



B3 Investigating the Identification of Vaginal Material Using Histological, Spectroscopic, and Molecular Methods

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The goal of this presentation is to inform attendees of the recent advancements in three emerging techniques for the identification of vaginal material in a forensic context.

This presentation will impact the forensic science community by providing insight into multiple techniques — histological, spectroscopic, and molecular — to identify and distinguish vaginal material from other biological fluids. Vaginal material does not yet have its own validated, specific, and reliable test for identification.

Body fluid identification is a crucial component in the forensic investigations of sexual assault. The recovery and identification of vaginal material has been a significant challenge for forensic scientists for decades. Forensic Short Tandem Repeat (STR) DNA profiles can be used to identify the donor; however, a crucial facet is to identifying the body fluid source of that DNA, thereby elucidating the circumstances of the case. The identification of vaginal material present on penile swabs, hand swabs, or foreign objects can be used to confirm or refute a victim's or suspect's statement. Several methods have previously been proposed, but none have garnered widespread acceptance. Traditional methods such as histological staining face challenges due to similarities in the cellular makeup of vaginal, buccal, and skin epithelial cells. In recent years, Raman spectroscopy has gained much attention for its use in the identification of body fluids; however, vaginal material has to date been overlooked. Finally, microbial profiling of vaginal material has most recently been proposed as a potential molecular biomarker; however, this work is still very much in its infancy. Further investigation of both traditional and emerging techniques to identify vaginal material is absolutely necessary to address the challenges faced in the industry today.

Following informed consent, this study investigated three techniques: histological, spectroscopic, and molecular methods. Histological methods involved staining skin ($n=10$), buccal ($n=10$), and vaginal ($n=10$) epithelial smears with six histological stains: hematoxylin and eosin, crystal violet, Lugol's iodine stain, Csaba stain, Dane stain, and the Ayoub-Shklar stain. While some of these stains are not considered novel, the last three have recently been identified as having the potential to identify vaginal epithelium. The DXR Raman microscope was used to analyze the spectra for vaginal material, buccal cells, skin epithelial cells, venous blood, menstrual blood, saliva, and semen ($n=10$ for each). Molecular methods involved relatively quantifying the expression of *Lactobacillus gasseri* and *Lactobacillus crispatus*, two common vaginal bacterial species. Real-time Polymerase Chain Reaction (PCR) was used with TaqMan® Universal PCR MasterMix and a primer-probe pair designed for each bacterial species. Cycle threshold values were used to determine expression levels.

For the histology test, skin cells were easily distinguished from vaginal and buccal cells when stained using any method due to the contrast in morphology; however, the Dane stain was found to be superior for the differentiation of all three cell types, as each cell type resulted in a different color, thereby reducing subjectivity. Using Raman spectroscopy, averaged spectra for each body fluid were taken and significant peaks were noted and identified. The resulting peaks from the previously researched body fluids such as venous/menstrual blood, semen, and saliva, were as expected, while the resulting spectra from the vaginal, buccal, and skin epithelial cells showed variation in peak presence and intensity. Preliminary results obtained from the molecular investigation of the vaginal bacteria indicate



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higher expression levels in vaginal material when compared to other body fluids; however, further experimental investigation and data analysis is required.

This research has highlighted the value of traditional methods as well as the merit of emerging methods for the identification of vaginal material in forensic investigations. Each method has been shown to have potential for use in casework, albeit each with their own shortcomings, thereby reinforcing the need for continued research and validation in this field.

Vaginal Material, Body Fluids, Sexual Assault