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Discriminating Peripheral Blood and Menstrual Blood with Different Methods and Instrumentations

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Abstract: ***The blood that flows throughout the body is referred to as peripheral blood. Erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes are the biological elements that can be extracted from human peripheral blood (platelets). Human peripheral blood-derived goods are crucial beginning points that are used in all areas of research and development. These products are used in numerous scientific disciplines, such as transplant and regenerative biology, microbiology, virology, oncology, vaccine development, toxicity, and immunotherapies. Regular vaginal bleeding that occurs as part of a woman's monthly cycle is referred to as her period, also known as menstruation. A woman's body gets ready to get pregnant every month. If there is no pregnancy, the lining of the uterus, often known as the womb, is lost. Blood and uterine tissue together make up the menstrual blood. The vagina is where it exits the body. Body fluid residues can yield incredibly insightful clues for forensic investigations. Bloodstains are particularly frequent in criminal investigations, and distinguishing between menstrual and peripheral blood is an important step for work on rape and sexual assault cases. This review study aims to outlines the criteria that forensic experts utilize to differentiate between menstruation blood samples and regular blood samples using various instruments and techniques.***

Keywords: Peripheral Blood, Toxicology, Menstruations, Bloodstains, Sexual Assault.

I. INTRODUCTION

This A unique bodily fluid is blood. Red blood cells, white blood cells, platelets, and plasma make up its four basic parts. Blood has a variety of purposes, including transferring waste items to the kidneys and liver for cleaning and filtering, carrying cells and antibodies that fight infection, preventing excessive blood loss, creating blood clots to prevent loss of blood, and controlling body temperature.

Whole blood, a mixture of roughly 45 percent blood cells and 55 percent plasma, is the blood that flows through the veins, arteries, and capillaries [1]. Your blood makes up roughly 7– 8% of your total body weight. A guy of average height has about 12 pints of blood in his body, whereas a lady of similar height has about 9 pints. Haematopoiesis, a tightly controlled process that occurs in the bone marrow, is how blood cells arise from hematopoietic stem cells. Red, white, and platelet-producing hematopoietic stem cells have the capacity to differentiate into these different blood cell types. All ages of people have these stem cells circulating in their blood and bone marrow, as well as in the umbilical cords of newborns [2]. The complex iron-containing protein called haemoglobin is what gives blood its red colour.

The tetramer of two alpha and two beta chains that make up haemoglobin is a globular protein [3]. One of the most significant biological clues that are frequently discovered at crime scenes is blood. It is regarded as a very useful forensic tool because of the important information it contains. Bloodstain analysis can help to shed light on the circumstances surrounding the commission of some violent crimes. Such important details can drive criminal investigations in the proper directions and aid in the criminal case's resolution. It is frequently found in situations of murder, sexual assault, and terrorist attacks, such as those involving bomb explosions [4].

The uterine lining is shed on a monthly basis during menstruation. Menstruation is also referred to as menses, menstrual period, menstrual cycle, and period. Menstrual blood, which is made up of both blood and tissue from the uterus, exits the body through the vagina after leaving the uterus. Vaginal fluids, blood, cervical mucus, and endometrial tissue all make up the menstrual fluid [5]. In the cases of Sexual Assaults or Rape, Blood is common evidence that can be found on the crime scene, there is a possibility that blood found on the crime scene can be menstrual blood or else, it is normal blood.

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II. PRELIMINARY EXAMINATION FOR THE CONFIRMATION OF BLOOD

An After forwarding the evidence in the Laboratory, experts analyses the evidence to confirm whether the present sample is blood or not. Firstly, physical examination is done in which colour, odour, pattern (in case of stain present on a cloth) and other physical characteristics are examined. Secondly, to establish the presence of blood, microcrystalline assays like the Takayama test and the Teichmann test are employed. Some other tests such as immune-electrophoresis, RNA based assays, Ion exchange chromatography methods can be conducted for the confirmation of blood in the sample. Following the confirmation that the suspected sample is blood, additional analysis is performed to determine whether the blood sample is menstrual or normal [6].

