The interpretation of body fluid mixtures using Raman Spectroscopy in forensic investigations

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ABSTRACT

Raman spectroscopy is becoming a popular technique in the forensic science discipline, and research is beginning to focus on its use in serology. This proof-of-concept study investigated Raman spectroscopy's ability to identify individual body fluids and differentiate between fluids in a mixed sample, while not damaging the sample, allowing for post-Raman DNA profiling. Four types of body fluid were analyzed at 780 nm, both separately and in mixtures, on aluminum slides and two types of cotton. Five areas were scanned on each sample, with each run consisting of five 20-second accumulations. DNA profiling was then performed on the samples. The individual fluids gave unique Raman spectra, and four of the six mixtures, consisting of two fluids, gave positive results for the presence of two fluids. Full DNA profiles were then obtained from a selection of samples after being analyzed. This study reveals the potential use of Raman Spectroscopy for body fluid identification as well as identification within mixed samples, which is crucial in some forensic investigations.

INTRODUCTION

In forensic investigations, stains recovered from crime scenes can often be a combination of different body fluids e.g. semen and saliva, or blood and saliva. However there has been little research into effective analytical methods to confirmatively identify the fluids contained within the these mixtures. Recently there has been a growing interest in Raman Spectroscopy for this purpose. Raman spectroscopy is built on the process of Raman scattering. In this form of scattering, molecules gain or lose vibrational energy from a photon, causing a photon to be released at a wavelength different from the original one (Berger, 2011). It is a fast-emerging technique in the forensic science field due to its easy and reagent-free process, as well as its non-destructive nature (Virkler & Lednev, 2010). One reason behind growing interest is its ability to work with trace samples, as it only requires a few picograms or femtoliters of sample to obtain a reading (Virkler & Lednev, 2009). The development of portable Raman spectrometers has further increased the technique's popularity, as it can now be a tool for crime scene investigators, as opposed to being a technique that is restricted to a laboratory environment (Eckenrode et al., 2001).

OBJECTIVE

To determine if Raman spectroscopy is capable of:

1. Identifying individual body fluids
2. Differentiating between two body fluids in a mixed sample
3. Obtaining a full DNA profile post analysis

METHOD & MATERIALS

All body fluids were collected from volunteers with IRB approval. Twenty total body fluid samples were collected: 7 venous blood, 4 saliva, 4 semen, and 5 urine. After being collected all samples were stored at 4°C.
10 microliters of individual fluids were placed on aluminum slides or fabric, and for mixtures 20 microliters were used. All possible two-fluid combinations were used. Four samples of each mixture were prepared: two of equal ratios and two of unequal ratios. All slides and mixtures were prepared in a sterile PCR hood. Samples were given time to dry overnight.
Spectra were collected using a Thermo Scientific DXR Raman Microscope equipped with a 10x objective and the Thermo Scientific OMNIC™ software. The laser was kept at a constant power of 10 milliwatts and wavelength of 780 nanometers. Each sample was run 5 times at different areas on the sample, with five 20-second accumulations per run. The five runs for each sample were averaged, and all the samples for an individual fluid were then averaged to create the final averages.
DNA was extracted using the QIAgen Swab Protocol. It was then quantified using the Quantifiler Human DNA Quantification Kit, and amplified with the Identifiler Plus Kit. It was then subjected to capillary electrophoresis to profile the DNA.

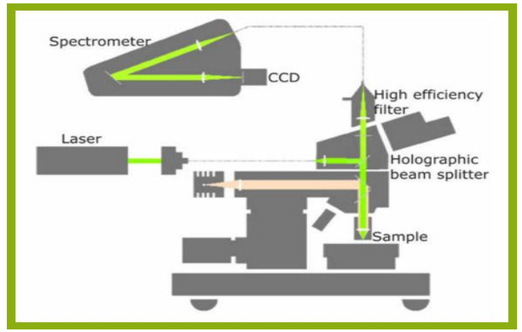

Figure 1: The process of Raman spectroscopy. The laser hits the sample focused under the microscope, and is scattered. That scattered light is collected and read by the spectrometer.

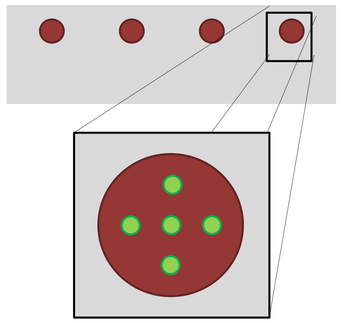

Figure 2: An example of a slide used in this study. The red dots represent the sample, and the green dots in the enlarged section represent the sampling areas.

