Abstracts of poster presentations

FORENSIC GENETICS

Forensic and comparative genetics

Presentation number: FG 1

VALIDATION OF SERATEC HEMDIRECT ON ANIMAL BLOOD

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The validation study of the SERATEC HemDirect blood test has been performed and the test is validated for forensic use. Initial validation test animal blood but all animal blood samples used in this study were from mammalian species and animals from other systematic groups were not included. This study aimed to determine whether the SERATEC HemDirect immunochromatographic test is highly specific for forensic identification of human blood, and whether there are non-mammalian species whose blood would show a false positive result on this test. The preliminary study included 8 samples of venous blood, human and 7 different animal species. All animal blood samples were obtained from Zagreb Zoo during the regular examination of animals. We used the blood of 7 animals who are commonly involved in car accidents or illegal hunting. All blood samples were analyzed by the SERATEC HemDirect test. A dilution set (1/4, 1/8, 1/10, 1/16, 1/20, 1/24) was prepared from whole blood by adding double-distilled water. A 50 µL sample of whole blood and each dilution were placed on a white cotton cloth and left to dry at room temperature. After drying, 1/4 of the sample was cut out and extracted in HemDirect buffer for 30 minutes. After extraction, three drops of each sample were added to the test well and the results were read and recorded after 10 minutes. The SERATEC HemDirect test showed positive results in all analyzed human blood samples (whole blood and dilutions 1/4, 1/8, 1/10, 1/16, 1/20, 1/24). In the analyzed blood samples of all animal species, the test gave negative results except in one case where a diluted blood sample of Rook (Corvus frugilegus L.) showed a weak positive result. The one weak positive result is ground for extending the study to more species of birds and reptiles since those can be found as pets in homes and can be involved in forensic investigations.

Key words: HemDirect, validation, forensics, blood test

Presentation number: FG 2

GENERATING HUMAN Y-STR HAPLOTYPES FROM MEDICINAL LEECHES

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Leeches are annelids which have been used for many centuries in the field of medicine. These worms convey an anticoagulant, known as hirudin, in their salivary glands. Once they hook onto human flesh, they make a small incision, and ingest 5 mL to 15 mL of blood from one human. In this study, 420 samples from 35 leeches were analyzed using Copan microFLOQ® Direct swabs, and 4N6 FLOQSwabs® Crime Scene collection devices, as well as the Yfiler® Plus, and the PowerPlex® Y23 amplification kits. The Yfiler® Plus amplification kit is a 27-plex Y-STR system which includes seven rapidly mutating Y-STR loci which allows for discrimination among related individuals, while the PowerPlex® Y23 System includes 23 Y-STR loci. North American medicinal leeches, Macrobdella Decora, obtained from a commercial source, were fed human blood meal from a male donor, and euthanized by freezing at specific times from 0 hour to 24 hours. The first method involved collecting minute amount of blood on the tip of Copan microFLOQ® Direct swab from the midgut of these organisms and the amplification of the blood while the swab remained in the reagents during thermal cycling. In the second method, 5 µL of blood from each midgut was concentrated to approximately 0.5 µL to 2.0 µL. The microFLOQ® Direct swabs were used to collect the concentrated blood and amplified directly. Finally, the 4N6 FLOQSwabs® Crime Scene swabs were utilized to collect the blood from the midgut, extracted with the DNA Investigator Kit from Qiagen. Extracted samples were quantified with the Quantifiler Trio DNA Quantification Kit. Y-STR profiles were obtained from known blood samples (reference samples) using the same methods. The Y-STR profiles generated from the blood ingested by the medicinal leeches using the three methods were consistent with the reference profiles of the donors. The results of this research reveal that the Copan microFLOQ® Direct swab is an excellent way to generate Y-STR profiles from the blood ingested by medicinal leeches. The extracted samples using 4N6 FLOQSwabs® Crime Scene swabs also yielded consistent and concordant profiles within and between samples and thus can be utilized to extract ingested blood. The direct amplification process bypasses the time-consuming, labor-intensive extraction and quantitation steps.

