogs is demonstrated. While this research focuses on a specific structural class, the protocol can be applied to other classes for fast and confirmatory identification.

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Multiple Transfers of Drug Contaminated Fingermarks and Their Analysis with Raman Spectroscopy

Victoria DePrimo, University of New Haven; Kenneth Zercie, University of New Haven, Pauline Leary, Smiths Detection, Nicholas Petraco, John Jay College CUNY, Lisa Dadio, University of New Haven, Brooke Kammrath, University of New Haven

The aim of this research was to determine if substrate, enhancement technique, and multiple transfers have an effect on the detection and identification of drugs in fingermarks using Raman spectroscopy. The ability to associate illicit drugs with a specific fingermark has great potential for forensic science, as it can associate the drugs with a specific individual.

Trace amounts of illicit materials within fingermark friction ridge deposits have been identified from single, secondary transfers using various substrates and enhancement techniques. However, questions remain about the amount of transfers drug contaminated fingermarks can leave on different substrates and still be detectable and identified.



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Raman spectroscopy has been utilized successfully in the forensic field to confirm the presence of substances such as drugs in fingerprint friction ridge deposits. Additionally, its ability to differentiate distinct features between other substances and those of a crystalline nature makes Raman a powerful tool in identification of trace amounts of drug materials. In utilizing benchtop Raman Spectroscopy, identification of drug-contaminated prints is possible over multiple transfers with considerable limitations in its application to casework, including on-site analysis and contamination. Portable Raman Spectrometers have been useful for rapid in-situ analysis of drugs, explosives, and other materials. While their portability is beneficial for on-site analysis of these materials, limitations of small spot size and reduced sensitivity were observed when used for testing transfers of drug-contaminated fingerprints.

This research examined the number of transfers a drug contaminated fingerprint could leave where cocaine was able to be detected using Raman spectroscopy. Ten participants imparted drug-contaminated prints 20 successive times on a series of five different substrates with specific enhancement techniques most commonly seen at crime scenes. Portable and Benchtop Raman Spectrometers were employed to assess the number of successive transfers from which drug contaminated prints can be detected from different substrates with enhancement. Results varied based on the substrate and enhancement technique. It was concluded that cocaine was detected in as many as 20 successive fingerprints after substrates were enhanced with cyanoacrylate or enhanced with black or fluorescent powder and lifted.

By understanding how these illicit materials transfer between individuals, and substances handled, as well as their persistence and limitations associated with its identification, greater assistance can be offered for investigations. Additionally, information on transfer and persistence is vital for the proper interpretation of this type of evidence, should it be used in a court of law. Using Raman Spectroscopy to associate illicit drugs with a specific fingerprint has the potential to put the drugs in the hands of a specific individual, aiding forensic scientists two-fold.

Kratom Identification by Portable GC/MS Instrumentation

Brooke W. Kamrath, PhD, University of New Haven; Zachary Lawton, MS, Sarah Goda, BS, Peter Massey, MS

Kratom is a relatively new “legal high” in the United States that is easily purchased over the internet, and its federal legality is under considerable debate. It has been linked with at least 44 deaths which have triggered significant attention by the FDA and DEA. Kratom comes from the *Mitragyna speciosa* leaves which is part of the *Rubiaceae* family native to South East Asia. Originally the plant was ingested for its stimulant properties, and it is advertised today as a concentration booster, a workout enhancer, a replacement for opioid painkillers and as a way to treat opioid addiction. The main active compound in kratom is mitragynine which in low doses binds to the delta-opioid receptors where it acts as a stimulant, but as the dose increases it binds to the mu-opioid receptors and becomes a sedative. It is in the same category as morphine but is 13 times more potent of an agonist for the receptors, thus it is similarly addictive. Consequently, in February 2018, the FDA determined that kratom was more than just a plant and classified it as an opioid. Although it has not yet been scheduled by the DEA, its ban appears to be imminent with this new FDA classification.



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Law enforcement officers commonly rely on the field identification of illicit drugs for arrests. The most commonly employed field test is a presumptive colorimetric test called the Narcotics Identification Kit (NIK) which relies on a reaction with the provided reagents to produce characteristic color changes. To date, no colorimetric test has been found to identify Kratom or its active compounds. In the lab, chromatographic tests (GC/MS and HPLC/MS) are used for the confirmatory identification of mitragynine in Kratom. A gap has thus been identified because there is a need for the on-scene identification of Kratom by the law enforcement community. This research reports on the positive identification of mitragynine in Kratom by portable GC/MS instrumentation. The Torion T-9 portable GC/MS using SPME sampling with direct injection via thermal desorption successfully detected and identified mitragynine in 14 samples of kratom: 10 powders, 3 pills, and an oil extract.

The Role of Technology in the Possible Demise of the Forensic Science Laboratory

Brooke W. Kammrath, University of New Haven; David SanPietro, University of New Haven, Peter De Forest, John Jay College of Criminal Justice

There are two emerging technologies that have an impact on the field of forensic science: laboratory automation and field deployable instrumentation. These two technologies can each have profound implications on the effective practice of forensic science in all areas of the physical evidence continuum.

Laboratory automation has already impacted analytical methods in forensic laboratories, improving efficiency and casework flow, thereby decreasing turn-around times. This has enabled an increased time for the more critical front-end issues of informed specimen selection. If this advantage of the role of the scientist in front-end evidence assessment is not appreciated, and this step is omitted, then we risk the misapplication of this technology rendering any perceived advantage doubtful.

Field deployable instrumentation (i.e. rapid DNA analysis, hand-held infrared and Raman spectrometers, portable gas chromatography/mass spectrometers) has provided the potential for on-the scene analysis by non-scientist investigators. The perceived advantage of these technologies is the analysis of materials with immediate and easy-to-understand results that can be used in real-time investigations. These instruments have utility in the relatively simple and straight-forward analyses of materials such as illicit drugs, however we have concerns with their use in complex crime scene investigations and reconstructions. There are numerous potential dangers with this tempting approach, including evidence destruction, the failure to recognize critical traces, an absence of scientifically informed specimen selection process, and the production of computer algorithm-based unverified conclusions and identifications. There is evidence of this already taking place with respect to scene investigations with connection to the rote use of swabbing in lieu of scientifically-based selection.

This directed discussion with potential audience participation will focus on suggestions for the proper implementation of these high technology **tools**. It is easy to lose sight of the fact that these technologies are just tools, and their utility



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is only as good as the scientific basis for their utilization. Although these technological capabilities are useful, they cannot supplant the need for scientific problem solving by educated and experienced criminalists.

This discussion will be fostered by the use of “Poll Everywhere”, a web-based audience response system. The audience will be able to respond in real time to the questions posed during this presentation via the web or via SMS texting on their phones. The authors request that attendees download the Poll Everywhere free app onto their mobile devices prior to attending this presentation in order to facilitate discussion. This is not mandatory because attendees will be able to respond via texting.

Analysis of Evidence

Ben Landas, CDS Analytical

Complex solid materials like black rubber, automobile paint, printing ink and duct tape may be examined using a variety of techniques, including microscopy, but present a real challenge for chemical analysis. Although GC/MS can provide a wealth of information about complex chemical systems, the components usually must be volatile to be compatible with the technique. Analytical pyrolysis expands the scope of GC/MS analysis by creating volatile samples from seemingly incompatible materials like tires, documents and glue. Precise heating of these samples at the GC inlet produces chromatograms representing the various constituents of the evidence, and require only microgram sized samples.

This presentation highlights analysis of typical forensic materials, including tapes, inks and paper, automobile paint, and rubbers, representing polymers and copolymers such as polyolefins, polyurethanes and acrylics, together with additives like plasticizers and antioxidants, all of which help differentiate and identify such multi-component materials.

Quantofix Nitrite Test Paper Validation for GSR Distance Determination

Beth Saucier Goodspeed, MA State Police Crime Laboratory

The purpose of this study was to evaluate and compare the performance of the Quantofix Nitrite test paper against the paper currently being used in the Massachusetts State Police Crime Laboratory Criminalistics Unit for GSR-distance determination. The Criminalistics laboratory’s gunshot residue protocol contains a procedure for porous (Modified Griess test) and non-porous (Reverse Modified Griess test) surfaces that require nitrite testing. The procedure presently employed by the laboratory utilizes hp Premium Presentation Paper – Matte and requires the laboratory to chemically treat the paper prior to use. This process takes approximately a day to prepare, coat, dry and QC and involves placing the paper in large buckets of chemicals, which present safety hazards.



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The Quantofix product is manufactured by Machery-Nagel and is available for purchase through CTL Scientific Supply Corp. This test paper purports ease of use, clear results, and that it can replace the current Griess test procedure. If the Quantofix paper is deemed to be a suitable replacement, benefits to the laboratory include no required preparation, thereby reducing the time for analysis and reducing the safety hazard involved in the preparation process. Additionally, the current laboratory prepared paper expires 30 days after preparation while the Quantofix paper's shelf life is reported to be 24 months from production if stored according to manufacturer's recommendations for light, heat and humidity levels.

This study evaluated the effectiveness of this paper with both Modified Griess and Reverse Modified Griess methods. Distances of contact, 3", 9", 18", 24", 36", 48" and 60", using the same firearm and ammunition, were tested using the laboratory prepared paper and the Quantofix paper with the Modified and Reverse Modified Griess testing procedures used by the MA State Police Crime Laboratory Criminalistic's unit procedure for gunshot residue. The results of the lab prepared and the Quantofix paper were then compared for effectiveness.

Trigger or Triggered; Which is More Deadly?