RESULTS

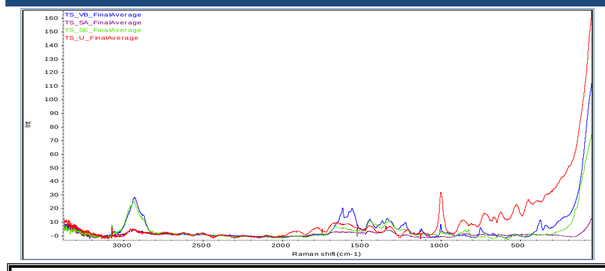

Figure 3: Comparison of final spectral averages of venous blood (blue), saliva (purple), semen (green), and urine (red).

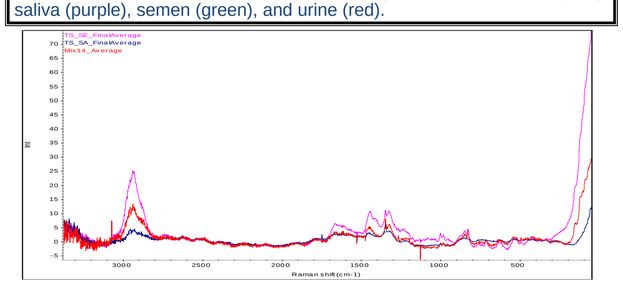

Figure 4: Comparison of a mixture of semen and saliva (red) with the final spectral averages of the two fluids (semen in pink, saliva in blue).

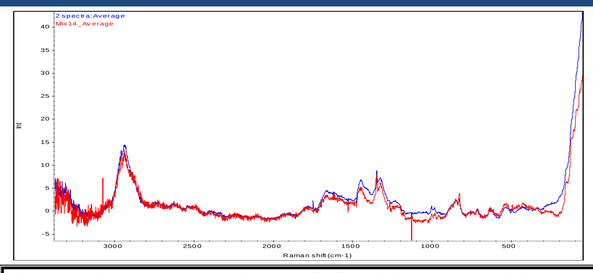

Figure 5: The mixture from Figure 4 (red) with the average of the semen and saliva final spectral averages (blue).

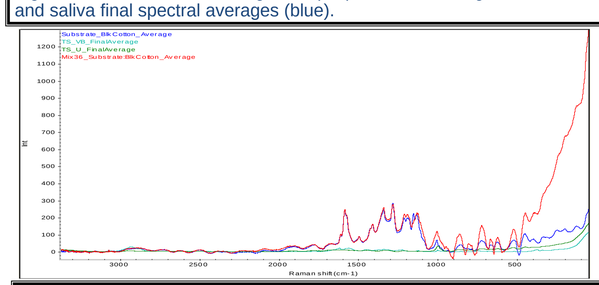

Figure 6: The spectra for a mixture of blood and urine on black cotton (red) compared to the averages of neat black cotton (blue), blood (light blue), and urine (green).

DISCUSSION

The individual fluids were able to be differentiated from one another while on the aluminum slides, which can be seen in Figure 3. Once placed on the substrates tested in this study, only the venous blood was able to be partially detected, and even then only on the white cotton substrate, limiting the techniques potential applications, as the aluminum slides are an ideal laboratory condition. For the mixture testing, detection of mixed samples only occurred on the aluminum slides. The mixtures that were detected on the aluminum slides were semen and saliva (in two of the four samples tested) (Figures 4 & 5), semen and urine (in three of the four samples), saliva and urine (in two of the four samples, partial detection in a third), and blood and semen (partial detection in all four samples), showing the technique may have limitations when differentiating body fluids in mixed samples. The two fabric substrates tested introduced too much interference for detection of the body fluids, as seen in Figure 6. This limits the techniques applications to the field of forensic science, as most body fluid evidence is found on some form of substrate. Further research could demonstrate efficient ways to remove that interference, allowing for the mixed samples to be detected clearly.

CONCLUSIONS

This proof of concept study has shown that Raman spectroscopy does have the ability to identify individual fluids by comparing their spectra. It has demonstrated that this technique is capable of identifying body fluids in mixed samples, while preserving the samples to allow for DNA profiling after the identification of the fluids present.

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