B80 The Evaluation of a Quantitative Proteomic Seminal Fluid Assay for Assessing Lateral Flow Test Error Rates and Predicting Downstream DNA Profiling Success

Heather E. McKiernan, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Catherine O. Brown, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Kevin M. Legg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Phillip Danielson, PhD, University of Denver, Denver, CO 80210*

Learning Overview: After attending this presentation, attendees will understand how a quantitative proteomic workflow for seminal fluid can be utilized to both estimate the rate of authentic false positive results associated with immunochromatographic assays as well as identify appropriate samples for subsequent genetic profiling.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a potential alternative workflow for sexual assault kit sample analysis while providing further information regarding false positive rates associated with currently employed immunochromatographic assays designed to target seminal fluid markers.

Forensic practitioners have long sought efficient and reliable means for identifying those samples that are best suited for successful genetic profiling. Traditional workflows for sexual assault kit sample processing rely upon enzyme activity and antibody-based serological tests for the detection of seminal fluid and/or saliva as well as microscopy for the detection of spermatozoa. This workflow can be laborious and costly while reliance on antibody-based serological testing can be prone to error. This has contributed to the popularity of Y-Screen assays as an alternative workflow for prioritizing sexual assault kit samples. While these Y-Screen approaches achieve rapid screening of samples for the presence of a detectible male contributor, they do not provide any serological information. As a result, samples lack what can be a critical investigative/biological context. Particularly with the introduction of more sensitive DNA profiling kits, this lack of context opens the door to alternative explanations for the presence of trace levels of male DNA (e.g., secondary/indirect transfer of skin cells).

A more sensitive and accurate technology for the confirmatory identification of seminal fluid would provide important physical evidence of sexual contact to greatly bolster the weight of even partial male DNA profiles. A particularly promising approach combines high-specificity protein biomarkers with a target-ion mass spectrometry. Applying absolute quantitation of the prostate specific antigen (PSA) and semenogelin (Sg) protein targets in the biomarker panel will enable forensic practitioners to make fuller use of serological information in their decision making on downstream analyses in order to improve the successful analysis of challenging sexual assault samples.

This research evaluated the relationship between quantitative levels of target seminal fluid peptides and the ability to generate Y-STR profiles from vaginal swabs collected at various post coital intervals. Seminal fluid protein content as determined by a proteomic assay was compared to the percent of Y-STR loci detected following genetic analysis. Based on preliminary data, there appears to be almost full concordance between target peptide concentrations and the ability to produce Y-STR profiles. These results indicate it may be possible to develop a proteomic SAK screening workflow– one which simultaneously provides a reliable means both for identifying samples likely to yield an interpretable DNA profile as well as providing serological information that speaks to the biological context of the sample.

Additionally, this quantitative proteomic assay was used to estimate the rate of authentic false positive results associated with immunochromatographic assays targeting seminal fluid proteins. Self-collected vaginal swabs collected from participants **not** engaging in barrier-free vaginal intercourse with male partners were tested using various immunochromatographic assays designed to detect both Sg and PSA. Any samples producing positive results on either immunochromatographic assay were evaluated to determine whether the target protein was present at levels above the reported sensitivity limits of the lateral flow tests. Out of the fifty (50) samples analyzed to date, 7 produced false positive results for ABAcard p30 while 2 produced false positive results for RSID Semen resulting in a 14% and 4% false positive rate respectively. Interestingly, in no instance did a sample produce a positive result on both immunochromatograpic assays. All cellular components of the 9 samples producing positive immunochromatographic results were confirmed sperm free utilizing Sperm Hy-Liter and fluorescent microscopy. As the proteomic assay has already been demonstrated to be more sensitive then lateral flow tests, negative results indicate that the positive immunochromatographic results were due to true false positive, non-specific binding events rather than the presence of these proteins at low levels. These data support the use of immunochromatograhic assay results as presumptive rather than confirmatory.

Forensic Science, Proteomics, Sexual Assault