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A Pilot Study On The Identification Of Blood Using Raman Spectroscopy

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Abstract:

Blood is frequently found at crime scenes, either as dried stains or in liquid form, yet it's often in limited quantities. Traditional tests for blood can sometimes damage the sample, hindering deeper analysis. Recent scientific work has highlighted Raman spectroscopy as a non-invasive method for identifying bodily fluids. This study delves into the sensitivity and accuracy of Raman spectroscopy in identifying blood, using the Raman peakseeker™-PRO785 for our analysis. Our study demonstrated that Raman Spectroscopy can detect blood even when diluted up to 1:250 with water. Additionally, this technique successfully identified blood in aged samples. In essence, this study underscores the importance of Raman spectroscopy in identifying various body fluids.

Keywords: *Raman Spectra, Human Blood, Sensitivity, Specificity*

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Introduction

Forensic serology is the application of serology in identification of body fluids such as blood, semen, saliva, vaginal secretions, etc., to legal matters for example in relating victim/preparator to the crime, to establish presence of victim or preparator during crime and also to produce body fluids as the evidence in court of law in order to solve the case. Of various body fluids blood is the most common evidence encountered in violent crimes such as assault, accidents, and murder **(Harris and Lee, 2007).** Blood consists of plasma, red blood cells (Erythrocytes), white blood cells (Leukocytes) and platelets **(Dean, 2005**). During examination of the blood evidence, forensic serologists confirm that the collected evidence from crime scene is blood. Another important aspect is to determine the species of origin of the blood sample and individualization **(Li, 2008).** Alternate Light Source such as Polilight is used for the identification of latent bloodstain in the crime scene. However, while using ultraviolet wavelength the DNA present in blood could get damaged and the sample cannot be used for DNA profiling **(Vandenberg and Oorshot, 2006**). Various presumptive tests are performed by forensic scientists to check particular stain or liquid might be blood. These tests are based on the peroxidase-like activity of heme group present in hemoglobin, a red-colored pigment present in erythrocytes **(Spalding, 2003).** The most confirmatory tests (for blood and semen) are destructive. It is necessary to apply different tests to confirm each type of body fluid; this limitation requires division of a sample into several parts, and a portion of the sample having to be kept for possible future analyses **(Zapata *et al.*, 2014).**

Raman spectroscopy has found increasing utility across diverse areas of forensic science, analyzing evidence like ink, lipstick, drugs, paint, and fibers. Its non-destructive nature allows for application in forensic serology, identifying bodily fluids such as blood, saliva, semen, urine, sweat, and vaginal secretions **(Virkler & Lednev, 2008).** This technique, based on the theory of inelastic scattering of low-intensity laser light, operates without damaging the sample **(Virkler & Lednev, 2009)**. It often requires minimal or no sample preparation, allowing direct analysis in some cases with quantities as minute as picograms or femtoliters. The advantage of detecting trace amounts of blood directly aids subsequent DNA analysis, a crucial step in investigations. Unlike conventional presumptive tests, Raman spectroscopy doesn't consume the sample and shows less interference with water, enabling analysis of blood in its fluid form **(Virkler & Lednev, 2008).** The unique spectral signature generated for various body fluids facilitates their distinction, even amid contaminants like sand, dust, or soil, and differentiation between different bodily fluids **(Harbison and Fleming, 2016; Sikirzhytskaya et al., 2023).** Raman spectroscopy identifies blood by discerning specific components such as hemoglobin, albumin, fibrin, and glucose based on their characteristic peaks **(Virkler and Lednev, 2008).** Its ability to measure individual erythrocytes allows the identification of minute blood particles present on tape or fibers, relying on the vibrational spectrum of hemoglobin **(De Wael et al., 2008).** The sensitivity of m RNA markers was similar to the sensitivity of presumptive blood tests such as tetramethylbenzidine (TMB) And Hexagon OBTI test but the disadvantage of this method is non-specificity with saliva due to trace amount of blood and mensural blood **(Harbison and Fleming, 2016**). UV-visible spectroscopy is considered highly reliable for confirming the presence of aged blood samples. Hemoglobin derivatives show characteristic sorbent band around 400 nm **(Virkler nd Lednev, 2009).** The advantage of this method is to estimate the time since deposition of blood stains **(Hanson and Bllantyne, 2010).** Blood may available in any form such as fresh liquid, stain, coagulated or dried. Hence requires different method of collection and preservation **(Kleypas and Badiye, 2023).**

This comprehensive overview underscores the efficacy of Raman spectroscopy in forensic science, particularly in identifying trace blood evidence. This study aims to utilize Raman spectroscopy to identify and determine the sensitivity and specificity of stored blood samples.

