A6 Human Body Fluid Identification by Matrix- Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

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After attending this presentation, attendees will have a better understanding of how Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF MS) was used for messenger ribonucleic acid (mRNA) profiling of blood, saliva, and semen.

This presentation will impact the forensic science community by introducing a new multiplex approach of mRNA profiling of body fluids of forensic interest.

It is now well established that different tissues have different genetic expression patterns. The discovery of mRNA biomarkers that are differentially expressed in three body fluids commonly found at crime scenes (blood, saliva, and semen) has contributed to the prospect of using mRNA as a tool for forensic investigation. The validation of these tissue-specific mRNA biomarkers by several research groups has paved the way for mRNA profiling as a potential supplemental method for human body fluid identification.

This presentation will introduce a new multiplex approach of mRNA profiling of body fluids of forensic interest. The most comprehensive methods for mRNA profiling tested to-date all share a common start which is total RNA extraction and complementary DNA (cDNA) synthesis. Diverging from this initial step are two frequently described and standard methods of mRNA profiling for forensic-like stains. The first one is qualitative in nature and combines end-point PCR and capillary electrophoresis (CE); whereas the second includes quantitative PCR chemistries. However, the drawbacks of these existing mRNA profiling methods are (a) the limited multiplex capabilities per reaction mainly due to a limited availability of fluorescent dyes/tags for both CE and qPCR, and (b) the use of internal sizing standards (CE). Sequenom's iPLEX® biochemistry on MALDI-TOF MS, commonly used for single nucleotide polymorphisms (SNPs) genotyping, was adapted for qualitative mRNA profiling. Briefly, an initial end-point PCR phase followed by a primer extension step was used with cDNA. A positive extension reaction was indicative of the presence of cDNA in the sample. This approach on MALDI-TOF MS has the ability to simultaneously determine the molecular weight of DNA fragments without the use of size standards, and thus provides multiple data points per experiment to enable analysis of high-level multiplex PCR reactions.

In this study, total RNA was extracted from mock forensic stains: blood (N=15), saliva (N = 13) and semen (N = 17), and converted into cDNAs. Multiplex primers (19 plexes composed of five blood, six saliva, four semen, and four housekeeping-specific primer sets) and corresponding extension primers/probes, were designed from the sequences of 12 mRNA markers. In general, the body-fluid specific primer sets correctly identified the targeted body fluid. The range of specificity of the primer sets was 93-100% for blood and semen samples and 88-100% for saliva samples. A multidimensional scaling (MDS) plot was constructed in which the gene expression data (obtained as genotypes and converted into numeric values) were projected onto two viewable dimensions representing linear combinations of markers that provide variation in the data set. MDS analysis of the samples based on expression of all 19 PCR assays demonstrated that the gene expression profiles of the three human body fluids clustered uniquely and were distinct from each other. The results of this study suggest that MALDI-TOF MS has potential for use as a method for the identification human body fluids of forensic interest.

mRNA Profiling, MALDI-TOF MS, Sequenom's iPLEX® Biochemistry

 ** Presenting Author*