Key words: Copan microFLOQ® Direct swabs, Y-STR, Leech

Presentation number: FG 3

THE ROLE OF GENETICS IN FATAL PULMONARY THROMBOEMBOLISM FORENSIC DIAGNOSIS

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The study aims to review the most recent and best evidence provided in the literature regarding the application of genetics methodology in the forensic diagnosis of fatal pulmonary thromboembolism (PTE) cases and the medico-legal issues involved. This review was conducted by performing a systematic literature search on online resources (PubMed Central database and Google Scholar) until 31st March 2022, using the following key terms: "((Genetics) OR (Forensic genetics)) AND ((pulmonary thromboembolism) OR (fatal pulmonary thromboembolism) OR (pulmonary thromboembolism diagnosis) OR (pulmonary thromboembolism deaths))." Reviews, abstracts, animal studies, articles regarding surviving subjects, and articles in which the correlation between forensic genetics and pulmonary thromboembolism diagnosis is not discussed were excluded. Only human studies of fatal PTE with genetic analyses were included. The characteristics of the identified articles will be summarized. Most of the articles were retrospective studies on autopsy samples. Pulmonary thromboembolism was identified as the cause of death in all cases. Epidemiological data and clinical history were available in all studies. The most frequently used samples for genetic analyses were blood and postmortem tissues. Genetic testing for common prothrombotic variants included FV Leiden, FII G20210A, and methylenetetrahydrofolate reductase (MTHFR). Trauma and immobilization were the most frequent risk factors for PTE, and in most cases, the clinical and epidemiological analysis showed patients' risk conditions. However, the identified articles show the importance of thrombophilia genetic screening in PTE cases with no significant risk factors. Moreover, unique characteristics were observed among different gender, age, and ethnic groups. This study highlights an association between genetic differences in different loci and PTE risk. Clinicians must be aware of the role of genetics in PTE epidemiology to undertake the proper preventive measures. In the forensic field, genetic testing is mandatory in selected fatal PTE cases, especially in medical malpractice cases. Genetic screening in selected cases should become a routine diagnostic test for PTE prevention.

Key words: forensic genetics, fatal pulmonary thromboembolism, pulmonary thromboembolism deaths, forensic diagnosis

Presentation number: FG 4

MASTR: AN EFFECTIVE PROBABILISTIC GENOTYPING TOOL FOR INTERPRETATION OF 2, 3, AND 4-PERSON STR MIXTURES ASSOCIATED WITH DIFFERENTIALLY DEGRADED DNA

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The goal was to evaluate the impact of differentially degraded 2, 3, and 4-person STR mixtures on likelihood ratios (LRs) generated by the probabilistic genotyping software, MaSTR (SoftGenetics, LLC). Mixtures were prepared from donors with known Fusion 6C profiles. Ratios considered were 1:1, 1:3, 1:6, and 1:10 for 2-person (n=144), 1:1:1, 1:1:3, 1:3:6 and 1:6:6 for 3-person (n=102), and 1:1:1:1, 1:1:1:3, 1:1:3:3, 1:3:3:6, 1:3:6:6, and 1:6:6:6 for 4-person mixtures (n=52), with combinations of pristine and degraded sources of DNA rotated between major and minor contributors. Shearing of buccal DNA extracts was achieved mechanically using a Covaris S220; 150 and 250 bps to simulate severe and moderate levels of DNA degradation, respectively. Data were analyzed using GeneMarker HID (SoftGenetics, LLC). Results were imported into MaSTR and analysis performed using a model panel of Fusion 6C data run on a 3130xl Genetic Analyzer. A subset of LRs were generated using 40,000 versus 10,000 iterations per chain (eight chains total, with a burn-in of 8,000 iterations), and with or without a conditioning profile. MaSTR performed as expected. The log(LR) values for mixture samples containing high quantities of pristine sources of DNA were at optimal levels. Lower-quality mixture data associated with sources of DNA at <0.05 ngs for each contributor resulted in peak imbalance and allelic dropout which reduced the weight in support of a contributor. This was exacerbated by higher levels of degradation, with some instances resulting in log(LR) values in support of an exclusion. As expected, LRs were lower when a known contributor was not provided, especially for samples containing degraded DNA. There was no appreciable difference in LRs when comparing 10,000 and 40,000 iterations per chain for 2-person mixtures. In all cases, findings were consistent with expectations associated with CE-based profile information. Overall, MaSTR proved to be a reliable tool for the analysis of STR mixtures of differentially degraded sources of DNA. The points of view in this abstract are those of the authors and do not reflect the views of their respective agencies. In addition, this abstract in no way reflects an endorsement of products, instruments, or software.