Peter Diaczuk, D&H Criminalistics Agency; Xiao Shan Law, Pedico Research Institute, Andrew J. Winter, Centenary University, Samantha Deibel, Penn State University

The Las Vegas Mandalay Bay hotel shooting last October (2017) brought attention to a device that was used by the deranged shooter. Deemed originally by the Bureau of Alcohol Tobacco and Firearms (BATF) as a legal modification to the firearms for which they were designed, the "Slide Fire" attachment enabled the owner of such a device to fire their semi-automatic rifle similar to a full-automatic rifle. Traditionally, full-automatic rifles and machine guns are regulated by the BATF as dictated by the National Firearms Act (NFA) enacted in 1934. The NFA mandated that firearms which discharged more than one cartridge with a single pull of the trigger required special registration and included an additional fee payable to the Treasury Department. This system was created in an effort to curtail the prominence of a certain class of firearms (notably the Thompson submachine gun) that had historically been favored by gangsters of the era, and to prevent shootings like the infamous "St Valentine's Day massacre." The Slide Fire attachment did not alter the internal mechanism of the firearm, and when used, did not allow more than one cartridge to be discharged with each pull of the trigger. Instead, it allowed the operator to engage the trigger at a much faster cyclic rate than would have been possible using muscle dexterity alone. Consequently, from the legal standpoint, firearms with the Slide Fire attachment installed were not considered any different from the semi-automatic rifles prior to their modification. If a semi-automatic rifle was already illegal in a certain jurisdiction, because of restrictive laws in that jurisdiction, it remained illegal. Conversely, if the same rifle was completely legal in that jurisdiction, it remained completely legal after the Slide Fire attachment was installed.

The question being addressed in this short project is whether or not a sound recording can be used to distinguish between an actual full-automatic firearm and a semi-automatic firearm equipped with the Slide Fire attachment. This



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question arose pertaining to the Mandalay Bay incident. The three firing mechanisms are compared; unaltered semi-automatic, semi-automatic with the Slide Fire attachment installed, and full-automatic.

The Slide Fire device will be examined as it came in the box from the manufacturer, how it is installed onto a factory semi-automatic rifle, and how it is used. A combination of high speed photography, a digital audio software program and a shot timer was used to gather data on the three firing platforms.

The Geometric Analysis of the Inherent Inaccuracies Found in Linear Measurement of Curved Bite-Mark Surfaces

Henry J. Dondero, BS, MS, DDS, Nassau County Medical Examiner

The Forensic Odontologist relies on highly accurate measurements to facilitate evidentiary quality bite mark analysis. Reasonably accurate Alginate, or the more stable and accurate polyether or polyvinyl siloxane impression materials, are capable of producing measurement friendly dental stone study models. All these measurements are usually taken in a flat plane linear environment. For example, the inter-canine cusp measurement is accomplished by simply placing the standard ABFO #2 ruler across the model and recording the appropriate dimension. Such data accurately translates to photographs (both film and/or digital) through specialized scanning techniques and photo processing software. The resultant images are generally accepted as evidence in litigation. Analysis of the bite mark is more problematic. Bite marks by their very nature are subject to either in vivo healing or post mortem decomposition. Elastomeric impressions, methacrylate tissue excision techniques, and specialized 1:1 close-up photographs or digital images all serve to preserve the bite mark as evidence. Measurement problems occur because bite marks are rarely made in a truly flat plane environment. It is the natural curves of the human body that lends itself to exhibiting a bite mark that has been made around a curved surface. If one should photograph the bite mark with the #2 ruler in view all objects are in a two dimensional posture and all measurements taken of a curved surface with a straight ruler will have some inherent inaccuracies. It is this inaccuracy that this paper will address.

Methodology for this analysis is based on the geometric relationships present between a straight ruler and a curved body part. If, for example, one considers a ruler resting on an arm in cross section the geometric shapes represented here are a straight line drawn tangent to a circle. It is obvious that the measurement on the line from the point of tangency towards the periphery would by nature be shorter than the arc length circumscribed by the resultant curve of the circle. It is this difference that will be calculated to determine if it is significant.

The #2 ruler is marked in five centimeter divisions with millimeter markings, therefore all measurements will be in millimeters. Incremental measurements were made for each angular degree from the center of the circle. Both the arc length and the tangential distance from the origin to a point formed by the intersection of a line drawn from the extended radius perpendicular to the tangent will be calculated and compared. An Excel spreadsheet was created to perform the numerous calculations. Five columns were established: "0" – the angle of the radius in degrees, "Arc



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Length in mm," "Tangent Length in mm," "Error in mm," and "% Error." If one should envision the focal plane of a camera to be placed above and parallel to the tangent line the resultant photographic image would not show anything passed the 90° arc. Recognizing that it is only this "quarter circle" that is being analyzed, calculations were made for "θ" values of 1 through 90 in one degree increments.

Initial results verified the known geometric relationship between the arc length and the tangent length as evidenced by the constant % Error fixed at 36.31%. The results also show significant differences in mm measurements as θ increases. A one degree deviation from the perpendicular results in an arc length (the arm and embedded bite mark) of 0.87mm and a tangent length (the ruler) of 0.56mm with an error of 0.317mm. While 0.3+mm error may not be significant at that level, a fifteen degree angulation results in a 4.75mm error on a 13.8mm arc length (arm & bite mark) with an 8.33mm tangent length (ruler). As one would expect the greatest discrepancy occurs at 90°. Here, an error of 28.5mm is seen. These measurements are accurate only for a perfect circle. In vivo measurements will more likely be taken on some elliptical form. If the greater diameter of the ellipse is perpendicular to the tangent then a proportionately greater error is seen. In the case of the lesser diameter being perpendicular to the tangent the resultant circumference would present a flatter and arguably more accurate measurable surface.

This analysis suggests that measurements along a curved surface should be made by rotating the ABFO #2 ruler along the arc or by using some flexible measuring device. Use of a Vernier caliper would offer the ability to measure "through" rounded soft tissue.

The Independent Crime Laboratory: The Good, The Bad and the Unexpected

Dennis C. Hilliard, MS, Rhode Island State Crime Laboratory – University of Rhode Island

For the most part crime laboratories throughout the United States are administered by law enforcement agencies at the municipal, county, state or federal level. According the United States Department of Justice's 2014 survey of Publicly Funded Forensic Crime Laboratories, there are 409 publicly funded forensic crime laboratories in the United States: 79 municipal, 98 county, 193 state and 39 federal. It is estimated that between 80 and 90% of these laboratories are administered by law enforcement agencies or prosecutors' offices.

In the 2009 National Academy of Sciences Report on Forensic Science, entitled "Strengthening Forensic Science in the United States: A Path Forward", recommendation 4 states: "To improve the scientific bases of forensic science examinations and to maximize independence from or autonomy within the law enforcement community, Congress should authorize and appropriate incentive funds to the National Institute of Forensic Science (NIFS) for allocation to state and local jurisdictions for the purpose of removing all public forensic laboratories and facilities from the administrative control of law enforcement agencies or prosecutors' offices." (2)



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This presentation will explore the RI State Crime Laboratory as a model for a Forensic Crime Laboratory administered by the University of Rhode Island and located on the main campus of the University



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Poster Session Abstracts

Split Second Decision - Is It Real Or Is It Fake?

Det. Andrew J. Winter, Middlesex County Prosecutor's Office; Peter Diaczuk, PhD., Xiao Shan Law, Penn State University; Katherine O'Connell, Centenary University

Blank (imitation) firing guns can appear remarkably realistic. Many blank guns look like authentic firearms, however blank guns are not designed, at time of manufacture, to fire a projectile (bullet) from the barrel. In fact, at time of manufacture, many of these barrels are sealed or plugged, the chamber may be designed shorter so not to allow a live cartridge to seat properly and vent holes are sometimes located on the barrel to disperse the energy that results from firing a live cartridge (preventing this energy from being dispersed from the end of the barrel). Blank ammunition is designed with a case, propellant and primer, but no projectile (bullet). When discharged, the blank cartridge has the potential to produce a flash and an explosive sound and the blank gun cycles in similar fashion to an authentic firearm. These blank guns are also sometimes referred to as imitation guns, starter guns, or even "Hollywood" prop guns. In this research project, we examined two blank firing guns and two authentic firearms using a sound meter and high speed photography.

Cartridge Case Ejection Angles Discharged from Semi-Automatic Pistols

Peter Diaczuk, PhD., Ashley D'Alessandro, Penn State University; Det. Andrew J. Winter, Middlesex County Prosecutor's Office

Crime scene investigators are regularly asked where a shooter was standing when a firearm was discharged. Firearms are designed to discharge a projectile (bullet) by use of a propellant. In a dynamic and rapidly changing crime scene, these spent projectiles or bullet fragments are difficult to locate and to associate to a shooter's origin without a bullet strike present at the crime scene. The semi-automatic pistol is commonly used and discharged in the commission of a crime and can potentially leave additional evidence behind at the scene as the spent case is extracted and ejected from the firearm. The spent case can provide a plethora of useful information. In addition to the final resting place of the spent case within the crime scene and its potential significance in determining where the shooter was at discharge, the angle at which the spent case ejects and travels from the ejection port of the firearm may also be significant. Various factors including ejection port location, the design of the firearm, and the way in which the firearm is held may influence the angle of ejection. This research project examines the angle of ejection of the spent case from several types of semi-automatic pistols utilizing high speed photography. Previous research by the authors examined where the spent cases landed on a grid pattern at discharge. This research examines the angle of ejection of the spent case at discharge and is a continuation of prior research.



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Forgery or Not: Laser-Induced Breakdown Spectroscopy (LIBS) for Oil Paint Analysis.

Amber Malloy, Alexandria Aloia, Madison Keiser, Rosemarie C. Chinni, Peter Rampson, Alvernia University

In laser-induced breakdown spectroscopy (LIBS), a laser pulse is focused on a surface. This heats, ablates, atomizes, and ionizes the surface material and results in the formation of a plasma. The light emitted by the plasma is collected, spectrally resolved, and detected. Elements contained within the samples can be identified by their unique spectral signatures. LIBS provides qualitative and quantitative results and has many applications from environmental to defense including metal sorting, explosive and fissile material detection, lead in paint determination, heavy metal contamination in soil, identification of cultural heritage objects, etc.

