B96   The Development and Testing of a Top-Down Proteomic Method for the Confirmatory Identification of Saliva

Kevin M. Legg, 2300 Stratford Avenue, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Shanan S. Tobe, PhD, Arcadia University, Dept of Chemistry and Physics, Forensic Science, 450 S Easton Road, Glenside, PA 19038; Phillip Danielson, PhD, Department of Biology, 2101 E Wesley Avenue, Lab 223, Denver, CO 80210; and Brianna L. Robbins, BA*, Arcadia University, 450 S Easton Road, Glenside, PA 19038

After attending this presentation, attendees will understand some of the issues with current techniques for the identification of saliva, how protein mass spectrometry can be used to identify a panel of salivary protein biomarkers that are specific to saliva, and why protein mass spectrometry is a more sensitive and reliable method for confirmatory identification of saliva than methods currently in use.

This presentation will impact the forensic science community by providing a confirmatory method for identifying saliva in forensic casework, thereby potentially aiding in sexual assault investigations.

While DNA analysis is often considered the "gold standard" of forensic biology, being able to determine the source fluid(s) in addition to a DNA profile can provide critical context to a case. Immunochromatographic and enzyme-based tests are commonly employed for screening evidentiary material for saliva; however, neither of these assays provide true confirmatory results as false positive results with non-target fluids and false negative results with dilute and/or degraded samples are well known. Several emerging techniques for confirmatory body fluid identification seek to remedy this gap by developing more reliable analytical techniques based on protein mass spectrometry, messenger RNA (mRNA), micro RNA (miRNA), and epigenetic profiling of body fluids.

Several studies employing protein mass spectrometry have been successful in identifying saliva; however, serious throughput limitations exist, which can prohibit the adoption of these techniques for routine serological screening. For example, multi-day sample preparation workflows coupled with 60+ minute analytical runs are not uncommon.

The goal of the current research is to create a faster, laboratory-compatible workflow for saliva identification. This was achieved by direct enrichment for low molecular weight salivary proteins and analysis on an AB SCIEX™ TripleTOF® 5600 platform. Using this approach, sample preparation can be completed in less than two hours from collection to injection, therefore allowing same-day analysis of evidentiary samples. Initial studies have identified several saliva-specific candidates, such as basic salivary proline-rich protein 1/2, salivary acidic proline-rich proteins 1, 2, 3, and 4, and submaxillary gland androgen-regulated protein 3B. Further studies are underway to identify additional biomarkers and to identify the most reliable biomarkers for identifying saliva by characterizing the fluid in the population by analyzing 50 unique saliva samples.

In conclusion, this study illustrates that protein mass spectrometry is a sensitive and specific method for the confirmatory identification of saliva, providing a truly confirmatory method for the detection of saliva in sexual assault evidence.

Saliva, Confirmatory Identifiation, Proteomics