B84 Surface-Enhanced Raman Spectroscopy for Forensic Analysis of Human Semen

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The goal of this presentation is to provide attendees with a general understanding of a common vibrational spectroscopic technique known as Surface-Enhanced Raman Spectroscopy (SERS) and how it can be used to identify an unknown stain such as semen and mixtures of semen with other body fluids. The protocols established in order to obtain reproducible results and the reasoning behind variations present between liquid and dried samples will be presented.

This presentation will impact the forensic science community by discussing how these preliminary studies and methodology development for semen identification via SERS demonstrate a potential new tool for the analysis of stains relevant to sexual assault cases. As research in this field progresses, reliable identification of additional body fluid stains is highly likely. This could greatly reduce the effort expended to analyze unknown samples, save the forensic community time and money, and establish a single confirmatory identification method for all human body fluids.

Identification of an unknown stain encountered at a crime scene, especially where the context of the case does not provide an indication of the identity of the stain, currently requires a number of time-consuming and costly presumptive and confirmatory tests be performed. SERS is a vibrational spectroscopic method that could allow crime scene analysts to rapidly identify unknown stains both in the laboratory and in the field. The SERS technique utilizes a laser which interacts with molecules applied to a gold nanoparticle chip (SERS substrate) that enhances the normal Raman signal, producing a shift in energy characteristic of the vibrational modes present. These shifts are detected as peaks and the combination of peaks provides the analyst with a unique spectral fingerprint of the molecular components of the sample. The advantages of this method include its high sensitivity, speed, non-destructive nature, ease-of-use, minimal sample preparation requirement, portability, and multiplexing capabilities.

In contrast to conventional Raman spectroscopy, SERS offers higher sensitivity resulting in small sample volumes (~1µL or less) being required for sample identification and the ability to process dilute solutions. This allows for the remaining sample to be utilized for other forensic tests, making the SERS technique an ideal analytical method for use at a crime scene.

It is hypothesized that SERS can be coupled with multivariate statistical methods and established as a confirmatory technique in the analysis of human body fluids encountered at a crime scene. While SERS has been explored for other applications such as analysis of drugs, bacterial diagnostics, and detection of single molecules, little research has been conducted to apply it to forensic analysis of human body fluids.[1-3] This investigation identified and characterized semen from a single donor: neat and neat stained on cotton swatches and swabs. All samples were measured in triplicate to ensure reproducibility and the SERS spectra were acquired using a 785nm laser excitation exposed for 10 seconds at 0.6mW. Ten spectra of each chip were taken, averaged, and then compared to one another. Analysis revealed two components of the semen spectra as hypoxanthine and xanthine. Using ordinary least squares analysis, the abundance of each was determined to be 0.34±0.10 and 0.76±0.16, respectively; residuals revealed the possibility of an unaccounted-for component(s).

A protocol was designed for the extraction of dried semen stains and application to the SERS chip, in which approximately 150µL of semen was pipetted onto a cotton swatch and allowed to dry for 24 hours. The semen was then extracted under various conditions by adjusting both the volume of water and the time elapsed of the cutting's exposure to water. This resulted in extractions using 5µL of water sitting for one, two, five, and ten minutes, and 10µL of water sitting for five and ten minutes. Optimal results were obtained when a small cutting of the stain was extracted in 5µL of water for five minutes.

Additionally, 1:1 mixtures of semen in combination with blood, saliva, urine, and vaginal fluid were evaluated. Resulting spectra were determined to be combinations of the body fluids present which can be procedurally pulled apart using ordinary least squares analysis. Overall, it was concluded that semen produces a spectral pattern that is consistent and readily distinct from blood, saliva, urine, and vaginal fluid.

References:

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Raman Spectroscopy, SERS, Semen Identification