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RESEARCH ARTICLE

ROLE OF RAMANSPECTROSCOPY IN BODY FLUID IDENTIFICATION- A REVIEW

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ABSTRACT

Detection and identification of body fluids, encountered at the crime scene are very important aspects of forensic science today. Identification can be difficult because many of the current techniques are specific to one body fluid, and typical biochemical methods are destructive – preventing any further analysis. To overcome these limitations, and by the potential use of Raman Spectroscopyfor non-destructive and confirmatory identification of body fluids at the crime scene, body fluids are successfully discriminated. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids.

Key words: Body fluids, Raman spectroscopy, Forensic science, Identification, Detection

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INTRODUCTION

Body fluid identification is an essential element in forensic science and has been frequently carried out at the crime scene and in laboratories for many years (Harbison, 2016). Their presence within the crime scene usually gives a lot of valuable and reliable information to forensic practitioners concerning not only just insights about the causes and results around the proof, yet in addition learning in regards to the identity of their owners (Zapata Felix, 2015). A body fluid may be defined as the fluid contained in three fluid compartments of the body i.e. the plasma of the circulating blood, the interstitial fluids between the cells and the cell fluid within the cells (https://medical-dictionary.thefreedictionary.com/body+fluid) Body fluid is composed of both organic and inorganic substances. Blood, Saliva, Semen, Urine and Vaginal secretions are the most commonly encountered body fluids at a crime scene and these body fluids (especially blood) as biological evidence plays a significant role in the reconstruction of the crime as well as in the identification of a suspect or victim as DNA can be extracted from them.These biological fluids have been studied in greater details throughout the years due to their consistent and observable closeness in both victim and scene of crime (Zapata Felix, 2015). Thus, the analysis of body fluids is a key factor during forensic investigation of a crime. Several preliminary and confirmatory biochemical tests are currently utilized for the detection of these body fluids:

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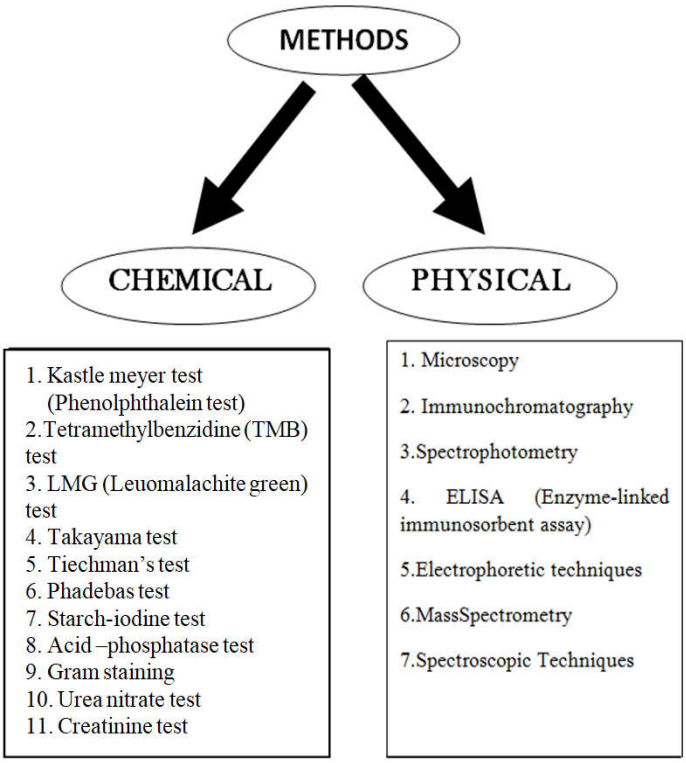
Most of the presumptive tests can be performed in the field; however sample preparation is often required whereas the confirmatory tests must be done in the laboratory, so forensic experts responding at a crime scene will not know the confirmed identity of fluid traces until much later on. The main problem with these tests is the destruction of the sample. There is a great need of reliable and ultimately on-field method that can completely differentiate body fluids from one another, although not destroying the sample in the process.Among the above mentioned physical methods, spectroscopic techniques are gaining popularity now days to identify the body fluids (4).