Key words: probabilistic, genotyping, STR, mixtures, degraded

Forensic dna databases

Presentation number: FG 5

CROATIAN DNA DATABASE

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The Croatian DNA database was legally formed at the beginning of the 2000s with new Police regulation. Firstly, it contains DNA profiles of persons in general and crime stains/unknown persons stored in home-made database. In 2006 database migrated to CODIS (FBI) platform, where DNA profiles of known persons were distinguished into suspects, victims, and laboratory staff. With the new system, DNA profiles originated from unknown persons also distinguished into DNA profiles originated from crime scene/stains and those originated from unidentified bodies/persons. Since the migration to CODIS, the Croatian DNA database recorded more than 2.500 matches of unsolved crime stains with suspect DNA profiles. With new regulation that came to force in late 2011 storing DNA profiles into the database, the time of retention and deletion especially DNA profiles from individuals involved in crime act was regulated more precisely. In 2018, because of Croatia's obligation to EU directives, DNA profiles were made available to EU member states through so-called Prüm decisions that enabled 400 unsolved domestic crimes to be linked to known persons. Before the start of automated exchanging DNA profiles and data, the DNA database and all stored profiles were thoroughly revised in which DNA profiles of suspects were differentiated into Suspects (general name for suspects, arrestees, and offenders not yet convicted) and Convicted Offenders according to revised legal acts. DNA profiles of crime stains also were revised in accordance with a number of loci and statistical significance. Today Croatian DNA database counts approximately 15.000 DNA profiles of which 11.000 are daily exchanging with other EU member states.

Key words: Keywords: DNA database, CODIS, DNA profile, crime stain, suspect

Presentation number: FG 6

A CASE FOR INTERNATIONAL ETHICAL GOVERNANCE OF FORENSIC DNA DATABASES

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The United Nations Educational, Scientific, and Cultural Organization has issued no fewer than three declarations on ethical conduct and genetics since 1997, but each of those declarations leave the ethical governance of forensic DNA databases to the country in which the database exists. In 2015, Kuwait attempted to create a universal DNA database, which was met with resounding international condemnation, including from the United Nations. Without international ethical governance, and the decision of the constitutional court in Kuwait, the database was not ‘unethical’ by the current standards. Without a cohesive, international ethical code, forensic genetics has strayed into the world of 'can we do?' often without asking the question of 'should we do?' Since 1995 when the first forensic DNA database was created, governments have amassed large databases of genetic information on a subset of their population and the use of those databases has gone largely unchecked. The international community cannot expect these databases to be used ethically if they do not provide ethical guidelines for their use, this presentation considers the creation of ethical guidelines for the creation and use of forensic DNA databases as well as a path forward for the declaration, implementation, and enforcement of those guidelines.

Key words: Ethics, Forensic Genetics, International Ethics, Forensic DNA Database

Forensic dna phenotyping

Presentation number: FG 7

EFFECT OF OVER-THE-COUNTER DRUGS ON DNA ANALYSIS OF BLOOD INGESTED BY MEDICINAL LEECHES

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Painkillers, such as aspirin (acetylsalicylic acid), are used by many to alleviate the pain associated with common ailments. Although the half-life for aspirin is only 2-5 hours, it is possible that there may still be residual drug in the body post-mortem. Hence, if a medicinal leech, Macrobdella Decora, feeds on the blood of a victim or a suspect who had recently ingested an analgesic, it may be possible to simultaneously obtain a complete DNA profile and drug identification from the blood extracted from the crop (midgut) area of the leech. The potential for generating a DNA profile depends on whether or not acetylsalicylic acid inhibits amplification due to the acid having a phenol group. Human blood was spiked with 25 ppm and 50 ppm aspirin solutions followed by DNA extraction at 0, 24 and 48 hours. The extracted DNA was quantified and amplified using the PowerPlex® Fusion 6 C for autosomal STR and PowerPlex® Y-23 system for Y-STR analysis. HPLC was used to determine the presence of aspirin. These amplified products were analyzed by capillary electrophoresis and fragment analysis of these reference samples were completed using appropriate software. Blood mixed with the aspirin was fed to the leeches which were then euthanatized by freezing at 0, 12, and 24 hours after being fed. Human blood from their crop was extracted and analyzed for the presence of aspirin and for generating the DNA profiles. Since complete autosomal and Y-STR profiles were obtained from blood spiked with aspirin and ingested by the leeches, it is concluded that inhibition was not caused by acetylsalicylic acid. No inhibition was noted in the quantification data and DNA amplification resulted in complete profiles from the reference blood samples mixed with aspirin and from the blood stored in the midgut of the medicinal leeches. All profiles were consistent within and between samples, thus indicating that medicinal leeches can be a valuable source of forensic evidence.

