B5 The Effect of Washing and Blood-Enhancement Reagents on the Use of Raman Spectroscopy for Human Blood Identification

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After attending this presentation, attendees will gain insight into both the advantages and disadvantages of Raman spectroscopy in reference to its use in the identification of human bloodstains under a variety of conditions frequently encountered in a forensic setting. These conditions include blood present on a number of fabrics, varying dilutions of blood, and the effect of washing and enhancement reagents on the analysis.

This presentation will impact the forensic science community by disseminating results of novel research regarding the effect of washing and subsequent enhancement of bloodstains, which according to this study has not yet been reported. This research also highlights alternative approaches to the methodology and the importance of investigating the effect of variables.

In forensic investigations, determining the identity of an unknown biological stain can aid both in reconstruction and identification of an individual. Human blood is commonly found at crime scenes, which is first presumptively identified at the scene, then confirmed in a laboratory setting; however, many of the tests used, both presumptive and confirmatory, consume the sample in question, preventing further analysis, namely DNA profiling. Raman spectroscopy has been gaining interest as a new method of body fluid identification, partly due to its non-destructive nature. Prior research has demonstrated that Raman spectroscopy provides a unique spectrum for blood, while also preserving the sample for DNA analysis. The goal of this study was to further investigate the use of Raman spectroscopy for human blood identification in simulated crime scene samples, including bloodstains on a variety of fabrics, at varying dilutions, following washing, and finally post-enhancement.

After obtaining informed consent from volunteers, venous blood was collected in sterile vacutainer EDTA vials. Using a 780nm wavelength laser and a controlled laboratory setting, Raman spectroscopy was performed on samples of blood under various conditions. These conditions included five fabrics (black and white cotton, black and white polyester, and denim), a series of dilutions (1:10 to 1:10^6, both wet and dry), and after washing and treatment with three enhancement reagents (Leuco Crystal Violet (LCV), Coomassie blue, and luminol).

A method of extraction of the stain from the fabrics was also tested. Baseline corrections for fluorescence were performed as necessary.

The results obtained from the bloodstains on a variety of fabrics illustrated that by using spectral subtraction, a signal similar to blood could only be recovered from the white cotton and white polyester samples. The results obtained from the diluted bloodstains revealed that only the neat blood gave a signal while wet. When dried, the neat blood, as well as the 1:10 and 1:100 dilutions, gave a signal with peak shapes similar to the blood reference; however, the peaks became significantly less intense after each successive dilution. The results obtained from the bloodstains that were washed and subsequently enhanced revealed luminol to have no effect or interference on the ability to obtain a clear blood spectrum following spectral subtraction; however, LCV and Coomassie blue introduced interference, giving indeterminate results. The results obtained by utilizing an extraction method of the stain from the fabric revealed a spectrum with similar peak shape and location, but lower intensity, resulting in a weak match to a library reference for blood, regardless of the substrate from which the stain was extracted.

This study demonstrated the capabilities of Raman spectroscopy as a means of identifying human blood in a variety of situations common to forensic investigations. The impact of washing and blood enhancement reagents reveals the importance of the choice of method and its bearing on subsequent Raman analysis.

Raman Spectroscopy, Blood, Enhancement