B6 The Development and Validation of a Multiple Reaction Monitoring (MRM) Mass Spectrometry (MS) Assay for Confident Identification of Protein Biomarkers for Blood, Semen, and Saliva

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Learning Overview: After attending this presentation, attendees will understand the components of a protein MS assay for identification of body fluids as well as the necessary controls and data analysis requirements for use of targeted MS proteomics in a forensic context.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a complete assay for the identification of body fluids by protein markers for implementation in forensic casework.

As the amount of DNA needed for identifying individuals from forensic evidence becomes increasingly small, the need to know the source of that DNA (e.g., blood, saliva, or semen) becomes increasingly important—affecting both the defense and prosecution, as well as the public's confidence in the accurate presentation of evidence and the impartial administration of justice. While this need is well recognized in the forensic community, currently there are no confirmatory body fluid assays in routine use in forensic laboratories. Proteins offer several advantages as body fluid biomarkers. The protein markers selected for this assay have tissue-specific functions (e.g., hemoglobin in blood to carry oxygen, amylase in saliva to digest carbohydrates, and semenogelin in semen for reproduction) and are therefore predominately and abundantly expressed in their respective body fluids, giving them both specificity and sensitivity as markers. Proteins are relatively stable to time and environmental insult, and because proteins markers do not require amplification, they can be detected even when partially degraded. MS-based protein assays are unbiased—the same protocol is applied to all unknown samples. Finally, protein and DNA can be separated from the same sample and, therefore, a protein body fluid assay does not consume DNA evidence that can be used for Short Tandem Repeat (STR) identity testing.

Previous work on proteomic body fluid identification established proof of concept and selection of specific protein biomarkers using an untargeted, discovery proteomics approach.[1] Discovery proteomics methods prioritize detection and identification of as many proteins as possible, with resulting limitations in sensitivity and reproducibility. In contrast, targeted proteomic techniques require the desired analytes to be defined in advance but are fast, sensitive, and reproducible, and thus more suited for standardization, validation, and routine implementation. Presented here is a detailed explanation of the steps taken to develop discovery study results into a targeted proteomics assay with standardized protocols and decision criteria suitable for thorough validation and implementation in routine forensic casework. This approach takes into account both the requirements of a sound targeted MS analysis method, supported by the large body of work in medical and pharmaceutical research, and the specific requirements for forensic testing of unknown samples and production of legally defensible results.[2-5]

The targeted assay uses MRM, carried out on a triple quad MS and involves targeting and isolating pre-selected peptide masses, fragmenting the selected peptides, and measuring the intensity of the expected fragment ions. Criteria for selection of peptides for each protein marker include stability, consistent ionization, avoidance of post-translational modifications, specificity to the target protein, and in the case of forensics, specificity to the target species. Fragment ion selection requires ruling out any masses that could be confused with interfering signals from the sample matrix, which in forensic samples may be unusually complex.

Using data collected from known samples of blood, semen, and saliva, as well as negative controls, a set of standard operating procedures was developed for the laboratory processing of forensic samples for analysis by High-Performance Liquid Chromatography (HPLC) and MRM MS. These include a quality monitoring system of known standards, controls, and expected instrument metrics. Similarly, a data analysis pipeline was developed, and statistical analysis of known samples was used to create robust decision criteria for the confident identification of at the level of targeted peptides, protein biomarkers, and, ultimately, the presence of a body fluid in an unknown sample.

Reference(s):

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