Key words: Medicinal leeches, Aspirin, Autosomal STR, Y-STR

Genetic analysis of forensic non-human material

Presentation number: FG 8

ANALYSIS OF CANNABINOID PROFILES AND THCAS SEQUENCES IN SEIZED CANNABIS RECOVERED FROM GROWING FARM

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The aim of this study was to assess the genetic and chemical profiles of the seized cannabis obtained from the growing farm. According to the farmer, certified variety Santhica 27 was sown. However, police officers recovered 118 plants under the suspicion of drug production. Quantitative cannabinoid content was determined using GC-FID on Agilent 7890A GC System. Extraction of DNA from plant material was performed using DNAeasy Plant Mini kit (Qiagen) following the manufacturer's protocol. DNA quantity was determined by Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). A portion of the tetrahydrocannabinolic acid synthase (THCAS) gene was amplified using Qiagen Multiplex PCR kit. Sequencing was performed according to the laboratory standard operating procedures using Big Dye Terminator v.3.1 Cycle Sequencing Kit on AB3500xl GA (both from Life Technologies). Data analysis was accomplished in Sequencher v.5.4.6 (Gene Codes). Sequences were aligned against the THCAS coding sequence of the drug-type Skunk deposited in NCBI (KJ469378). Based on qualitative chemotype, 61% of total samples were cannabigerol (CBG) predominant, and 39% were THC dominant. Alignment of the obtained sequences correlated with their cannabinoid content, clustering in two distinct groups. Eleven identical sequences differed from variants in hemp Finola and probably Santhica 27 that showed undetectable THCAS. Moreover, several variants were revealed including 1064A that had been previously reported to be responsible for THCAS inactivation and cannabigerolic acid (CBGA) precursor accumulation. This finding was in correlation with the average CBG content of 2%. The remaining three sequences were identical to those obtained in drug-type. The average proportion of THC in these plants was 2.5%. According to chemical and genetic results, we conclude that on the industrial hemp farm was growing THC and CBG predominant cannabis varieties under the guise of certified variety Santhica 27.

Key words: Cannabis sativa L, chemotype, THCA synthase, growing farm

Presentation number: FG 9

DNA ANALYSIS OF SCARCE FUNGAL SAMPLES INDICATES THEIR PSYCHOACTIVE ORIGIN

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The majority of psychoactive fungi contain psilocybin (PY) and psilocin (PI), latter causes LSD-like psychoactive effects but lesser in intensity. PY and PI are illegal in Croatia and most of EU countries, while fungi as their source are mainly accepted. Since PY/PI are not yet synthesized in fungal spores and sclerotia (“truffles”), drug users increasingly buy them via Internet without legal consequences. Furthermore, established toxicology and morphology methods are useless on these kind of samples. Hence, main goal of this study was to present the usefulness of DNA analysis on abovementioned forensic samples in order to confirm its psychoactive origin. Study addressed sequencing of internal transcribed spacer (ITS) of nuclear DNA and large portion of 28S gene (nuclear-encoded large subunit ribosomal RNA genes, nLSU-rDNA). ITS region enables unambiguous identification of fungal species while part of LSU gene from 5' end enables distinguishing between closely related psychoactive and non-psychoactive species. DNA from total of 14 “spore-print“and „truffle“ samples seized on Croatian territory (2010-2014), were isolated and ITS region and large portion of 28S gene (nLSU-rDNA) were sequenced. ITS and LSU sequences were obtained from 11 out of 14 tested samples with haplotypes congruent to GenBank database sequences of psychoactive species Psilocybe cubensis and Psilocybe mexicana. One out of 14 samples most probably represents a mixture of these two, and for the two remaining samples only ITS haplotype was obtained, matching those of P. cubensis. As previously published, P. cubensis varieties are most popular among users due to their proven potency and are easy growing. Applied DNA method can be easily implemented into routine forensic workflow. The latter legislation update, i.e. to include fungi species/body parts as the source of psychoactive substances could lead to continuing decrease of illegal fungi drug abuse.