The purpose of this project is to show LIBS's application for indirect dating, authentication, and identification of oil paint pigments. Pigments are the organic or inorganic substances that give paint their colors. The samples consisted of ten oil paint pigments from traditional and modern palettes. The oil paint pigments were analyzed in pure form and binary mixture form using the LIBS system. The data was taken in multiple ways: single shot analysis, ten shot accumulation analysis, and depth profiling. The results showed that LIBS can be used in single shot mode for data collection and can distinguish between the oil paint pigments using the ten shot accumulation data. It was also possible to determine the binary mixture composition by focusing on one or more wavelengths. Depth profiling was accomplished and showed the multiple layers from the paint, primer, and canvas. Since synthetic paints (modern) were not available prior to the 19 century, the presence of a synthetic paint could allow for determination of whether the artwork was conserved in the past or if it was a forgery. Conservation could be proved by doing a depth profiling analysis because the components of the traditional pigment would appear as the same sample spot was interrogated with the laser.

Quantitation of Cathinone and Cathine in a large Khat Seizure

Susan Neith, NMS Labs.

Khat is a flowering plant with evergreen leaves (*Catha edulis*) native to the Horn of Africa and the Arabian Peninsula. Khat contains the alkaloid cathinone (DEA Schedule 1), a stimulant, which causes excitement, loss of appetite, and euphoria similar to amphetamine. It has been grown and chewed for use as a stimulant for centuries.

The analysis of Khat leaves for the purposes of identification and/or quantitation of cathinone and cathine can be challenging due to several factors. The low level of active ingredient(s) found in the plant, concentration variation within different parts of the plant, age of the leaves and the potential degradation cathinone to cathine. Separation of isomeric forms and proper sampling protocols for plant material should also be considered. Because of these factors, sensitive and selective methods are required to provide accurate quantitation of cathinone and other Khat alkaloids.



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Liquid Chromatography/Tandem Quadrupole Mass Spectrometry (LC/MS/MS) was utilized for quantitation of Cathine, cathinone and phenylpropanolamine in plant material. This methodology can also separate the cathine from its isomer phenylpropanylamine that may not be possible in traditional GC/MS methods. These analytical techniques were used to confirm the presence of these substances in dried plant material and provide a quantitative value. Data will be presented using a combination of the above referenced analytical techniques.

Fingerprint Analysis: Extraction Modification and Evaluation via Bioanalytical Assays

Erica Brunelle, Morgan Eldridge, Brenna Thibodeau, Jan Halamek, University at Albany, State University of New York

Fingerprints have been a valuable piece of evidence in many criminal investigations. However, one key element that is often overlooked is that sweat and sebum create the fingerprint image. Sweat contains varying amounts of metabolites produced by the body due to metabolism. In general, sweat is continuing to attract attention from the scientific community. Although difficult to detect at a crime scene, sweat has the advantage of being a noninvasive sample.

To date, there are few known methods for extracting the metabolic content from sweat and fingerprints. Currently, most methods require the use of solid phase extraction (SPE) and/or gas chromatography (GC). However, this only works if the metabolites are volatile – exogenous metabolites. In the case of the endogenous metabolites such as the ones used for biological sex identification, additional sample preparation would be needed to derivatize the metabolites for GC.

In 2015, the Halamek Lab developed a straightforward extraction protocol for use with chemical and biochemical analysis methods which do not need derivatization. The initial protocol was employed for extracting amino acids from fingerprints. Polyethylene film and acid hydrolysis – elevated temperature and mildly acidic conditions – were used to trap the fatty content of the fingerprint and extract the water-soluble metabolites, respectively. This aqueous solution was then used as the sample for analysis.

Despite this protocol working well for our purposes these past few years, there is no way to accurately determine precisely how well it is working – do we actually have the maximum yield of extracted metabolites and are we actually extracting the most efficiently? The ultimate goal is to have an efficient and, most importantly, a specific extraction protocol that can be used to extract specific types of metabolites. If specific groups can be targeted, the concept of identifying multiple originator attributes from a fingerprint because more achievable.



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Semen Identification at a Crime Scene: Using Bioaffinity-based Assays for Analysis of Rape Kits

Elizabeth Weiss, Amy Mascorro, Jan Halamek, University at Albany, State University of New York

According to the Department of Justice, an American is sexually assaulted every 98 seconds, leading to upwards of 300,000 sexual assault and rape cases annually. In sexual assault investigations, rape kits are one of the most backlogged pieces of evidence along with DNA. The traditional methods of rape kit analysis are time consuming and expensive, therefore they sit in evidence rooms for years and many times the assailant is never caught. While nothing can replace a traditional DNA analysis, there is a need for a method that affords the ability to determine the presence of semen in order to conclude if a sexual assault may have occurred. This concept will not directly identify an assailant's identity but will confirm that a sexual assault may have taken place.

In previous research, the Halamek Lab has focused on generic biological evidence that is likely to be found at a variety of crime scenes. Currently, however, the focus has shifted to expanding the portfolio of biological evidence that can be analyzed with similar methods to those that have been used for analyzing blood, fingerprints, and sweat. This new area of research focuses on the development of modified rape kit where a biological sample is analyzed for metabolites that are specific to semen. To determine if the body fluid found at a crime scene is semen, a bioaffinity-based cascade to detect fructose can be used. This is possible because fructose is only found at high levels in semen.

This new area of focus is centered around evidence that is likely to be found at a crime scene where a sexual assault may have occurred. As with our previous research, the ultimate goal is to develop a straightforward analysis method that can be utilized by all members of law enforcement directly at a crime scene, without the need for intensive scientific training.

Investigating Commercially Available microRNA Extraction Kits for Use with Forensically Relevant Body Fluids

Autumn T. Muise, Karly Johannsen, and Claire L. Glynn, PhD. University of New Haven

While current research is highlighting the potential of miRNAs for body fluid identification, little research has been performed to investigate the best method for extracting the miRNA content from forensically relevant body fluids. There are over a dozen commercially available miRNA/RNA extractions kits, with new kits regularly being released onto the market. All miRNA extraction kits currently available, however, have been designed for use with pristine clinical laboratory samples, such as cell cultures, primary tissues, plasma/serum, etc. A kit designed specifically for use with forensic samples –venous blood, semen, saliva, menstrual blood, and vaginal material – is not yet commercially available. The aim of this research was to select four commercially available miRNA extraction kits, and to assess their ability to extract the miRNA content from forensically relevant body fluids in sufficient quantity and quality for downstream analyses.



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Following Institutional Review Board (IRB) approval, body fluids were collected from volunteers with written informed consent. Venous blood was collected by a licensed phlebotomist into EDTA vials. Semen and saliva were collected into sterile conical tubes. Menstrual blood and vaginal material were collected using sterile cotton swabs. All samples were stored at -20°C until extractions were performed. The miRNeasy Mini Kit (Qiagen®), PureLink™ miRNA Isolation Kit (Invitrogen™), magMAX™ mirVana™ Total RNA Isolation Kit (Applied Biosystems™) and High Pure miRNA Isolation Kit (Roche) were the chosen kits in this study. Each miRNA isolation was performed according to the manufacturer's protocol. The extracts were quantified using the NanoDrop™ One UV/Vis spectrophotometer (Thermo Scientific™), and the Qubit 3.0 fluorometer (Invitrogen™), with the RNA HS assay kit. As each kit has a different final elution volume, all results were converted from $\text{ng}/\mu\text{L}$ to total RNA (ng) obtained.

Quantifiable amounts of miRNA were collected from all samples. The quantitation results generated using the NanoDrop™ One UV/ Vis Spectrophotometer showed the miRNeasy kit to be the optimal kit in the majority of the body fluids with yields ranging from 2844-6279 ng total, with the exception of vaginal material, in which magMAX™ mirVana™ Total RNA isolation Kit yielded higher results. The results were verified using the Qubit 3.0 Fluorometer. This quantification method further confirmed that the miRNeasy was the superior kit, however the Qubit showed that the High Pure Isolation Kit was preferred for the extraction of vaginal material. While some kits resulted in extracts that were too low to produce quantitation values, every sample extracted using the miRNeasy kit resulted in quantifiable yields. miRNeasy's miRNA concentrations ranged from 487-12749 ng total RNA across all body fluids. When comparing the cost per sample, ease of use, and additional resources required, per kit, the miRNeasy kit was found to be the most user friendly, least time consuming, and required minimal additional resources. The cost per sample was in the mid-range of all 4 kits. The results of this study contribute to the growing understanding of the potential of microRNAs as a novel tool for forensic scientists.

Following the Transfer of Touch DNA Evidence in Simulated Car Jackings

Amanda G. Araujo, Bay Path University

Touch DNA left on handled surfaces due to the shedding of skin cells has been studied extensively and has even been proven to transfer to a subsequent handler of an item in question. Although touch DNA transfer has been proven in a controlled laboratory setting, there has been very little research on uncontrolled DNA evidence in a crime scene-type setting. It is important to understand how DNA behaves in realistic, or crime scene scenarios, in order to better decipher the evidence in such cases. This study set out to show a difference in relative DNA concentrations between regularly shared vehicles and a simulated car theft, in which an unrelated individual drove another vehicle for a short period of time. By comparing the allele peak heights of each user relative to one another and the amount of time the steering wheels were handled, patterns of DNA transfer were examined. While the results proved a difference in the peak heights of profiles, distinct patterns could not be established due to such a small sample size ($n=5$). Ultimately, this information can aid in establishing the events that took place in criminal acts such as vehicle theft by use of touch



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DNA samples collected. Further research with a larger sample size and more variables tested may yield results in which touch DNA evidence tells a story of the events that occurred to create the profile amplified.

What Can be Done with a Bag of Bone: Utilizing DNA and Osteological Analysis on Human Skeletal Remains from the Flevaeis Plot in Rhodes

McKenna Lohr, B.S., Lisa Ludvico, Ph.D., Duquesne University

When human skeletal remains are found at a crime scene or archaeological site, there are several forms of analysis that can be performed to determine identifying characteristics of the individual(s), including osteological and DNA analysis. In the case of the Flevaeis Plot in Rhodes, two graves were uncovered containing six to seven skeletons each. The dates of the objects buried with the remains range from 3200 BCE to 650 CE, indicating potential occupation of this site by several cultures. Osteological analysis was performed on the remains brought to Duquesne University to determine size/intactness of each bone, the type of bone, side of origin, and in some cases sex, age, and overall health. Based on this data, it is estimated there are twelve bones total from at least four individuals, two males and two females. To determine if this estimation is accurate, DNA analysis was performed in conjunction with osteological analysis. A decalcification and extraction protocol obtained from the Human Identification Center at the University of Northern Texas was utilized to isolate human DNA from the skeletal remains. A mitochondrial DNA (mtDNA) mini primer set created by the Armed Forces DNA Identification Lab (AFDIL) was used on the extracted DNA to amplify and sequence hypervariable region 1 (HV1). This data was used to determine haplogroups which can be used to differentiate between the skeletal samples as well as determine maternal lineage.

