B9 Assessing Potential Inhibitory Effects of Personal Lubricant on the Ability to Detect Biomarkers Consistent With Semen, Saliva, and Vaginal Fluid

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

Learning Overview: After attending this presentation, attendees will have gained an understanding of the effects of personal lubricant on the ability to detect and identify protein biomarkers using protein mass spectrometry for the analysis of sexual assault evidence.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an in-depth scientific evaluation describing the adverse effects of lubricant on serological identification and how modified procedures using advanced instrumental techniques can mitigate deleterious effects.

Sexual assault evidence remains one of the most commonly encountered types of evidence. This has prompted extensive research and development in advancing confirmatory serological techniques in order to complement the sensitivity obtained with modern genetic testing. Protein mass spectrometry has been demonstrated as an attractive alternative to traditional testing methodologies, allowing for the confirmatory identification of trace levels of fluid-specific biomarkers. In order to further evaluate the implementation of protein mass spectrometry in relation to sexual assault kit workflow testing, an extensive study has been conducted to evaluate the effects of lubricants on the ability to detect target biomarkers. Lubricants have the potential to inhibit protease activity, displace hydrophobic markers during Solid Phase Extraction (SPE), and suppress ion detection during mass spectrometry analysis.

A previously established assay containing protein biomarkers consistent with seminal fluid (semenogelin 1, semenogelin 2, prostate specific antigen, prostatic acid phosphatase, and epididymal secretory protein), saliva (α-amylase, statherin, submaxillary gland androgen-regulated protein 3B, and cystatin-SA), and vaginal fluid (small proline rich protein 3, cornulin, neutrophil gelatinase, Ly6/PLAUR, suprabasin, periplakin, and involucrin) was utilized during the course of this research. Lubricant types assessed included water-based with glycerin, water-based without glycerin, silicon-based, hybrid silicon, and natural oil-based lubricants. Three studies were performed for the completion of this research: (1) determining the ability to detect vaginal fluid biomarkers from vaginal swabs fortified with lubricant: (2) establish the effect of lubricant types on the ability to detect biomarkers of seminal fluid and saliva; and (3) assess the ability to identify biomarkers on pre-lubricated condoms.

Vaginal swabs free of semen or saliva were collected, solubilized, and pooled to create a single-source matrix. The extract volume was normalized and applied to clean cotton-tipped swabs prior to fortification with lubricant and/or seminal fluid and saliva. For the first portion of this research, vaginal swabs were enriched with 1µL, 5µL, or 15µL of lubricant. Samples for the second portion of this study were prepared in a similar manner, with swabs fortified with lubricant and either 1µL of seminal fluid or 10µL of saliva. Last, for the third portion of this study, prepared swabs were moistened and used to sample the external and internal portions of a pre-lubricated condom. This sample set was designed to simulate an authentic vaginal swab recovered from an individual who had been assaulted with a barrier form of contraception.

Swabs were solubilized and protein material was separated from cellular material through centrifugation. Samples were then pre-treated prior to enzymatic digestion using SPE with Waters® Oasis™ HLB 1cc vac cartridges as an additional cleanup to reduce lubricant impact. An Agilent® AssayMAP Bravo automation platform was employed for tryptic protein digestion and micro-SPE clean-up. An Agilent® 6495 triple quadrupole mass spectrometer coupled to a 1290 series liquid chromatograph was utilized for this study. Data was interpreted according to three criteria: (1) the overall Peak Area Response (PAR) of the target biomarker; (2) biomarker PAR in relation to internal standard; and (3) PAR of digestion control protein. PAR response was dependent on the type of lubricant assessed, with greater effects observed on hydrophobic biomarkers. Water-based lubricants had little-to-no effect on biomarker detection. As hypothesized, increased lubricant volumes resulted in greater PAR loss. Select hydrophobic biomarkers experienced a >80% decrease in PAR with silicon and natural oil lubricants; however, this issue was resolved with additional sample preparation procedures. Furthermore, select hydrophilic biomarkers exhibited ion enhancement in the presence of silicon-based lubricants, the effects of which were also decreased with the additional sample pre-treatment.

In conclusion, lubricant type does affect the ability to accurate identify protein biomarkers. A sample preparation method was developed in order to eliminate a majority of the deleterious effects observed on the consistency of biomarker detection.

Serology, Sexual Assault, Proteomics

*Presenting Author