Key words: „spore print“, „truffles“, DNA sequencing, ITS, LSU

Presentation number: FG 10

IDENTIFICATION OF GENETIC MATERIAL OF WATERBORNE MICROORGANISMS FROM STERNAL ASPIRATE

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The most widely used complementary method for the diagnosis of drowning is the detection of waterborne organisms in the organs of systemic circulation. In this study, we adopted sternal bone marrow aspiration – which in hematology a routine method – instead of femoral bone marrow opening, to prevent the possible contamination during autopsy. During autopsy, besides sternum aspirate, lung and spleen tissues, and bone marrow of the femur were also taken for test the presence of diatom shells by microscopy, and cyanobacterial DNA by PCR. From all tissue samples, 2 g were mixed with 6 ml digesting buffer (0.01 M Tris, 2 % SDS) and 20 µl Proteinase K. After 2 days of incubation at 56°C, microscopic slides were prepared for diatom test, furthermore, 400 µl aliquots were saved for cyanobacterial DNA detection. DNA extraction was carried out with Macherey-Nagel NucleoSpin Soil kit, followed by amplification of a segment of cyanobacterial 16S rRNA gene. The amplicons were separated in 2 % agarose gel and visualized with SYBR Gold intercalating dye. We were able to obtain sternal bone marrow aspirate in all the tested seven suspected drowning cases. In four of the sternal samples, both diatoms and cyanobacterial DNA were detected, while in one additional case, sternum was tested positive by PCR, but no diatom shells were identified. Femoral bone marrow was positive for diatoms only in one case, and by PCR in two cases. The rest of the cases – drowning into a bathtub, falling into a cistern -, all tissue samples were negative. According to autopsy, heart failure, and the high fall was the cause of death, respectively. Sternal bone marrow aspiration can be simply performed in the beginning of the autopsy, before the body cavity is opened, therefore minimizes the chance of contamination. Our results showed that diatom test has low sensitivity, which can be increased with PCR-based methods. Sternal bone marrow was found to be a better source for detection of waterborne organisms in our pilot study than the femoral bone marrow.

Key words: Drowning, Cyanobacteria, DNA

Genome-based applications in forensic science

Presentation number: FG 11

DETECTION OF 13 HYPERVARIABLE REGION 1 (HV1) SNPS USING SINGLE-BASE EXTENSION (SBE) PRIMERS IN PARALLEL WITH SANGER SEQUENCING

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The aim of this study was to determine whether single-base extension (SBE) chemistry can be applied to the forensic practice of testing the target single nucleotide polymorphisms (SNPs) of the mitochondrial DNA (mtDNA) Hypervariable Region 1 (HV1). Despite its rather weak discrimination power compared to the short tandem repeat (STR) markers, high copy number of mtDNA per cell and its stability against degradation still guarantee mtDNA testing a place in modern forensic genetics. Buccal swab samples were obtained from 294 unrelated individuals from Bosnia and Herzegovina, following signing of the informed consent form by all participants. After DNA isolation, full sequencing of HV1 was done using chain-termination Sanger sequencing method. SBE reactions were then performed by targeting 13 SNPs that were identified to be the most frequent in the study population. Uniplex SBE reactions for each individual SNP, as well as two multiplex reactions were prepared for both pure and mixed samples, and results thus obtained were compared with those obtained by Sanger sequencing. The results showed complete agreement of the Sanger sequencing results with SBE reactions for both uniplex and multiplex reactions. No significant differences in signal intensity between reactions with forward and reverse SBE primers were observed. The results obtained with SBE were encouraging in regard to multiplexing and processing of the mixed samples, since the allele of minor contributor to the sample was observed in SBE electropherogram in all prepared mixtures. SBE method is limited by the fact that only target SNPs of interest will be analyzed, meaning that they must be carefully selected and curated for each population. However, typing with SBE protocol is accurate, as compared to the golden standard of Sanger sequencing, but was more time- and labor-efficient and simpler to analyze.

