Body Fluid Identification from Sexual Assault Evidence

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Salient Points

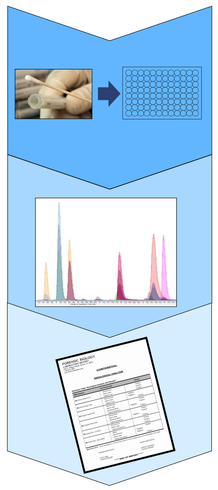
- **Current serological methods** of stain identification for sexual assault screening consume valuable evidence but yield only presumptive results.
- **A confirmatory** mass spectrometry-based assay has been developed to detect body fluid specific proteins in saliva and seminal fluid.
- **A high throughput sexual assault** backlog workflow has been designed and successfully tested. This technique provides practitioners with a more accurate, reliable, and sensitive assay when compared to traditional methods.

Introduction

Serological characterization of biological material can provide critical information to prioritize samples for microscopic and DNA analyses. Currently, the technologies used for the analysis of seminal fluid and saliva (critical for screening sexual assault evidence) is based on presumptive indication of a body fluid with many tests yielding false positive. Under four NIJ awards, high-specificity protein biomarkers have been compiled and validated. These biomarkers have been incorporated into mass spectrometry-based workflow for screening sexual assault evidence.

Improved Backlog Workflow

This workflow is well suited to batch analyses in plate formats; provides true confirmatory stain characterization and automated data processing.



Batch processing of sexual assault swabs in 96 well plate format.

Confirmatory mass spectrometry analysis

Automated reports to direct further processing

Experimental Design

To assess this workflow, simulated sexual assault swabs were prepared by spotting varying quantities of semen and saliva (**Table 1**) onto blank (i.e., semen free) vaginal swabs that had been previously collected and dried.

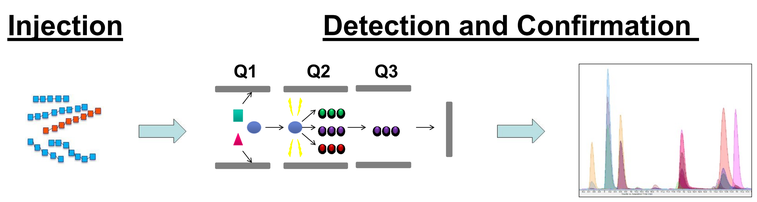
Table 1: Body Fluid Content of Simulated Sexual Assault Swabs

Body Fluid	Swab Set 1	Swab Set 2	Swab Set 3	Swab Set 4	Swab Set 5	Swab Set 6
Saliva(µl)	1	1	2.5	5	10	25
Semen(µl)	1	2.5	5	10	20	25

Each swab was added to 500µl of Buffer and incubated at room temperature for 30 minutes. The swabs were then placed in spin baskets and centrifuged for 3 minutes. **Immunochromatographic Assays:** The resulting extract was added to an ABA p30 card (200µl), an RSID Semen card (100µl) and an RSID Saliva card (100µl). The results were interpreted per the manufacturer's instructions. **Mass Spectrometry Assay:** 15-30 µl of extract was denatured, reduced and alkylated before digestion with 1µg of trypsin. Samples were analyzed on a ABSciex API 4000 triple quadrupole mass spectrometer running in multiple reaction (MRM) mode.

Analysis by Mass Spectrometry

Body Fluid	Protein Biomarker
Seminal Fluid	Semenogelin
	Prostate Specific Antigen
Saliva	Alpha Amylase
	Statherin



All targeted biomarkers for both seminal fluid and saliva were reliably detected (i.e., based on response ratios, retention times, peak shape and symmetry as compared to a known positive control) at all sample quantities and ratios tested. Of particular note, is the fact that robust results indicating the detection of both seminal fluid and saliva were obtained down to 1µl each of semen and saliva – the level at which the RSID Semen immunochromatographic system began to fail. Representative results can be seen in **Figure 2** where 1 µl of both saliva and seminal fluid were spotted onto blank (semen free) vaginal swabs.

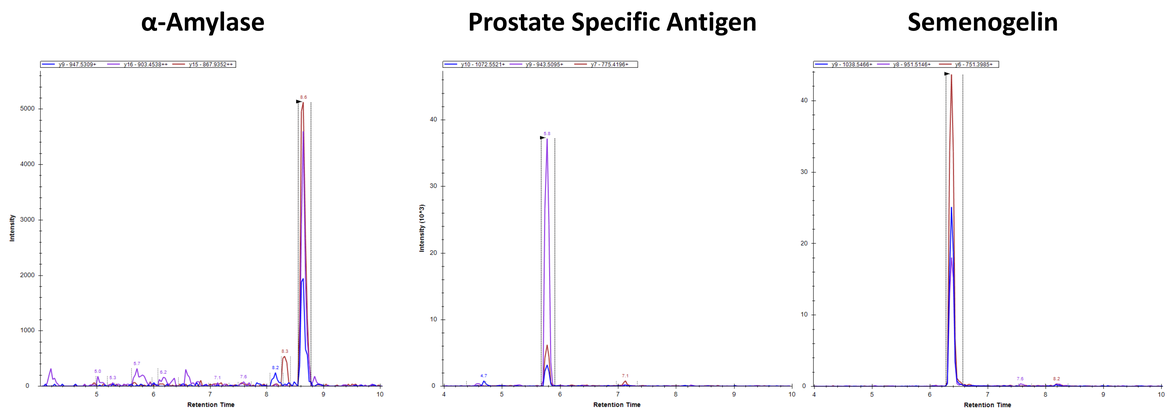


Figure 2: protein identification from simulated sexual assault swab containing just 1µl semen and 1µl saliva. Body fluid confirmation is based on the detection of alpha amylase, prostate specific antigen and semenogelin using the triple quadrupole mass spectrometer based screening method.

Analysis by Immunoassay

Additional blank (semen free) vaginal swabs were processed using three antibody based methods. ABAcard® p30, RSID Semen and Saliva. Positive results were seen from all kits tested. However, at high concentrations of body fluids (**Figure 3**) the hook effect produced erroneous weak positive results. The RSID semen test kit also approached the Limit of Detection (LOD) with 1 µl of each fluid.

Hook effect
25 µl semen and saliva

Weak positive
1 µl semen and saliva

Figure 3: Weak positive results with RSID Semen Immunochromatographic test strips

Concluding Remarks

A mass spectrometry approach to serological screening offers several advantages when compared to traditional screening methods:

- Mass spec results obtained are confirmatory for human stains.
- Detection is far more reliable since it is based on the unique mass spectra of each protein not the visualization of a test line.
- Improved sensitivity when compared to antibody based kits.
- Workflow is amenable to batch processing with automated prep, analysis and reporting.
- Well suited to multiplexing. Multiple body fluids can be screened in a single analysis.
- Assay consumes less evidentiary material. Less than 10% of swab extract.

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