Extraction and Sequencing of Human Environmental mtDNA from Natural, Aqueous Environments

Shannon Mahoney, Duquesne University

The identification of human remains belonging to missing persons has long been considered a fairly challenging and universal problem in the field of forensic genetics. However, the recovery of DNA from several different forms of human tissue, bones, and hair has allowed researchers and forensic professionals to further the general effectiveness of missing persons investigations. Despite of this, DNA has still had little implementation as the primary component in the process of locating missing persons. The application of DNA technologies in these investigations would provide a more empirical-based method for the location of drowning victims, persons missing in bodies of running water, or victims of certain mass casualty events. In this study, a protected sample of human epidermal tissue would be submerged in a small body of running water to stimulate a natural decay within the tissue. From the decaying tissue, an expulsion of epidermal cells and other biological matter are to be collected at different incremental distances downstream, such that we would be able to successfully link said downstream material to our source DNA. This study is developed to act as a proof-of-concept study to determine to what extent human DNA can be pulled from natural environments and sequenced from mixed DNA samples amplified using AFDIL mitochondrial mini-primer sets. The utilization of mitochondrial DNA permits us to obtain a higher specificity for mixed or low concentration samples, due



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to the application of the set on multiple hypervariable regions. This effect, matched with the biological material's naturally high copy number, should hopefully produce a high quantity yield of mtDNA from the aquatic matrix. This project will utilize the commonly used Qiagen extraction method in order to demonstrate a universal application of human-based environmental DNA for crime lab use. This form of DNA research will hopefully revolutionize the application that modern eDNA analysis has on science, shifting it away from a purely ecological and anthropological background and permitting its function in the applied forensic sciences.

Using Cell-Free DNA to Improve STR Analysis of Sweat Samples

Nathan McFadden, B.A. Dr. Lisa Ludvico, Ph.D., Duquesne University

Touch DNA (tDNA) samples in the forensic community are known to be difficult samples to obtain ample amounts of template DNA and produce DNA profiles using STR analysis. Cell-free DNA (cfDNA) is DNA present outside the cell and found in bodily fluids such as sweat, saliva, and blood serum. Previous research has thought cfDNA could be used to enhance DNA profiles obtained from tDNA samples. However, they have not been conducted with a cell-free DNA extraction kit. In the first phase of this study, Qiagen extraction kits were used to extract DNA from blood serum samples. The two Qiagen kits used were the QIAmp DNA Mini Kit and the QIAmp Circulating Nucleic Acid Kit. The Circulating Nucleic Acid Kit uses vacuum filtration through a silica membrane with different lysis and wash buffers than the DNA Mini Kit. DNA yields and STR profiles were compared between the two kits and confirmed the Circulating Nucleic Acid kit provided larger amounts of extracted DNA and improved profiles. In the second phase, sweat samples were collected on glass beads then extracted with the Circulating Nucleic Acid Kit and genotyped with Promega Fusion 6C to validate the use of cfDNA for forensic use. If cfDNA can be validated using sweat samples, cfDNA could be a key component in the genotyping of touch DNA samples.

Forensic Characterization and Discrimination of Manila Envelopes

Maria Isabel Sanchez-Melo Virginia Maxwell, D.Phil., Brooke Kammrath, Ph.D., University of New Haven

Envelopes can be found at crime scenes when ransom, threat letters or potentially harmful substances are sent to victims. Therefore, they are important probative items of evidence analyzed by forensic document examiners. Manila envelopes are commonly used in the United States to transport or send documents as they are made of thick and durable paper. Although there are many studies on the forensic analysis of office paper and paper-based banknotes, and only a few on white envelopes, there does not exist previously published research on the physical or chemical characterization of manila envelopes. The goal of this research was to analyze manila envelopes using analytical methods normally applied for the analysis of paper and adhesives with the purpose of characterizing and comparing those sold by different manufacturers as well as the envelopes included in the same and different batches from the same manufacturer.



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Samples from five manila envelopes, size 9x12", from each of the three boxes purchased from ten different brands were examined in order to evaluate whether there are significant differences that can be used for forensic discrimination and/or identification of the manufacturer. The analytical methods were also evaluated to determine the discriminating potential of each when applied to manila envelopes. The analytical methods considered in this research consisted of physical measurements of the envelopes and its folds, color examination, the use of Alternate Light Sources (ALS), and chemical analysis using Thin-Layer Chromatography (TLC), Attenuated Total Reflection Fourier-Transform Infrared (ATR FT-IR) Spectroscopy, Raman Spectroscopy, X-ray Fluorescence (XRF), and X-ray Powder Diffraction (XRD). An analytical protocol for the forensic analysis of manila envelopes was developed, beginning with the non-destructive ones, using the most discriminating techniques previously mentioned.

Comparison of Periodic Acid-Schiff Staining with Lugol's Iodine for the Detection of Vaginal Fluid in Dried Material

Melissa Rogers, Dr. Lawrence Quarino, Cedar Crest College

Forensic Science literature presents a variety of tests to confirm the presence of various bodily fluids; however, such a test for vaginal fluid is still needed. A confirmatory test for vaginal fluid will provide critical context for forensic casework, especially in sexual assault cases where no semen is present. In the past, Lugol's iodine was used as the main method for determining the presence of vaginal fluid by staining glycogen (1). This type of staining however has been shown in the present study to produce positive results for both saliva and urine demonstrating its lack of specificity. Although not frequently used as a test for the presence of vaginal fluid, the Periodic Acid-Schiff (PAS) reagent has been reported to be effective in staining epithelial cells high in glycogen (2). This study aims to compare the ability of these two stains to detect the presence of vaginal fluid in dried material.

Following Institutional Review Board (IRB) approval, multiple vaginal swabs, saliva swabs, and urine samples were obtained from female participants who varied in age, menstrual cycle, and birth control methods. The first stage of this study involved extracting the glycogen from these samples and then adding a 3% solution of Lugol's Iodine to each extract to sample wells in a microtiter plate. The absorbance of each sample was then measured with subsequent quantitative analysis for glycogen using a standard curve. The results of this method indicated that the concentration of glycogen in urine was very similar to that of vaginal fluid, and so could not be distinguished from each other. Concentration values in saliva were also not appreciably lower. Staining of vaginal smears on microscope slides with Lugol's iodine also did not show observable differences between the three fluids. Conversely, for the second stage, vaginal smears showed significant observable differences in glycogen staining with PAS when compared to smears made from saliva and urine. Differences were so pronounced that absorbance assays would simply be redundant. Results between women of varying age were comparable.

Not only do these results bring to light a more effective stain for vaginal fluid, but also provides the foundation for a potential confirmatory test that could be used in serology laboratories. Development of such a test for vaginal fluid will help to provide context for sexual assault investigations.



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The Evolution of Heroin Constituents in Seized Drug Casework

Nicole Lattanzio, Melissa Johnson, NMS Labs

The primary goal of our work is to show the evolution of heroin constituents in seized drug casework over time. This was done by looking at the frequency of detection of adulterants and fentanyl compounds found in seized drug cases that were analyzed by NMS Labs within the period of April 2016 to June 2018 and tested positive for heroin. The change in complexity of heroin samples over time has had and will continue to have a profound effect on all members of the community. This involves an impact on everyone from the users of the drug to the forensic scientists performing an analysis on samples submitted.

NMS Labs performs seized drug testing for many different jurisdictions throughout the nation. Seized drug testing includes gas chromatography mass spectrometry (GC/MS) testing of seized pills, plants, and powders. Cases analyzed between April 1st, 2016 and June 30th, 2018 were reviewed for positive findings. A limitation, especially in seized drug analysis, is that adulterants and other components in the mixture may not be reported. Therefore, the data-mining process included looking at all compounds indicated and not just those reported.

Of the 91,975 exhibits tested over this time period, 23,225 (25%) of the samples were positive for heroin. The relative rates of adulterant and fentanyl compound detection in these heroin-positive samples were compared.

An upward trend for all adulterants and fentanyl compounds within the scope of analysis was observed. The changing composition of heroin samples in casework has led to a need for chemists to modify sampling protocols to deal with these complex mixtures, and a requirement to continue expanding the scope of analysis to include all the new compounds being introduced.

Another trend on the rise is overdose deaths. According to the CDC, heroin-related overdoses increased by 19.5% from 2015 to 2016, with 15,500 deaths in 2016 (1). In 2017, heroin-related overdose deaths were documented at 15,958, according to NIDA, continuing the incline (2). Our data reflects the growing complexity of heroin, which could be a major contributor in the continued rise of heroin-related overdoses.

The Effect of Latent Fingerprint Processing on the Recovery of Gunshot Residue Particles from Latex Gloves.

John Biello, Lynn Schneeweis, Massachusetts State Police Crime Laboratory; Kelsie Jenquine, Boston University

Gunshot Primer Residue (P-GSR) is produced when a firearm is discharged. The primer is contained in the primer cup within the ammunition. When the firing pin strikes the primer cup, an initial explosion occurs which ignites the



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propellant and ejects the projectile. The primer composition is transitioned to a gaseous product released from all openings of the firearm and re-solidifies in the form of rounded, microscopic particles. These microscopic particles are readily distributed on surfaces, including individuals, in the vicinity of the discharged firearm. A particle having a specific morphology and containing lead, antimony and barium is characteristic of a Gunshot Primer Residue particle. Detecting and analyzing P-GSR using the Scanning Electron Microscope coupled with Energy Dispersive X-Ray Spectroscopy has been used and accepted in the scientific community since the late 1970's.