Key words: hypervariable region 1 (HV1), mtDNA, mtDNA control region, single-base extension (SBE), single nucleotide polymorphism (SNP)

Presentation number: FG 12

SINGLE CELL TRANSCRIPTOME AND GENOME SEQUENCING FOR GENETICALLY SEPARATING, CHARACTERIZING AND IDENTIFYING INDIVIDUALS FROM BIOLOGICAL MIXTURES

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Separating individuals who contributed to biological mixtures and their genetic identification are crucial in forensic investigations where mixed crime scene traces are often encountered, but remains largely unsolved despite several attempts. Here, we present a single cell transcriptome sequencing approach with a novel bioinformatics pipeline aiming to solve this long-standing, societally relevant problem. Our pipeline extracts different sets of single nucleotide polymorphisms (SNPs) from single cell RNA sequencing (scRNA-seq) data we obtained from biological mixtures and uses them for the different purposes. Our approach also allows determining the tissue(s) of origin of the cells present in the mixture. We validated our approach using de novo generated scRNA-seq datasets from multi-person blood mixtures and in-silico mixtures generated from individual scRNA-seq datasets involving different numbers and bio-geographic ancestries of contributors and different ratios. For all up to 9-person balanced and imbalanced mixtures with ratios up to 1:60, we achieved a clear single cell cluster separation. Sex, bio-geographic ancestry of the maternal, paternal and bi-parental sides as well as individual identification were genetically determined correctly for all separated contributors. To further increase the number of captured SNPs, thereby increasing the ability of deconvoluting more complex mixtures including minor contributions based on less cells, we additionally investigated single cell genome sequencing by applying the single cell chromatin accessibility assay (scATAC-seq) to various biological mixtures. Our single cell omics approach has the potential to solve forensic mixture deconvolution for genetically separating, characterizing, and individually identifying perpetrators from multi-person biological mixtures found at crime scenes and can also be applied for detecting and resolving contamination in cell cultures or to separate cancer cells from normal ones.

Key words: mixtures, single cell, transcriptomics, SNPs

Presentation number: FG 13

EVALUATING THE USE OF ANCIENT DNA LABORATORY PROTOCOLS IN THE DOWNSTREAM DNA IDENTIFICATION OF BURNED FORENSICALLY-DERIVED SAMPLES

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DNA analysis is a pivotal tool in the identification of human remains recovered from forensic contexts. Under ideal conditions, DNA is sufficiently preserved for DNA identification, including short tandem repeat (STR) profiling. However, natural exogenous factors can limit the amount of recoverable DNA from skeletal material. Where skeletal tissues are exposed to more severe insults, such as fire, obtaining adequate quantities of DNA for downstream analysis has proven challenging. As ancient DNA (aDNA) research is already heavily invested in optimizing the recovery of DNA from challenging samples under similar contexts (e.g., low yields of highly fragmented/degraded DNA), we evaluate DNA yield, quality, usability in STR analyses, NGS library preparation, mitochondrial DNA (mtDNA) recovery, and targeted single nucleotide polymorphism (SNP) assays using protocols adapted from aDNA and forensic analyses. Initial STR and NGS analyses showed that the aDNA extraction protocol recovered higher quantities of shorter DNA fragments at temperatures >350°C. Additionally, our results suggest that there may be an acute point of DNA degradation at temperatures >350°C resulting in a drop in observed STR allele recovery, mtDNA genome read counts and depth of coverage, and SNP calling efficiency. We continue this research using samples obtained via controlled burning of ~10 donor cadavers to evaluate DNA recovery and useability across multiple skeletal elements by comparing methodologies in samples from the same sampling locations across individuals. Preliminary results from this second study show that aDNA extraction protocols appear to provide adequate DNA yields more consistently for initial STR analyses, especially at higher levels of thermal exposure. Our results demonstrate that by optimizing our laboratory protocols to recover DNA efficiently from highly degraded bone samples, we greatly enhance individual identification possibilities in such challenging forensic contexts.