Methods:

Protocol used for sample collection: Blood samples (3 ml) would be collected from the volunteer, chicken (Gallus gallus domesticus), and goat (Capra hircus). The samples were collected in EDTA vacutainers and kept stored at a temperature of 4 degrees until they were required for analysis

Preparation of Blood dilutions: Dilutions of human blood in the water at a ratio of 1:50,1:150,1:250

Sample analysis using Raman spectrometer: In the present study the spectra of blood samples would be obtained on excitement within a built 785 wavelength laser. The blood samples are analyzed by using Raman Peakseeker™ -PRO785From was conducted at BMS Institute of Technology and Management, Bengaluru, Karnataka. The Raman spectra obtained would be plotted using Origin 8 software.

Result:

Blood consists of several components such as hemoglobin, albumin, and glucose. Among these components' hemoglobin constitute the major part of the blood. Therefore, the determination of hemoglobin peaks in the Raman spectrum helps in the identification of blood samples. Further to distinguish human blood from non-human blood, blood samples of goat and chicken are analyzed with Raman spectrometer. The prominent peaks of hemoglobin at 1368, 1576 and 1619 per cm were seen in human blood till the dilution of 1:250 with distilled water (Figure 1-5). Other components such as phenylalanine and glucose show peaks at 1001 and 1123 per cm respectively. Raman spectrum was unable to differentiate human blood from goat and chicken blood since it shows same peak as of human blood samples. (Figure 6-7)

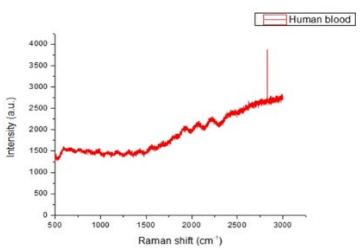

Figure No. 1: Human blood Raman spectrum

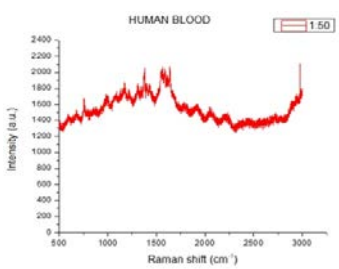

Figure No. 2: Human blood Raman spectrum at dilution 1:50

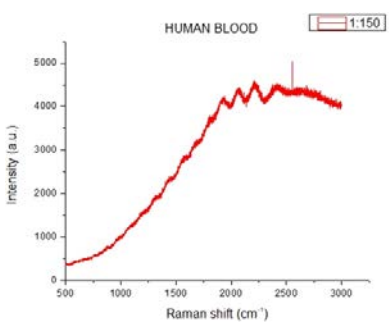

Figure No. 3: Human blood Raman spectrum at dilution 1:150

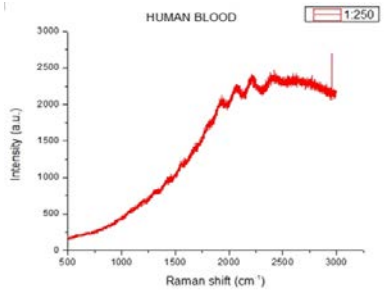

Figure No. 4: Human blood Raman spectrum at dilution 1:250

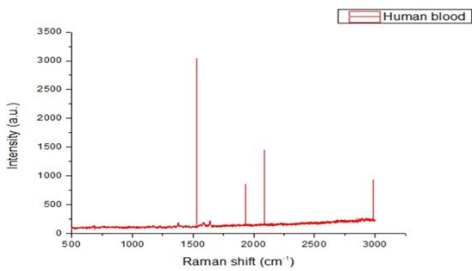

Figure No. 5: One month aged human blood Raman spectrum

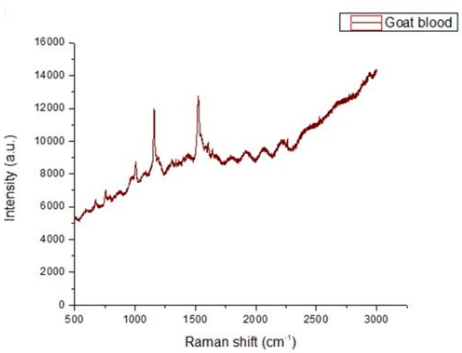

Figure No. 6: Goat blood Raman spectrum

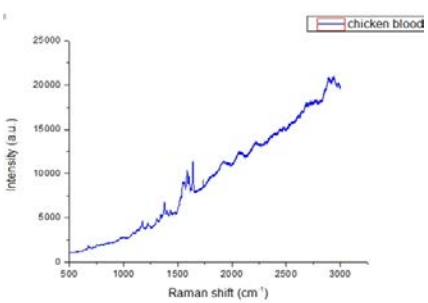

Figure No. 7: Chicken blood Raman spectrum

Discussion:

Forensic serology plays a critical role in identifying bodily fluids at crime scenes, which might not always be visibly present. Once detected, establishing the nature of these fluids becomes crucial for subsequent DNA analysis, aiding in investigations. The presence of blood, common biological evidence, often signifies a violent crime, whether in liquid or stain form. To identify blood effectively, the method used should meet specific criteria, including non-destructiveness, minimal sample requirement, preservation of DNA evidence, immediate result feedback, field-testing capability, reliability, and suitability for courtroom testimony.