The Massachusetts State Police Crime Laboratory (MSPCL) routinely receives requests to test items for the presence of P-GSR. Periodically, requests to examine the same item for both P-GSR and latent fingerprints are received by the Laboratory. In order to ensure the latent prints are not damaged during the P-GSR process, the fingerprint analysis is routinely performed prior to the item being sampled for P-GSR. However, during the fingerprint analysis process, some mechanical manipulation of the item is necessary and it is known that P-GSR particles are easily transferable or lost from an item, particularly when the substrate item has a non-porous surface texture such as a latex gloves. Consequently, it is possible that some loss of P-GSR material may occur during the latent print analysis process.

The purpose of this study was to evaluate the effect of processing a non-porous item for latent fingerprints on the subsequent recovery and detection of P-GSR. Data collected from this study may be used by the MSPCL to advise law enforcement agencies requesting both P-GSR and latent print analysis on items as to the potential effects that one type of analysis may have on the results of the other type of analysis. In circumstances where one type of testing may preclude obtaining results from the other and an agency must designate which analysis is more forensically relevant to their investigation, these data will assist agencies with making an informed request.

Results of this study and the potential impact, if any, on the testing scheme at MSPCL for these types of requests for analysis will be discussed during this poster presentation.

Examining the Use of Spectroscopic Techniques for Discriminating Cosmetic Foundations

Jessica McFarland, B.S., Thomas A. Brettell, Ph.D., D-ABC, Megan Zellner, M.S.F.S., and Lawrence Quarino, Ph.D., D-ABC, Cedar Crest College

Cosmetic foundations and other personal care products can be found at a crime scene in the form of a smear on clothes or other fabrics. This type of trace evidence could then be used to corroborate accounts of the event, if an unknown sample and a known sample could be compared. There is a wide variety of components that make up foundations, which vary based on the manufacturer, color, and type of foundation. However, little research has been conducted in the way of distinguishing between foundations for forensic purposes. This study examined 34 cosmetic foundation samples from a variety of types and manufacturers using Raman microspectrophotometry, attenuated total reflectance (ATR) infrared spectroscopy, and scanning electron microscopy/energy dispersive x-ray spectroscopy (SEM/EDX). All of the methods used required little to no sample preparation. For Raman analysis, each sample was



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placed directly onto a glass slide, where three different locations of the sample were scanned sixteen times. Using the ATR, each of the samples was analyzed in triplicate using 32 scans at a resolution of 4 cm^{-1} . A background was taken prior to each run. For SEM/EDX analysis, each sample was placed on a piece of carbon tape in order for it to be analyzed. Three different locations on each sample were analyzed at 200 times magnification. A 20 kV beam with a 4.4 spot size was used. The data obtained from all three techniques was determined to be reproducible. For the Raman and ATR data, each sample spectrum was compared to every other sample spectrum in pairs. Of the 561 pairs compared of the Raman data, 90.55% of them could be discriminated. Of the 210 pairs compared of the ATR data, 95.23% of them could be discriminated. Principal component analysis (PCA) was used to analyze the elemental concentrations obtained from the SEM/EDX. Principal components 1, 2, and 3 were found to account for 40.60%, 22.55%, and 14.76% of the variance respectively. Visual examination of 3-dimensional plots showed that replicates of each sample clustered together and could be distinguished from others. These results show that using a combination of the three non-destructive techniques shows the potential to differentiate between 34 cosmetic foundation samples.

Species Identification of Necrophagous Beetles by Chemometric Processing of DART-HRMS Chemical Signatures of Ethanol-Insect Suspensions

Amy Osborne, Justine E. Giffin, Rabi A. Musah Ph.D., University at Albany, SUNY; Jennifer Y. Rosati, Ph.D., John Jay College of Criminal Justice.

One of the most important aspects of a death investigation is estimation of time since death, also known as postmortem interval (PMI). This can be difficult to assess for remains that are in advanced stages of decay. Carrion insects found on and near the body can assist forensic investigators in accomplishing this task, as there is a well-established correlation between a given stage of decomposition, and the insect species that colonize the remains. Since the timeline associated in the insect progression through various life stages is well established, knowledge of the species of the retrieved entomological evidence can be used to calculate PMI. For this reason, accurate species identification is critical. However, this process is challenging because different insect species are often morphologically similar at a given life stage. Thus, species identification often requires the time-consuming exercise of rearing eggs or larvae to maturity so that the species can be identified by examination of the gross physical features of the adult. Although species identification can be accomplished more rapidly through DNA analysis, the absence of mapped genomes for the vast majority of necrophagous insects makes this approach impossible in most cases.

It is demonstrated here that chemometric processing of direct analysis in real time mass spectrometric data acquired from analysis of insects, can be used to rapidly accomplish species identification. Eighteen species of necrophagous insects belonging to the Diptera and Coleoptera orders were studied, such as *Prochyliza xanthostoma*, *Necropilia americana*, *Creophilus maxillosus*, and *Necrobia violacea*. DART-HRMS analysis was performed on ethanol suspensions of each sample, in replicates of five. As adult specimens of entomological evidence are often stored in dried form, the optimal conditions for insect rehydration prior to mass spectral analysis were also investigated. With regard to sample preparation, while it was determined that species-specific DART-HRMS chemical fingerprints could be acquired using dried, non-hydrated samples, consistent results were obtained when dried samples were suspended in aqueous ethanol for at least 24 hours. Suspending the samples for more than 24 hours was found to confer no added advantage, nor was an advantage seen in rehydrating the insects with steam prior to placing them in suspension. The results showed



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that the ethanol suspensions of each species exhibited a unique chemical fingerprint, and that these fingerprints were consistent for members of the same species but different between species. The application of Kernel Discriminate Analysis (KDA) to the data revealed that clear differentiation is possible between insect species through the chemical fingerprints generated by DART-HRMS analysis. Further, the separation occurs even between insects belonging to members of the same family, such as the Silphidae family.

The results of this work form the foundation for the creation of a database of necrophagous insect chemical signatures that can be used to rapidly identify species in order to facilitate more rapid PMI determination.

The World of Psychoactive Peppers - The Quantification of Yangonin in *Piper methysticum* aka "Kava" by Direct Analysis in Real Time-High Resolution Mass Spectrometry

Megan I. Chambers, Amy M. Osborne, Rabi A. Musah, Ph.D., University at Albany - SUNY

Piper methysticum, referred to as Kava, is a mind-altering plant substance endemic to the Pacific Islands that has been identified by the United Nations Office on Drugs and Crime (UNODC) as one of the 20 plants of concern containing psychoactive molecules. A protocol has been developed for analysis of this plant material with direct analysis in real time-high resolution mass spectrometry (DART-HRMS). In order to develop a method to confirm the presence of Kava, as well as for the quantification of relevant psychoactive compounds, DART-HRMS can be used to rapidly screen for the analytes of interest. The presence of all six major kavalactones (yangonin, desmethoxyyangonin, kavain, dihydrokavain, methysticin and dihydromethysticin) in Kava products can be confirmed with this method. Because yangonin is a psychoactive component of Kava plant material, a standard curve developed using calibrators ranging from 5 to 200 ppm and an internal standard of yangonin- d_3 was used in order to quantify the amount of yangonin in commercially available Kava products. DART-HRMS allows for the peak integration of individual samples based on selected m/z values. Although yangonin and yangonin- d_3 have nominal m/z values of 259 and 262 respectively for $[M+H]^+$, the corresponding isotope m/z values of 260 and 263 were used instead. This was done to avoid the often observed overloading of the yangonin $[M + H]^+$ peak in non-isotopically-labeled samples, which lead to inaccurate peak area ratios that did not represent the true concentration of yangonin in samples. The ratio between the yangonin and yangonin- d_3 isotope peaks was preserved in all calibrators and Kava study samples, and could therefore be reliably used to develop a standard curve to confirm the yangonin concentration in quality control (QC) samples, and accurately determine the unknown yangonin content in Kava products. In accordance with guidelines set by the U.S. Food and Drug Administration (FDA), all calibrators and QC replicates at each QC level for each run of the standard curve were within acceptable percentages of their theoretical concentrations, with all standard curves producing R^2 values ≥ 0.9990 . With the success of three validated runs, the protocol was considered acceptable for application to study samples with unknown concentrations of yangonin. The samples consisted of a variety of 18 products including capsules, paste, roots, powders, and tinctures/liquids from a total of eight vendors. Sample preparation of Kava products involved lyophilization of the tinctures and liquid samples, followed by extraction of all study samples using ethanol with subjection to multiple rounds of sonication and centrifugation in order to optimize the extraction of yangonin. Although one tincture and one liquid sample fell just below the validated curve range, the remaining 16 Kava products analyzed contained yangonin concentrations that fell within the linear range of the standard curve. Tincture and liquid yangonin concentrations were



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between 1.0307 and 4.5863 mg/mL, while the solid samples exhibited a range from 2.7086 to 8.9940 mg/g. Future work for this project includes developing similar protocols to quantify the psychoactive components of other plant materials reported on the UNODC's 20 plants of concern list.

Optimizing a Method for DNA Recovery while Preserving Latent Prints on Paper

Niti Dalal, Patrick McLaughlin, Mechthild Prinz, John Jay College of Criminal Justice

Paper evidence is commonly encountered in cases like kidnapping, threatening letters, extortion and bank robbery and the optimal workflow between latent print processing and DNA collection is of interest for the forensic community. The overall aim of this project was to achieve optimal amounts of DNA for typing without destroying the fingerprint pattern. The first study compared two collection techniques—tape-lifting by Scotch Removable Poster Tape and dry swabbing with FLOQSwabs— using prints deposited in defined locations. Samples were extracted with QIAamp® DNA investigator kit (Qiagen) and tested using Quantifiler Trio and Identifiler Plus (Thermo Fisher Lifetechnologies); fingerprints were developed with 1,2-indanedione and examined with alternative light source at 455 nm. The second aim was to determine if visualizing print locations prior to DNA collection would impact success rates. We developed samples with fluorescent powder prior to tape lifting and 1,2-indanedione treatment. Each study involved 11 volunteers who were asked to wash and dry their hands, rub their face for 5 seconds and deposit both hand prints on copy paper for 5 seconds.