Key words: forensic DNA, burned bone, genomes

Presentation number: FG 14

EFFECT OF OZONE DISINFECTION ON FORENSIC SAMPLES

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In certain situations, disinfection of forensic case sample may be required, e.g., in the early stages of COVID-19 pandemic, or in case of objects which might have been related to biological warfare or terrorism. Our aim was to test the applicability of ozone disinfection technique on forensic samples. We examined the effect of ozone on mock casework samples. We collected samples from worn surgical masks. We investigated blood, saliva, and semen stains. Immunoassays were applied to detect special antigens in blood, saliva and semen samples. As supplementary test, luminol, Phadebas test and microscopy was applied, respectively, in addition to examination of body fluid specific RNA markers. In surgical masks, the detected allele counts between control and ozone-treated samples showed no significant difference ($p = 0.513$). However, the two tested sampling sites, the elastic earloop and the middle part of the nosepiece, showed significant difference in detected allele numbers ($p = 0.011$). Comparison revealed a statistically significant difference between the contributors ($p = 0.001$). Immunoassays were able to identify the sample type after the ozone-treatment. Phadebas showed that ozone-treated samples showed no or only very low enzyme activity. By RT-PCR, we could detect the specific markers in both ozone-treated and control samples. The STR profiles from the masks showed that sampling site and contributor had greater effects on profiling that the ozone treatment itself. Ozone does not damage the structure of hemoglobin, amylase and prostate specific antigen; however, it decreases the enzymatic function of salivary amylase. Detection with luminol was successful. Microscopic observation of sperm cells also showed no alteration between the ozone-treated and the control samples. RT-PCR was successful in all cases; therefore this disinfection method does not hamper the RNA-based biological fluid identification. According to our pilot study, ozone treatment does not encumber the routine forensic sample processing, so ozone treatment could become an accepted method to disinfect crime scene samples.

Key words: STR, ozone, biological fluid, RNA biomarkers

Presentation number: FG 15

AN ASSESSMENT OF PROBABILISTIC APPROACHES TO MTDNA MIXTURE INTERPRETATION

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Mitochondrial (mt) DNA plays an important role in the fields of forensic and clinical genetics, molecular anthropology, and population genetics, with mixture interpretation being of particular interest in medical and forensic genetics. In forensics, mixture deconvolution generally relies on genotyping of STRs, but this approach struggles to resolve samples with similar contributor proportions and degraded samples. The high copy number, haploid state (single haplotype contributed per individual), high mutation rate, and well-known phylogeny of mtDNA, makes it an attractive marker for mixture deconvolution in damaged and low quantity samples of all types. Given the desire to deconvolute mtDNA mixtures, the goals of this study are to 1) combine and assess two existing software tools, MixtureAce™ and Mixemt (1), to deconvolute mtDNA mixtures 2) create a dataset of in-silico MPS mixtures from whole mitogenome haplotypes representing a diverse set of population groups, and consisting of two and three contributors at different dilution ratios to test the combined tools, and 3) since amplicon targeted sequencing is desirable, and is a commonly used approach is forensic laboratories, create biological mixture data associated with two amplification kits: PowerSeq™ Whole Genome Mito (Promega™) and Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific by AB™) to further validate the software for use in forensic laboratories. Findings will include qualitative measures and statistical evaluation of forensic evidence and will significantly enhance the value of mtDNA testing in forensic laboratories through the assessment of software tools and best practices regarding the deconvolution of mtDNA mixtures. Overall, exact contributors were detected in 17.7% (42/237) of 2 and 3-person in-silico mixtures of small amplicon targeted MPS data, and increased to 70% when closely related haplogroups were included. Spurious haplogroups, most likely due to private mutations, were detected in addition to the contributing haplogroups in approximately 30% of the mixtures. Inclusion of a randomly selected, known haplotype in the analysis reduced the number of samples with spurious haplogroups from 32 (56.1%) to 15 (26.4%). Biological samples were also considered. 1. Samuel H. Vohr, et al. 2017. FSIG 30; 93-105.

Key words: mtDNA, mixtures, MPS