B6 Evaluating *In Vitro* Seminal Fluid and Saliva Biomarker Degradation Using a Mass Spectrometry-Based Serological Assay

Catherine O. Brown, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Janelle Leo, BS, Philadelphia, PA 19150; Phillip Danielson, PhD, University of Denver, Denver, CO 80210; Kevin M. Legg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090*

Learning Overview: After attending this presentation, attendees will understand the effects of endogenous degradation on the selection of protein biomarkers for seminal fluid and saliva using emerging body fluid identification methodologies.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by evaluating the stability of peptide biomarkers for serological testing of sexual assault evidence using protein mass spectrometry.

The use of protein mass spectrometry for serological testing of sexual assault evidence has demonstrated increased specificity and sensitivity over current antibody-based techniques. However, loss of sample due to natural removal of target material through vaginal drainage as well as breakdown of biomarkers by endogenous protease enzymes within the vaginal vault make the detection of seminal fluid and saliva in sexual assault samples more challenging.

Protein denaturation via naturally occurring protease activity is one limitation affecting post-coital detection intervals for immunochromatographic assays. These testing devices rely on the intact conformational shape of target proteins for antibody binding and detection. However, proteomic mass spectrometry-based approaches do not require conformation protein integrity as samples are subject to enzymatic digestion prior to analysis. It is important to understand whether proteolytic digestion of seminal fluid and saliva targets in the vaginal vault occurs within a target peptide sequence. This would provide additional information regarding biomarker stability ensuring accurate detection and identification in low level and degraded sample types (i.e., extended post-coital interval sexual assault samples).

Three seminal fluid biomarkers (Semenogelin 1, Semenogelin 2, Prostate Specific Antigen) and two saliva biomarkers (Alpha-Amylase, Cystatin-SA) were selected for evaluation. To eliminate the effects of natural drainage on target material and isolate the factor of interest, endogenous proteolytic degradation in the vaginal vault, *in vitro* incubations were performed. Seminal fluid- and saliva-free vaginal swabs were acquired from 10 female volunteers and extracted and pooled. Seminal fluid and saliva were obtained from a single male donor. Samples were prepared in triplicate by diluting neat seminal fluid 1:100 and neat saliva 1:50 with liquid vaginal extract. Seminal fluid samples were incubated at 37° C for 1, 2, 3, 6, 8, 10, and 12 days and saliva samples were incubated at 37° C for 1, 2, 3, 4, and 5 days. An immediate collection at Day 0 served as a positive control while blank vaginal extract served as a negative control. Samples underwent a tryptic protein digestion and C18 SPE clean-up on an Agilent® AssayMAP Bravo automation platform prior to analysis. An Agilent® 6495 triple quadrupole mass spectrometer coupled to a 1290 series liquid chromatograph was utilized for this study with an Agilent® ZORBAX 300 SB-C18 column. Separation was performed over 10 minutes with a 90% acetonitrile gradient.

Seminal fluid and saliva biomarkers were quantified using synthetic peptide standards (0.5 fmol/µL to 1 pmol/µL). Isotopically-labeled internal positive controls were used to evaluate sample response. For peptide LSEP (PSA), the initial concentration was 90 fmol/µL. Concentrations decreased in a linear manner, with calculated concentrations of 61 fmol/µL, 27 fmol/µL, and 6 fmol/µL at Days 1, 2, and 3 respectively. LSEP was not detected after three days of incubation. The QITI peptide (Sg 1) held an initial concentration of 2100 fmol/µL over three days of incubation, then began to decrease (1265 fmol/µL Day 6 and 488 fmol/µL Day 8). This peptide marker was not detected after eight days of incubation. Semenogelin 2 peptide GSIS exhibited a similar pattern to QITI. Peptide LSGL (Alpha-Amylase) had an initial concentration of 9 fmol/µL, decreasing to 5 fmol/µL at Day 1, and 3.5 fmol/µL at Day 2 where it remained for Days 3 to 5. Cystatin-SA peptide ALHF had an initial concentration of 2.5 fmol/µL which remained stable across the 5-day study.

In conclusion, endogenous breakdown of target material was evident for PSA and Alpha-Amylase peptide targets. Semenogelin 1 and 2 peptide targets appear stable for 3 days into the post-coital interval and are detectable at quantitative values 8 days into the post-coital interval. Cystatin-SA peptide targets demonstrated stability over the full 5 days of evaluation. The selected peptide amino acid sequence of target protein biomarkers should be considered, not only for specificity purposes, but to ensure the endogenous breakdown and cleavage of protein material does not negatively affect biomarker detection.

Forensic Science, Proteomics, Serology