The results showed that tape lifting gave full DNA profiles for all volunteers and 76% of tape-lifted fingerprint pattern when developed were of value for comparison. On the other hand, many partial profiles were seen with swabbing technique and 57% of swabbed fingerprints when developed were of value for comparison. DNA concentrations ranged from 0.01-0.39 ng/ul for tape-lifting and 0.0009-0.06 ng/ul for swabbing. The fluorescent powder developed prints showed reduced DNA concentrations (0.0007-0.21ng/ul) after tape lifting but still yielded 9 out of 11 DNA profiles. The study indicates that Scotch removable poster tape provides higher DNA yields than the FLOQSwabs and enough material is left to develop the prints with 1,2-indanedione even after treatment with fluorescent powder.

The Prevalence of Male DNA Under a Female's Fingernails

Alexis Baxter and Janine Kishbaugh, Cedar Crest College, Elayne Schwartz and Holly O'Connor, Westchester County Forensic Science Laboratory

The frequency of finding a male DNA profile under a female's fingernails is unknown. When a victim comes into contact with a male assailant during a violent or sexual assault, DNA genotyping is used to find male DNA on the victim. However, finding male DNA may not necessarily reflect the context on how the transfer occurred and may not be the profile of the assailant. Considering that DNA methodologies have improved with sensitivity, transfer may occur through casual contact. In addition, secondary and even tertiary transfer may be detected. Assessing the random nature of male to female transfer is the basis for this study. Female participants in this study either lived with or



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frequently encountered male individuals in their daily lives. A female that lives alone was used as a control. All samples were collected via cutting with fingernail clippers. All nails from one hand were combined and analyzed together. DNA from these samples were then extracted using a QIAamp® DNA Investigator kit, followed by quantitation via real-time PCR using Promega's Plexor® HY. Amplification was performed with the PowerPlex® Y23 system. Genotyping was performed using an Applied BioSystems 3130XL and fragment analysis performed with GeneMapper® IDX v1.5. Of the 42 samples tested, 90% produced partial profiles from single and multiple contributors. Over half of the samples had multiple contributors. In some cases, profiles were linked to male co-habitants. Since only partial profiles were obtained in this study, it is hypothesized that full male profiles obtained during casework is more indicative of close contact like that seen in assault cases.

Phylogenetic-Based Differential Extraction: A First Step to Leverage Universal DNA Profiles

Molly Dunegan, Michael Marciano, Assistant Research Professor, Syracuse University Forensic and National Security Sciences Institute

Forensic DNA analysis routinely focuses on human DNA analysis; however, an unknown biological sample contains more information than just that of a single organism. This project represents the first phase in developing a system of identifying multiple organisms in a sample including plants, animals and microorganisms. Differential lysis and extraction methods are currently being developed to separate the DNA present in an unknown sample into three respective categories at the kingdom level, based upon differences of the cellular components. In particular, the composition and "thickness" of the cell wall and cell membrane of animal, bacteria, and plant cells allow one portion of a mixed sample to be lysed while retaining the integrity of the remaining organisms. Separation of one phylogenetic component will then permit the remaining components to be lysed and extracted with minimal contribution from the preceding component. Each type of lysis in this differential method involves a slightly different chemical composition of lysis buffer and incubation that has been modified for the specific targeted organisms. The separation of DNAs from differing contributing phyla in unknown samples will increase interpretability through decreasing complexity in sequencing applications.

The end goal is to enable the identification of a select yet informative group of species present in unknown samples. Recent technological advancements such as global species geolocation databases will henceforth enable the possibility for this research to aid in tracking samples from locations in which they are obtained back to their origin. This potential could substantially impact innumerable scientific developments and various types of forensic trafficking casework, as an abundance of differing species may be present within each unknown sample.

Three kingdom-specific universal barcode markers for *E. coli*, *H. sapiens*, and *A. thaliana* were chosen to represent the three categories of organisms. Each representative was extracted and amplified with their respective markers separately, utilizing the portion of the differential lysis and extraction protocol designed for that organism, to ensure that the procedure was successful. The organisms were combined, and the full differential extraction results are currently pending.



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Examining the Possible Difference Between Venous and Menstrual Blood Through the Methylation Status of the hCG- α Protein as a Means of Identification

Alexandra Farah, Dr. David San Pietro, University of New Haven

In the field of forensic science, there is currently no field test that can be used to differentiate between venous blood and menstrual blood at a crime scene. However, it is well known that there are differences between the two kinds of blood. Menstrual and venous blood have different component proteins, which helps to explain their different functions in the human body. One of these proteins is Human Chorionic Gonadotropin (hCG), which is found during a women's menstrual cycle (Zimmerman et. Al) Using the beta form of this protein, this study focuses on finding the hCG- β protein in menstrual blood, seeing if it is also in venous blood, and comparing the level in which the protein occurs to see if it is possible to develop a differentiating assay for identification purposes.

After obtaining Institutional Review Board (IRB) approval, each volunteer was asked to sign a consent form and donate both venous and menstrual blood. The venous blood was collected by a certified phlebotomist in EDTA treated vials, and the menstrual blood was collected using sterile cotton swabs by the volunteers themselves. Both kinds of blood samples were then stored in a refrigerator at 8°C until used.

The venous and menstrual blood DNA was then extracted using the QIAamp® DNA Investigator Kit following kit protocols – *Isolation of Total DNA from Small Volumes of Blood or Saliva*, and *Isolation of Total DNA from Surface and Buccal Swabs* – for the venous blood and menstrual blood, respectively. The extracted DNA was then quantified using the NanoDrop™ One UV/Vis Spectrophotometer and the Qubit® 3.0 fluorometer. The extracted DNA was then treated to an EpiTect® Bisulfite Treatment according to the kit protocols. After, the converted DNA was prepped for qPCR using a Custom Taqman™ Gene Expression Assay and run through qPCR. The assay probe was created using a known hCG- β sequence found in the National Center for Biotechnology Information (NCBI) Database, with primer3 software.

Relative qPCR was run on the Applied Biosystems® 7500 Real-Time PCR System to determine each sample's cycle threshold (C_T) values. The results showed that not only did the protein present itself in menstrual blood, it also presented itself in the venous blood. Each blood sample was run with five replicates of venous and menstrual blood. The average C_T values of each type of blood was different – with menstrual blood having a C_T value range from 34-36 cycles and venous blood having a range from 32-34 cycles. When the standard deviation of each type of blood was calculated, it concurred with the idea that the C_T values were not overlapping. This proved to be statistically significant ($p < 0.01$) and will further be examined with a larger sample size and more testing. This research showed that there is a possible difference in menstrual and venous blood regarding hCG- β protein levels, as a result of bisulfite conversion. Future research will be to determine if these results are also shown when collecting samples from dried blood stains on different surfaces.



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The Necessity of an Objective Measurement for Rigor Mortis

Reed M. Flanders, Syracuse University

Rigor mortis is one piece of the puzzle that investigators use in order to determine the approximate postmortem interval of a decedent. Ideally, when estimating time of death, investigators will utilize both temperature and non-temperature based methods. The re-establishment of rigor mortis after mechanical loosening, or breaking, is one of the frequently used non-temperature based methods for determining time of death. However, there are currently discrepancies in studies in regard to the hours post mortem (hpm) at which re-establishment can occur. Past literature has primarily claimed 8 hpm as the final time point at which re-established rigidity can be present. Yet, two recently published articles provide evidence for this phenomenon occurring up to 20 hpm. While these recent articles provide important findings in regard to the re-establishment of rigor mortis, they have a key methodological limitation that may undermine their conclusions. This limitation is that the rigidity of rigor mortis in each of these studies was assessed subjectively (e.g., joints were flexed and the rigidity was labeled as negative, moderate, intermediate or strong, based on the authors own determination). Thus, the subjective nature of the assessment makes the evidence far weaker than if measured through the use of an objective measurement. Therefore, these discrepancies could be a result of the subjective nature for assessing rigidity. This review provides recommendations for strengthening the methodology of future of research into the assessment of the reappearance of rigor mortis. Several invasive and non-invasive objective measures of rigor mortis are presented. The hope is that this review will assist experts and researchers in the field to implement more rigorous, objective assessments when investigating the re-establishment of rigor mortis. In doing so, more definitive conclusions can be reached on an accurate time period for the presence of both rigor mortis in general, as well as its re-establishment.

Modeling Stutter and Pull-up using GlobalFiler in Forensic DNA Analysis

Angie Zhao, Michael Marciano, Jonathan Adelman, Syracuse University

Forensic DNA analysis has become an irreplaceable tool in the science field. Its use can convey information about its contributor, familial relationships, phenotypes and even ancestry. In order to utilize this information, however, biological samples must undergo processing such as DNA extraction, amplification and detection followed by interpretation. This manipulation can introduce non-biological, process related, artifacts that can complicate DNA profile interpretation, namely pull-up and stutter. This project focuses on developing detailed models of stutter product formation and pull-up, with the goal to enable automated identification and removal of non-allelic influence from the profile.

Stutter is defined as the process of slipped strand mispairing and leads to non-allelic peaks in a DNA profile that can be one or more repeat units than the true allele peak. Pull-up is a failure of the analysis software to differentiate between the various fluorescent dye colors used to generate the DNA replication results. It is due to spectral overlap where each dye's broad fluorescent spectrum can intersect with each other. This is quite prominent in multiplex



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systems where multiple reactions occur simultaneously and multiple dye colors must be used. Pull-up in DNA profiles can create additional peaks similar to stutter and can be mistaken as true allele peaks. Just like stutter, pull-up must be addressed to provide a more accurate DNA profile interpretation. This project will provide important insight into DNA interpretation through providing more accurate and reliable means of artifact identification and will also contribute to understanding the GlobalFiler system, a relatively new amplification kit that can be further for current and future users of DNA analysis. We analyzed previously amplified DNA data provided by many participating labs using the GlobalFiler system to model the stutter percentage, or the percentage of expected stutter product based on the height of the parent/true allelic peak, as well as pull-up. Stutter was modeled using non-linear equations such as the Weibull equation. Pull-up was modeled using a machine-learning algorithm known as Symbolic Regression. Our results found that over 90% of stutter and pull-up alleles were correctly identified and removed.

