B66 Front-End Fractionation of DNA and Proteins for the Simultaneous Genetic and Serological Analysis of Sexual Assault Case Samples

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Learning Overview: After attending this presentation, attendees will have gained insight into a workflow that permits the fractionation and simultaneous analysis of DNA and proteins in sexual assault samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing comparative data evaluating methodologies for the co-extraction of DNA and proteins. The results of this study demonstrate the compatibility of proteomic workflows and the importance of serological identification in sexual assault evidence testing.

In order to combat the sexual assault kit backlog in the United States, more efficient processes for serological and genetic sample preparation are needed. Currently validated serological methods (with the exception of microscopy) for semen/saliva identification suffer from limitations associated with specificity and sensitivity. By pairing simultaneous fractionation or co-extraction of cellular (DNA for genetic testing) and protein material for use with proteomic serological identification, these limitations associated with traditional antibody-based serological assays are overcome, providing practitioners and investigators with more actionable forensic results. Simultaneous preparation for proteomic and genetic analysis will also aid with expedited turnaround times.

Samples were prepared using single-source vaginal swabs from an individual abstaining from any form of sexual intercourse and single-source semen and saliva from a male donor. Vaginal swabs were solubilized and extract was pooled to normalize protein material. Vaginal extract was applied to clean cotton swabs and fortified with various dilutions of semen and/or saliva prepared in deionized water. Replicate sample preparations (n=10 per method) were co-extracted using one of three evaluated sample fractionation workflows. The first method was a previously validated in-house sample preparation workflow for the proteomic analysis of biological samples in which soaking and centrifugation are used to pellet cellular material for genetic analysis while proteins remain in the supernatant. Protein material underwent a tryptic digestion prior to analysis. A phenol/chloroform organic extraction was utilized prior to genetic analysis. The second co-extraction method developed by Kranes et. al. employed a simultaneous DNA extraction and tryptic protein digestion followed by subsequent fractionation utilizing a Molecular Weight Cutoff (MWCO) filter.[1] The third fractionation method evaluated utilized a commercial product, the QIAGEN® AllPrep® DNA/RNA/Protein Mini Kit, which separates protein and genetic material fractions using selective binding filtration chemistries and protein precipitation. All protein fractions, regardless of front-end prep method employed, were assessed using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). All DNA extracts were quantified using the Applied Biosystems® Quantifiler® Trio quantification kit, amplified using the Globalfiler™ Polymerase Chain Reaction (PCR) amplification kit, and analyzed on an Applied Biosystems® 3500 Genetic Analyzer.

The three sample fractionation protocols were compared according to the following criteria: (1) peak area intensity observed via UPLC-MS/MS analysis for target protein biomarkers; (2) DNA quantification values; (3) overall quality of genetic profile obtained; (4) consistency among preparation replicates; and (5) speed/cost and ease of workflow. These data demonstrate that sufficient DNA and protein can be obtained from simulated sexual assault samples to allow for the simultaneous forensic analysis of each fraction, eliminating the need for forensic analysts to prioritize one type of testing over another.

Reference(s):

1. Kranes, S., Sterling, S.A., Mason, K. et al. Simultaneous DNA and protein extraction using trypsin. *Forensic Sci. Int. Genet. Suppl. Ser.* 2017;6:e203–e204.

Proteomics, Serology, Genetic Analysis

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