Over the years other immunological presumptive tests are available for the identification of blood. Heme select, ABA card HemaTrace and Hexagon OBTI methods used to identify primate blood and works well on aged and degraded material. The main disadvantage of Hexagon OBTI and ABA card methods is they can produce false-negative results for neat blood due to high dose hook effect. There are also, emerging trends in field of body fluids identification and discrimination using molecular methods.

DNA based assays were made for the identification of blood. DNA methylation at 5' position of cytosine in CpG dinucleotide used to identify body fluids **(Frumkin *et al.*, 2011)** Specific DNA methylation markers cg06379435 and cg08792630 are used for the identification of blood **(Park *et al.*, 2014).** The mRNA markers such as porphobilinogen deaminase (PBGD), β-spectrin (SPTB) and hemoglobin beta (HBB) are used for the detection of blood due to abundance of these proteins **(Haas *et al.*, 2010).** Furthermore, different approach in RT-PCR was made to detect alpha locus1 (HBA) RNA marker to identify the blood **(Nussbaumer *et al.*, 2005).** In 2005 Juusola and Ballantyne developed Reverse transcriptase polymerase chain reaction (RT-PCR) capable of detecting ample amount of mRNA from the minimal amount of body fluids such as blood, resulting in the detection of blood-specific genes erythroid δ-aminolevulinate synthase (ALAS2), STPB and GAPDH (housekeeping gene) (**Juusola and Ballantyne, 2005)** Recently, RT-LAMP (Real Time Loop-Mediated Isothermal Amplification) was developed to identify blood by use of HBB (Hemoglobin Beta) as blood- specific marker **(Su *et al.*, 2015).**

Proteomic along with mass spectrometry allows the identification of blood by detecting blood specific proteins- alpha and beta hemoglobin and spectrin **(Yang *et al.*, 2013; Zubakov *et al.*, 2008)** identified nine stable RNA markers with the help of GNF SymAtlas tissue database for identification of bloodstains. Another alternative to proteomics, NMR (Nuclear magnetic resonance) was developed for the identification of blood. NMR combined with statistical analysis (includes multivariate statistical analysis, principal components analysis and hierarchical cluster analysis) can able to detect blood from mixture of body of body fluids **(Scano *et al.*, 2013).**

There is advancement in the development of portable devices and biosensors **(Trombka *et al.*, 2002)** has suggested the method which is different from the normal laboratory presumptive test. XRF a portable device used for the identification of blood at the crime scene. It measures iron content in blood. Biosensors is the one of emerging method in identification of blood **(Frascione *et al.*, 2013)** The device produces measurable signal when it recognizes the biological fluid. This optical sensing technology is highly specific to body fluids and non-destructive in nature. A recent study was made on immunofluorescent biosensors for detection of blood and it has positive results. For this study anti-glycophorin A was conjugated with fluorescent semiconductor quantum dots, it shows characteristic fluorescence emission spectra when it detects the blood samples. These spectra vary depending on the concentration of the sample **(Harbison and Fleming, 2016).** As quantum dot does not affect the sample, the same can be used for DNA profiling **(Frascione *et al.*, 2013).**

The non-destructive attenuated FT-IR used in biomedical science diagnostic purposes and even for the identification of body fluids such as blood, saliva, semen and vaginal secretions. Based on the spectra observed body fluid can be differentiated from each other **(Orphanou, 2015)** Raman spectroscopy, meeting these criteria, emerges as a potential technique for non-destructive identification of blood samples with minimal or no sample preparation. Studies conducted by Virkler & Lednev in 2008 and 2009 explored the capabilities of Raman spectroscopy in differentiating body fluids like semen, blood, saliva, sweat, and vaginal fluid based on their unique spectral signatures. However, limitations were observed in distinguishing between human, goat, and chicken blood samples. Additional research by Wael et al. in 2008 revealed challenges in differentiating human and non-human blood particles using Raman spectroscopy, while Sikirzhytska et al.'s work in 2013 demonstrated the identification of highly contaminated blood without prior knowledge of contaminants. Boyd et al.'s studies in 2011 and 2013 focused on identifying human blood samples and using surface-enhanced

Raman scattering (SERS) for blood stain identification, respectively.

The study in question, while successfully identifying human, goat, and chicken blood samples using Raman spectroscopy at 785 nm, had limitations in distinguishing between these sources. The technique displayed sensitivity up to a dilution of 1:250, yielding positive results even in aged blood samples. However, it fell short in differentiating between human, goat, and chicken blood samples.

In conclusion, while Raman spectroscopy presents promise in identifying blood samples, particularly in non-destructive ways and with minimal sample requirements, further research involving a larger sample size is necessary to better understand its capabilities, especially in differentiating between animal blood sources.

Conclusions:

Raman spectroscopy can be used for blood identification.

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