Confirming False Positive Rates in Immunochromatographic Assays Using Protein Mass Spectrometry and Microscopic Analysis

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Studies have highlighted a growing national problem regarding the number of untested Sexual Assault Kits (SAKs). A 2011 National Institute of Justice report revealed Los Angeles alone had 10,000 untested SAKs. This backlog has fueled the need for specific and efficient testing of SAK evidence. In traditional workflows, serology is used to indicate the presence of a targeted bodily fluid and prioritize samples for genetic analysis. However, given the lack of sensitivity and specificity of modern serological assays, current SAK workflows skip serological identification altogether for a “direct to DNA” approach. While these Y-Screen workflows achieve rapid screening of samples for the presence of a detectable male contributor, they do not provide any serological information. As a result, samples lack what can be a critical investigative context. Improved serological capabilities with enhanced sensitivity and specificity would provide greater confidence in results for the confirmatory identification of seminal fluid. At a minimum, forensic biologists should understand the limitations associated with traditional serological approaches to seminal fluid identification when processing SAK samples.

Current serological techniques based on antigen-antibody binding have exhibited not only sensitivity but specificity limitations. False positive results can be obtained by non-target biological fluids such as breast milk, urine, and vaginal fluid, or non-specific binding events. This study evaluates a promising emerging technique that combines high specificity protein biomarker detection with targeted mass spectrometry. Human-specific peptide markers for seminal fluid proteins were targeted and peptide standards were synthesized and used to perform quantification of seminal fluid peptide targets using an Agilent 6495 mass spectrometer coupled to a 1290 series liquid chromatograph. This approach has shown to be both more specific and sensitive in identifying a bodily fluid compared to current immunological based approaches. Thus, this proteomic workflow was used to evaluate authentic false positive rates of current immunochromatographic techniques for seminal fluid identification.



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Self-collected vaginal swabs collected from participants **not** engaging in barrier-free vaginal intercourse with male partners were tested using various immunochromatographic assays designed to detect both Sg and PSA (ABAcad® p30 Test, SERATEC® PSA Semiquant, and RSID™-Semen). Similarly, three seminal fluid biomarkers (Semenogelin 1, Semenogelin 2, and prostate specific antigen) were used for seminal fluid identification via mass spectrometry. Any samples producing positive results on any immunochromatographic assay were evaluated to determine whether the target protein was actually present at levels above the reported sensitivity limits of the lateral flow tests. Additionally, Sperm HY-LITER™ was used to microscopically confirm the absence of spermatozoa in all samples producing positive immunochromatographic results.

In a sample set of 100 vaginal swabs, a total of 9 false positives were observed, 7 of which were consistent with PSA and 2 with Semenogelin, for a corresponding total false positive rate of 9%. These samples were all confirmed negative with mass spectrometry and microscopic analysis. This data supports the use of current immunochromatographic assays for the *presumptive* detection of seminal fluid while also providing further support for the improved specificity of alternative serological approaches using mass spectrometry identification of biological targets.

Recovery of touch DNA: a comparison of four collection methods on various substrates

Rachel Gilmore, Emily Neverett, Claire L. Glynn, Ph.D., Department of Forensic Science, Henry C. Lee College of Criminal Justice and Forensic Science, University of New Haven

When a person comes in contact with an item, epithelial skin cells are transferred from person to surface in varying amounts. Therefore, it can be suggested that victims of forced child labor inadvertently shed their epithelial skin cells onto the items they are manufacturing. These cells can be recovered from protected interior surfaces where only the person manufacturing the item would have touched. DNA isolated from shed cells is commonly known as touch DNA. Donor age estimation of touch DNA samples is currently being researched using DNA methylation analysis and shows great promise. It is crucial to choose a collection method that optimizes the recovery of as many cells as possible. There are several methods currently employed for touch DNA collection within accredited crime laboratories; however, there is no globally accepted standard for recovery from different substrates. An extensive search of published literature revealed the wet/dry double swab method, the sodium dodecyl sulfate (SDS) swab method, and the mini-taping method to produce the most consistently high yields of touch DNA. More recently, a novel gel film was suggested as an ideal method for touch DNA collection, with the added benefit of visualizing the cells microscopically on the gel surface prior to extraction. The aim of this research was to investigate these methods on a variety of substrates selected to be representative of products manufactured by child laborers.

Following approval from the Institutional Review Board, with informed written consent, one volunteer was selected to deposit touch DNA on all samples to ensure consistency. Eight substrates were chosen: cotton, denim, felt, polyester, plastic, ceramic tile, wood, and cardboard. To mimic the manufacturing process, the volunteer sewed a double seam on each fabric sample using a sewing machine, thus trapping the volunteer's epithelial skin cells in the seams. For the



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other surfaces, flat 4" x 5" sections were rubbed by the volunteer's hand five times with approximately the same force each time. Following deposition, the four collection methods were used: wet/dry double swabbing, SDS swabbing (2% SDS solution), mini-taping (Scenesafe FAST™ Pack), and gel film (Gel-Pak®). The QIAamp DNA Investigator kit (Qiagen®) was used to extract DNA from collected samples, following the manufacturer's protocol. All samples were eluted in a final volume of 50 µL. Quantitation was performed using the Qubit 3.0 Fluorometer (ThermoFisher Scientific) using the double stranded (ds) DNA High Sensitivity (HS) assay kit. Varying DNA concentrations were obtained from all surfaces with each collection method. On the fabric samples, the mini-tapes recovered the most consistently high concentrations (0-0.314 ng/uL) of DNA. On the other surfaces, the wet/dry double swab method recovered the most consistently high concentrations (0-2.68 ng/uL) of DNA. These yields obtained are sufficient for downstream processing, including DNA profiling and methylation analysis.

The results of this study provide a valuable contribution to the forensic science industry by highlighting optimal touch DNA collection methods for particular surfaces. Additionally, this research contributes to the ongoing efforts for age-estimation of touch DNA samples to combat forced child labor.

Developing a Method to Quantitate Carboxyhemoglobin Using Raman Microspectrophotometry

Haley Ann Melbourn Marianne Staretz, Ph.D., Thomas Brettell, Ph.D., D-ABC, Cedar Crest College; Heather Maldonado, M.S., State of Delaware Division of Forensic Science

Carboxyhemoglobin quantitation is a routine toxicological analysis performed in cases of acute carbon monoxide poisoning, which could be helpful, or even critical, in the processing of both civil and criminal cases. Quick and easy to use methods of carboxyhemoglobin quantitation include spectrophotometry and CO-oximetry, although these methods tend to be inaccurate and imprecise both at low concentrations and in putrefied post-mortem specimens. Reliable gas chromatographic quantitation methods are available as well, but these require extensive sample preparation and therefore more time for analysis. The development of a Raman spectrophotometric quantitation method has the potential to provide more reliable results with a shorter acquisition time without destroying the sample.

Carboxyhemoglobin and oxyhemoglobin samples were generated by bubbling carbon monoxide and free air, respectively, through whole bovine blood preserved in K2EDTA for 30 minutes to ensure complete saturation. Using the appropriate mixtures of 100% carboxyhemoglobin and 100% oxyhemoglobin, standards ranging from 10% carboxyhemoglobin to 90% carboxyhemoglobin were made. A 30 uL aliquot of each standard, 100% carboxyhemoglobin, and 100% oxyhemoglobin was deposited onto an aluminum foil covered glass microscope slide. All samples were left to air dry completely prior to analysis.

In this study, a Thermo Scientific DXR2 Raman Microscope was utilized and samples were viewed under 100x magnification. Samples were interrogated using a DXR 785 nm laser with a laser power of 4.0 mW to prevent photodegradation and damage to the sample. The acquisition of each sample included 20 exposures, each lasting 10



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seconds. For each sample, three Raman spectra were obtained from different locations to generate an average spectrum to represent that sample. A comparison of the average spectra of the 11 samples showed differences with changing carboxyhemoglobin saturation, indicating the potential for quantitation to be possible.

Forensic Body Fluid Identification with the Epiect® Bisulfite Kit and the Reliability of DNA Methylation with Real Time qPCR

Mandy Pascu, Dr. David San Pietro, University of New Haven

The goal of this presentation is to display potential advancements in forensic body fluid identification. The body fluids being examined were analyzed by their methylation differences. The body fluid DNA samples underwent a bisulfite conversion and were analyzed with real time qPCR. The data gathered may allow for the multiplexing of different body fluid primers to be incorporated into this technique for identification purposes.

Body fluid detection is useful in aiding in the interpretation of events at a crime scene. The identification of body fluids can aid in the possible reconstruction of the order of events, as well as the interpretation of genetic testing results (DNA). Discovering the origin of the body fluid is important to support statements made involving a crime. Currently, there is not a universal confirmatory test used to accurately and specifically identify these different body fluids simultaneously. Developing a technique that can meet these criteria can be vital in circumstances where mixtures are involved or when the amount of DNA is limited.

Methylation is the addition of a methyl group onto a cytosine base in DNA. DNA methylation effects the functional role of transcription that in turn influences gene expression. Due to this, various tissues throughout the body display diverse methylation patterns as they require different traits to be expressed for optimal functionality. Recent studies have found a variety of body fluids to have potentially differentiating methylation specificities.

Following the Institutional Review Board (IRB) approval, blood, saliva, vaginal fluid and menstrual blood were collected from five female participants with informed written consent. Genomic DNA was extracted from these body fluids with the QIAamp® DNA Investigator Kit. The genomic DNA underwent a bisulfite conversion with the Qiagen Epiect® Bisulfite Kit. This kit converts unmethylated cytosine bases into uracils. Uniquely colored florescent probes were created for previously determined methylation specific sequences for blood, saliva and vaginal fluid. The samples were analyzed with the Applied Biosystems® 7500 real time qPCR instrument with 7500 system SDS Software (v1.2.2f2) to determine cycle threshold values for each body fluid within their designated probe sequence. The CT values were also collected for each body fluid with the alternate probe sequences to compare methylation expression differences, as well as any possible cross reactivity.



