B77 A Comparison Study of a Mass Spectrometry (MS) -Based Serological Assay With Existing Casework Models

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After attending this presentation, attendees will better understand the advancements achieved with protein MS for the identification of five human body fluids and their impact on existing casework models.

This presentation will impact the forensic science community by assessing the performance of a validated MS-based serological assay in an operational environment. The results of this study demonstrate an increase in sensitivity and specificity over existing workflows.

Protein MS has emerged as a technique to supplant traditional enzyme- and antibody-based tests for the identification of body fluids. As part of a multi-year initiative, a robotic, fully automated sample preparation workflow coupled with protein MS has been developed and validated for the confirmatory identification of five biological fluids (saliva, blood, seminal fluids, vaginal fluids, and menstrual fluids). Sample preparation is conducted in 96-well plate format using the Agilent® AssayMAP Bravo automation platform with protein identification on an Agilent® 6495 triple quadrupole mass spectrometer coupled to an Agilent® 1290 series Ultra High-Performance Liquid Chromatography (UHPLC). Combining these two pieces of hardware, up to 100 samples can be prepared and analyzed in one day. A fit-for-purpose developmental validation has been completed by integrating appropriate studies according to guidelines set by the Scientific Working Group for Forensic Toxicology (SWGTOX) and the Scientific Working Group on DNA Analysis Methods (SWGDAM). Developmental validation studies assessed reproducibility/repeatability, sensitivity, stability, mixture analysis, specificity, carryover, ion suppression, and limit of detection.

In addition to the developmental validation studies, the workflow was assessed to demonstrate that the technology is "fit-for-implementation" in relation to existing casework models (e.g., immunochromatographic/antibody screening, and DNA/Short Tandem Repeat (STR) typing protocols) and the overall operational workflow of a forensic laboratory. A series of appropriate mock casework samples that assessed recovery of substrates, overall assay sensitivity, impact of potential contaminants, multi-fluid mixtures, sample degradation, and mock sexual assault kits were prepared and processed in tandem by MS, currently employed serological tests, and by standard DNA profiling methods.

Commercially available serological tests included antibody- and enzyme-based platforms targeting blood (RSID™ Blood, ABAcard® HemaTrace), seminal fluid (RSID™ Semen, ABAcard® p30), and saliva (RSID™ Saliva, SALIgAE®). DNA extracts were processed via organic/organic differential extraction, Quantifiler™ Trio DNA Quantification Kit, GlobalFiler™ Polymerase Chain Reaction (PCR) Amplification Kit/Y Filer® Plus PCR Amplification Kit, and analyzed using Applied Biosystems® 3500 Genetic Analyzer. Two hundred fifty samples were assayed across the three approaches. For serological analysis, the MS approach offered superior detection limits (e.g., human blood detection from less than 10nL of whole blood recovered from a swab) while also providing true confirmatory results. Furthermore, the MS method can reliably detect vaginal and menstrual fluids, for which commercial assays do not exist. While genetic testing of STRs has historically proven to be much more sensitive than traditional serological methodologies, MS and DNA analysis are more comparable in terms of sensitivity limits. For low-level samples (e.g., picoliter quantities of body fluid), a relationship between the number of protein targets identified and corresponding peak height intensities with the number of alleles detected from the known donor was observed. For example, a low-level seminal fluid sample produced two out of five target seminal fluid markers and generated 67% of an STR profile. Overall, the MS method allowed for clear, unambiguous, serological identification of body fluids to the point where the technology can be called "comparable" to STR testing.

In conclusion, the implementation of the MS approach offers comparable sensitivity to current genetic testing methodologies, providing an advantageous relationship between a positive body fluid identification and the likely success of downstream DNA analysis. This provides a greater tool to forensic examiners seeking to perform sample prioritization and deliver confirmatory contextual information in a criminal investigation.

Serology, Proteomics, DNA Testing

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