B106 Improving Seminal Fluid Detection Sensitivity in Extended Postcoital Intervals by Triple Quadrupole (QQQ) Mass Spectrometry

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After attending this presentation, attendees will better understand the use of protein mass spectrometry for the detection and quantitation of human seminal fluid during an extended postcoital interval.

This presentation will impact the forensic science community by illustrating how protein mass spectrometry can be used to enhance the potential for the successful detection and confirmatory identification of seminal fluid in an extended postcoital interval. The results of this study can provide the forensic science community with a powerful tool to aid in obtaining potentially probative evidence in sexual assault investigations.

This work adapted a fully validated multiplex QQQ Multiple Reaction Monitoring (MRM) method for multiple body fluid identification for the focused identification of seminal fluid protein biomarkers. The modified assay provides enhanced sensitivity for seminal constituents that surpasses the sensitivity currently achievable with commonplace serological screening techniques. This makes it possible to confidently detect the presence of seminal fluid in extended postcoital intervals.

Previous research has resulted in the development of a qualitative mass spectrometry-based assay for the confirmatory identification of human seminal fluid. The current study has demonstrated that Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) can be utilized to both detect and quantitate seminal fluid four days postcoitus in authentic samples. Initial limits of detection and quantitation were determined using heavy isotope-labeled peptide standards complimentary to the targeted peptide fragments of interest. By comparison, paired analysis using the ABAcard® p30 and RSID™-Semen immunochromatographic assays failed to produce a positive test result one and three days postcoitus, respectively.

The goal of the current research was to facilitate the analysis of challenging sexual assault evidence by optimizing the quantitative tandem mass spectrometry method to increase the detection and quantitation of seminal fluid protein biomarkers. Using an Agilent® 6495 QQQ mass spectrometer coupled with a 1290 UPLC system, seminal fluid-specific proteins (selected based on previously acquired time-of-flight data) were analyzed. The specificity of the protein biomarkers as well as the potential for matrix interference was evaluated through analyses of two-, three-, and four-component mixtures of seminal fluid in combination with commonly analyzed body fluids (i.e., peripheral blood, vaginal and menstrual fluids, saliva, and urine). The sensitivity achieved by the MRM seminal fluid assay was established using a serial dilution of semen spotted onto vaginal swabs. It was found that a 1:131,072 dilution of seminal fluid in a vaginal fluid matrix could be reliably detected by the assay. This translates into a doubling of the detection interval for authentic postcoital samples — enabling detection up to more than eight days postcoitus.

Additionally, intact synthetic proteins (prostate-specific antigen and semenogelin-1) were used to create a "standard detection curve." These data provide insight into the lower limits of detection and quantitation of natural seminal fluid proteins. The ability to quantify seminal fluid protein biomarkers can be used to establish background

interferences (semenogelin-1) and baseline levels (prostate-specific antigen) of the targeted proteins.

In conclusion, this study provides comprehensive data detailing the sensitivity and specificity of a novel serological assay approach utilizing protein mass spectrometry for the detection and quantitation of human seminal fluid.

Proteomics, Postcoital Interval, Sexual Assault