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Preliminary results indicate that saliva and vaginal fluid CT values are significantly different from each other (p -value < 0.014). The results were analyzed with GenStat 16th edition (v16.2.11713) using a two-way ANOVA. Statistical analysis is currently ongoing to determine if the body fluids can be successfully multiplexed and differentiated from each other. Further work will be conducted to analyze the multiplexing technique with mixture samples.

Screening of Selected Synthetic Cannabinoids in Human Urine Utilizing LC-(QqQ)MS/MS Direct Urine Analysis

Rahman Farzam, Woolaghan Valerie, Constantinou Pistos, Department of Chemistry, West Chester University

New synthetic drugs of abuse have become increasingly popular and are highly consumed in the last ten years, since their first identification in drug-like products in 2008. Many drug users consume herbal mixtures as a legal alternative to cannabis products to circumvent drug testing. The emergence of ever new designer drugs is an ongoing challenge for analytical toxicologists in forensics, because they are covered only by limited established analytical methods and these new drugs may cause serious toxicity or impairment.

The aim of the present study was to investigate a novel, simple and rapid Liquid Chromatography tandem mass spectrometry (LC-MS/MS) method of direct urine injection analysis, for the identification and quantification of selected synthetic cannabinoids (AB-CHMINACA, AB-FUBINACA, AB-PINACA, AMB-CHMINACA, ADB-FUBINACA, ADB-PINACA, APP-CHMINACA, APP-FUBINACA, 5F-AB-PINACA, 5F-ADB-PINACA, and 5F-ADB) in human urine. The main advantage of the proposed methodology is the minimal sample preparation procedure, as diluted urine samples are directly injected into the LC-MS/MS system after filtration. Agilent Jet Spray ionization in positive mode using Multiple Reaction Monitoring (MRM) was chosen for the identification and quantification of the analytes. After the initial dilution, the urine sample is injected into the LC-MS/MS, and the compounds are separated on a Poroshell EC-C18 (2.1 x 100 mm 2.7 μ m) reversed-phase column. Mobile phase consisted of a gradient program using ammonium formate 10 mM/formic acid 0.1% (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The method validation results for selectivity, specificity, linearity, precision, accuracy, recovery and ion suppression will be presented.

The method demonstrates simplicity and fast sample preparation, accuracy and specificity of the analytes which makes it suitable for preliminary screening procedures. Using this method, laboratories may overcome the cross-reactivity issues which are associated with immunoassays at low cost and comparable run time.



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Detection of Explosive Residue on Gloves Exposed to Environmental Conditions

Shannon Lamy, B.S., Alyssa Marsico, Ph.D., Department of Forensic Science, University of New Haven

Homemade improvised explosive devices (IEDs) are commonly used across the world, so identifying the type of explosive used and perpetrator are important. If gloves are worn while creating an IED, explosive residue could be present on the gloves and this can then be used as evidence to link the criminal to the device. The topic of this research project is the detection of explosive residues removed from various glove materials after exposure to a range of environmental conditions. This research will be used to determine how well different explosive compounds can be detected off of these surface types and to determine if this changes with different environmental exposures.

The detection limits of the explosives TNT, PETN, and TATP using Gas Chromatography – Mass Spectrometry (GC-MS) off of nitrile gloves, leather working gloves, cotton gloves, and mechanic gloves were investigated to determine how well each explosive can be detected on each glove. The explosives were deposited onto the gloves, then different swabbing methods were tested to determine what method is best able to extract the explosives from the gloves. The swabbing methods tested included rayon swabs, cotton balls, alcohol wipes, a solvent flooding method, and direct extraction from the glove itself. These methods were tested with two different extraction solvents; methanol and acetonitrile. The samples were then analyzed using GC-MS and the peak areas from the chromatograms were compared to determine which swabbing method was best by determining the extraction efficiency for each method. A preliminary test was conducted using the compound 4-Nitrophenol to gain insight into expected extraction efficiencies of the different swabbing methods. These results indicated that the highest extraction efficiency was from directly extracting the compound off of the glove in acetonitrile, followed by the flooding method and the cotton ball swabbing methods. Preliminary results using TNT suggest that using an alcohol wipe or a rayon swab are efficient methods to swab TNT off of cotton gloves. However, several unidentified peaks were also present in the chromatogram when using the rayon swab to extract TNT. These peaks could be trace material that was already present on the glove before the explosive was added, suggesting that the rayon swab method is an efficient extraction technique.

After determining the most efficient extraction method, the effect of cleaning products was investigated. Preliminary results lead to the discovery of new unidentified peaks present in mixtures of explosive compounds with Simple Green cleaning solutions. These unidentified peaks warrant further investigation to determine their identity and origin. Future studies will involve exposing gloves to environmental conditions including simulated wind, rain, shaking/movement, contamination, and time to determine the effect of each on the detection limits of the explosives and the identity of the compounds detected.

The results of this research indicate the optimal swabbing method for different explosive compounds on a variety of gloves as well as how various conditions affect how well they are detected. These results can provide useful information in cases involving explosive residue on gloves, or similar fabric.



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Plenary Session

What in the OSAC is Happening?

The Organization of Scientific Area Committees (OSAC) for Forensic Science works to strengthen the nation's use of forensic science by facilitating the development of technically sound forensic science standards and by promoting the adoption of those standards by the forensic science community. These standards are written documents that define minimum requirements, best practices, standard protocols, and other guidance to help ensure that the results of forensic analysis are reliable and reproducible. OSAC is administered by the National Institute of Standards and Technology (NIST), but the great majority of its more than 550 members are from other government agencies, academic institutions, and the private sector. These members have expertise in twenty-five specific forensic disciplines, as well as general expertise in scientific research, measurement science, statistics, law, and policy. OSAC members work together to develop and evaluate forensic science standards via a transparent, consensus-based process that allows for participation and comment by all stakeholders.¹

OSAC guidelines are vetted through standards developing organizations (SDOs) such as the AAFS Academy Standards Board, ASTM International or the American Dental Association. This Plenary Session will provide a clear understanding of the standard development processes as well as updates from various OSAC Subcommittees including developments at the SDO level.

Chair: Erin C. Luck, Pennsylvania State Police

8:30 – 8:50 American Academy of Forensic Science (AAFS) Academy Standards Board (ASB) Standard Development Process

Jennifer F. Limoges, M.S., New York State Police Forensic Investigation Center

The AAFS Standards Board, LLC (ASB) is an ANSI-accredited Standards Developing Organization (SDO). Its purpose is to develop American National Standards, Technical Reports and Best Practice Recommendations.

8:50 – 9:10 ASTM International Standard Development Process

Agnes D. Winokur, M.S., Drug Enforcement Administration, Southeast Laboratory

¹ <https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science>



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ASTM International is a globally recognized leader in the development and delivery of voluntary consensus standards. The Organization of Scientific Area Committees (OSAC) for Forensic Science through the National Institute of Standards and Technology (NIST) has entered into a contract with ASTM International that gives 30,000 public criminal justice agencies free access to standards published under ASTM Technical Committee E30 on Forensic Science.



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9:10 – 9:30 NIST OSAC Facial Identification Subcommittee Update

Todd Putorti, New York State Dept. of Motor Vehicles, Division of Field Investigations

The Facial Identification Subcommittee focuses on standards and guidelines related to the image-based comparisons of human facial features.

9:30 – 10:00 NIST OSAC Fire Debris and Explosives Subcommittee Update

Dennis C. Hilliard, M.S., Rhode Island State Crime Laboratory

The Fire Debris and Explosives Subcommittee focuses on standards and guidelines related to the scientific examination and analysis of materials associated with fire and explosion investigations.

10:00 – 10:30 Break

10:30 – 11:10 NIST OSAC Biological Data & Reporting and Biological Methods Subcommittees Update

Mechthild Prinz, PhD, John Jay College of Criminal Justice

The Biological Data Interpretation and Reporting Subcommittee focuses on standards and guidelines related to scientifically valid methods of interpretation, statistical analysis and reporting of biological results. The Biological Methods Subcommittee focuses on standards and guidelines related to molecular and biochemical methods used to analyze evidence and reference items.

11:10 – 11:30 NIST OSAC Toxicology Subcommittee Update

Sabra Botch-Jones, M.S., M.A., D-ABFT-FT, Boston University School of Medicine

The Toxicology Subcommittee focuses on standards and guidelines related to the analysis of biological samples for alcohol, drugs, or poisons, and the interpretation of these results.

11:30 – 11:50 NIST OSAC Seized Drugs Subcommittee Update

Agnes D. Winokur, M.S., Drug Enforcement Administration, Southeast Laboratory



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The Seized Drugs Subcommittee focuses on standards and guidelines related to the examination of evidence to identify drugs and related substances.

11:50 – 12:05 NIST OSAC Bloodstain Pattern Analysis Subcommittee Update

Peter Valentine, M.S., University of New Haven

The Bloodstain Pattern Subcommittee focuses on standards and guidelines related to the scientific detection and analysis of bloodstain patterns present at crime scenes and on associated evidence.

12:05 – 12:15 Questions/Discussion



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General Session

Building Generational Bridges Through Effective Communication

For the first time in history, there are five definable and different sets of “Generations” attempting to function within the workplace. This wealth of diversity can either be a tremendous benefit to the Forensic Science laboratory or a source of continual strife that can potentially paralyze even the most dedicated laboratory team.

This presentation provides the Forensic Science Professional the tools and techniques to bridge the gap between the seemingly insurmountable generational differences and allow the formation of synergistic teams that are more capable than the sum of their individual members.

The program will:

- Identify the five generations in the workplace, and define them by experiences and events.
- Compare and contrast the values and the potential outcomes of generational interaction.
- Consider and identify potential problems for an organization when people from different generations fail to communicate effectively.
- Compare and identify differing feedback styles and their impact as well as offer strategies for effective cross-generational communication.

Speaker: Dan B. Gunnell, Assistant Lab Director

Illinois State Police Joliet Forensic Science Laboratory



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