Limitations of Chemical and Physical Examination of Body fluids

A presumptive test is one that, when positive, would lead the forensic examiner to strongly suspect biological fluid is present in the tested sample. When negative, the test often helps to eliminate stains that need no further consideration. Presumptive may be recognized as those that produce a visible color reaction or those that result in a release of light (nordby). The chemical tests are not human specific and in general are applied sequentially when a mixed body fluid may be present. Many rely on the properties of enzymes in body fluids and many of the reagents are destructive to the samples and/or inhibit downstream processes (Tobe, 2007). Nonvisible stains or stains on dark surfaces are difficult to locate in situ and have been visualized with light sources that use the autofluorescence shown by some body fluids.Variability between body fluids and different surfaces can affect the usefulness of these

methods, and exposure to such light sources may cause damage to the DNA in the stain. The very sensitive luminol test is used in the dark to locate blood, but gives false positives with a wide range of chemicals and dilutes any stain that may be required for further analysis (Webb, 2006 and Quinones , 2006).



Significance of Raman Spectroscopy over Conventional Examinations

The above-mentioned limitations give rise to a rapid, multidimensional, easy-to use, non-destructive confirmatory technique called Raman Spectroscopy. It is a versatile method for analysis of a wide range of forensic samples. Raman spectroscopy was named in the honor of its inventor, C.V. Raman, who, along with K.S. Krishnan, published the first paper on this technique (Raman, 1928). It is a type of vibrational spectroscopy that is based on the inelastic scattering of laser light through its interaction with vibrating molecules (Vitali Sikirzhytski, 2016). Raman spectroscopy is a scattering technique. It is based on Raman Effect, i.e., frequency of a small fraction of scattered radiation is different from frequency of monochromatic incident radiation. It is based on the inelastic scattering of incident radiation through its interaction with vibrating molecules (New Jersey, 1997 and Chalmers, 2012). No sample preparation is required, and the signal can be collected from samples as small as a few femtolitres (10^{-12} gram or 10^{-15} liter, respectively) of liquid or picograms of solid. Raman spectroscopy has approximately10-fold better spatial resolution (~1lm or less) than mid-infrared (mid-IR) spectroscopy. Raman spectroscopy is not limited by physical state and can be performed on gaseous, solid, liquid, gelatinous, nontransparent, and heterogeneous samples with complex chemical compositions. Recent studies have demonstrated that under certain conditions (resonance and surface enhancement), Raman spectroscopy can be performed at the single molecule level (Vitali Sikirzhytski, 2016). Raman spectroscopy is a molecular spectroscopy based on the inelastic scattering of monochromatic light by a questioned sample. During the process the photon either transfers energy to the sample (Stokes) or receives energy from it (anti-Stokes). This difference in energy of the scattered photon corresponds to vibrational or rotational transitions in the sample. The information gained from the vibrational and rotational levels of the probed sample allows for positive identification of the sampled material. Raman scattering is a powerful qualitative and quantitative analytical method based on a process where incident monochromatic photons interact with a sample to produce scattered photons with an energy distribution characteristic of molecular structure (Yu , 1977 and Peticolas, 19751). Ultimately, Raman spectra provide information regarding molecular chemical structure, molecular conformation, interactions between molecules and the surrounding environment, and the physical state and condition of matter. Raman spectroscopy uses a variety of laser sources that provide excitation in a wide spectral range covering ultraviolet, visible, and near-infrared (UV-VIS-NIR).

A typical Raman spectrum consists of several narrow bands and is considered a unique signature of the material. Raman spectroscopy shows minimal interference from water which simplifies the analyses of biological fluids and their traces.Raman spectroscopy is a nondestructive test that relies upon the scattering of low-intensity laser light by compounds including biological materials. The resultant spectra are complex and require advanced statistical treatments to build a unique spectroscopic signature of the molecular structure of each fluid. This complexity is in part because dry body fluids are heterogeneous and there is additional variation between individuals. Typically, non-resonance Raman spectroscopic measurements do not damage the sample. The stain or swab could be tested in the field and still be available for further use in the laboratory for DNA analysis, and that is very important for forensic applications. Raman spectroscopy, when compared to fluorescence spectroscopy exhibits much higher selectivity and specificity to chemical and biochemical species despite having a lower sensitivity, and it could potentially be useful in resolving mixtures of multiple body fluids. The Raman spectroscopic signatures obtained from the samples are compared to reference Raman spectroscopic signatures for different body fluids. The signatures comparison permits the determination of the type(s) of body fluids present in the sample.It also seems to overcome the low specificity of UV-Vis spectroscopy.Raman signatures are sharp and narrow peaks observed on a Raman spectrum. These peaks are located on both sides of the excitation laser line (Stoke and anti-Stoke lines). Generally, only the Stokes region is used for comparison (the anti-Stoke region is identical in pattern, but much less intense) with a Raman spectrum of a known sample. A visual comparison of these set of peaks (spectroscopic signatures) between experimental and known samples is needed to verify the reproducibility of the data. Therefore, establishing correlations between experimental and known data is required to assign the peaks in the molecules, and identify a specific component in the sample. The Raman effect is obtained when a photon interacts with the electron cloud of a molecular bond exciting the electrons into a virtual state. Raman spectral acquisition is accomplished by moving the sample in a stepwise manner until the entire region of interest is characterized. The main disadvantages of Raman Spectroscopy are weakness of Raman effect in the absence of resonance and surface enhancement as well as the potential for fluorescence interference.

Table 1. Comparison between three spectroscopic techniques for potentialbody fluid identification

PARAMETERS	UV-VISIBLE SPECTROSCOPY	IR SPECTROSCOPY	RAMAN SPECTROSCOPY
Sample Preparation	Required	Required	Not Required
Types of Body Fluids Analysis	Semen, Saliva and Urine	Only Blood	Applicable for all body fluids
Nature of Examination	Destructive	Destructive	Non-Destructive
Frequencies	Limited to UV-Visible frequency	Limited to IR frequency	Uses UV, Visible and near-IR frequencies
Light Source	UV-Visible lamp	IR light	Monochromatic light
Effect of Drugs	Yes	Yes	No
Analysis of Degraded Samples	No	Upto a certain extent	Yes
Cost	Expensive	Highly expensive than UV-Visible spectrometer	Low in cost
Dynamic Range	2ml	10-20 microns	Not Specific

But, there are solutions to this problem. First, to use deep ultra-violet light and second, to use near IR-excitation for Raman spectroscopy measurements. This technique in particular, has proven to be a very promising analytical technique for several forensic applications (Zufang Huang, 2011). Raman mapping can be used to probe across a sample's surface, instead of a single point. Meanwhile, the selective nature of Raman spectroscopy enables it to discriminate between chemically analogous species (Igor, 2016). Its power is further enhanced when chemometric analyses are applied to spectroscopic datasets.Consequently, Raman spectroscopy has been used to study pure body fluids (Zhao, 2007), cells (Virkler, 2009), mixtures (Grasselli, 2002), and contaminated traces (De Wael, 2008). Raman spectroscopy can also discriminate between human and non-human animal blood (Eckenrode, 2001), as well as peripheral and menstrual blood (Kotowski, 1986). Thus, the technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids (Muro, 2016). Laboratory methods available today enable forensic scientists to detect and identify extremely small quantities of body fluids. Comparatively DNA analysis may then be used to unequivocally eliminate an individual as a possible source of the fluids or attribute the origin to a particular individual with practical certainty (James, 2009).

Conclusion

Body Fluids are the major part of the human body as well as an important trace biological evidence in the field of forensic science associating in crime scene reconstruction and identification of suspect or victim through DNA extraction. Their examination faces several problems in earlier times. A number of techniques like colour tests, microscopic examination, Immuno chromatography, Spectrophotometry etc have been employed but due to their destructive nature the spectroscopic techniques proves to be useful. Among the spectroscopic techniques such as UV spectroscopy, IR spectroscopy, Raman spectroscopy is found to be a universal, multi-dimensional, non-destructive confirmatory technique for forensic identification of body fluids. It is easy to use, versatile and trustworthy technique which can overcome all the limitations of the other spectroscopic techniques and is capable in quick identification of body fluids both individually as well as in mixtures thus assisting in crime scene management and legal system.

Future Suggestions

The Raman Spectroscopy proves to be a universal non-destructive confirmative technique for body fluid identification at a crime scene. In Future, advanced software can be adopted for a portable Raman Spectrometer due to its great potential and necessity in modern crime investigation system. Further, a methodology should be developed which can be potentially used for determining the age of body fluids. Determining the age of a body fluid stain on a crime scene, will have a major impact on the efficiency of crime scene investigation in at least two major aspects. First, determining the stain age could help to establish the time of the crime. Secondly, determining the age of numerous body fluid stains recovered at a crime scene should allow law enforcement agencies to prioritize the collected evidence to those, which are time-related to the crime. However, it also requires the understanding of how aging affects the spectral response from body fluid traces.

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