III.DIFFERENTIATING WHETHER THE PRESENT BLOOD SAMPLE IS MENSTRUAL OR PERIPHERAL

All Various tests are conducted to differentiate whether the present blood sample consists of menstrual blood or peripheral blood:

A. Crystal Test

This test is also performed to confirm the presence of blood in the sample. Under this, two types of reagents are used: Takayama reagent and Teichmann reagent. The given blood sample is treated with one of these reagents on a slide with a heat shock wave and formation of crystals are observed under the microscope. The crystals formed in the case of Takayama reagent are observed as pink in colour and needle-shaped whereas when Teichmann reagent is used, brown coloured rhombic- shaped crystals are observed under the microscope [7]. This crystal test will give positive results in the case of Peripheral or Normal blood whereas it will always give negative results with Menstrual Blood as very less amount of haemoglobin is present in menstrual blood as compared to peripheral blood.

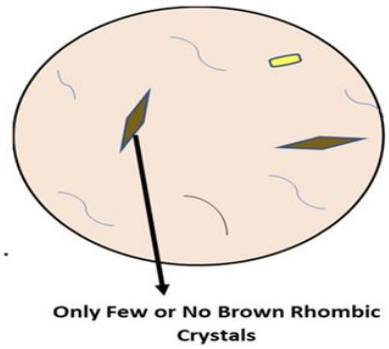


Fig. 1 Teichmann test shows negative result with menstrual blood.

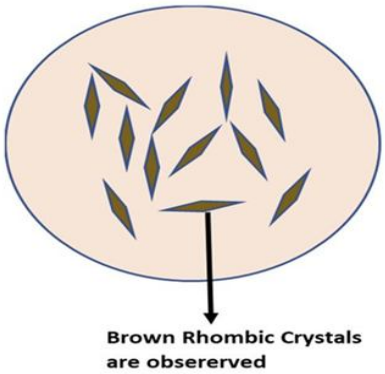


Fig. 2 Teichmann test shows positive result with normal blood.

However, the crystal test is not a reliable or accurate method for discriminating between peripheral blood and menstrual blood. It is a subjective test that is highly dependent on the observer's interpretation of the crystal formation [8]. The test can also produce false positives and false negatives, leading to incorrect results. Moreover, the crystal test does not provide any information about the molecular composition of the blood or its chemical properties.

B. Haemoglobin Estimation

A conjugated protein called haemoglobin makes over 90% of the dry weight of red blood cells. Two pairs of related polypeptide chains, known as globin chains, make up the tetramer haemoglobin, or haemoglobin. Each of the four chains has a heme linked to it, which is a combination of protoporphyrin and ferrous iron. It is basically a protein which is present in blood, is responsible for transporting oxygen molecules. There are various methods for the detection of haemoglobin:

1) *Sahli's Method:* The amount of haemoglobin in the supplied sample is estimated using this technique. Using the Sahli hemoglobinometer requires combining the blood sample with 1N HCl to create acid hematin, then adding distilled water until the calibration tube's color matches the testing tube's colour. The Hb value is what is seen once the colors of the calibration tube and testing tube are matched [9]. This method has various advantages like it is inexpensive, easy to use and it does not require any technical skill. Sahli's method is a type of "Visual Method". Some other visual methods are Dares method, Haldane method, Tallquists method, WHO haemoglobin colour scale method and Spencer method.

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2) *Cyanmethemiglobin Method:* This method is considered as the most accurate and widely used method. The principle of this method is that all forms of haemoglobins aside from sulfhemoglobin are transformed to cyanmethemoglobin when blood is dissolved in a solution containing potassium ferricyanide and potassium cyanide. With a photoelectric colorimeter, the color's intensity is proportional with levels of haemoglobin and contrasted with a known cyanmethemoglobin standard at 540 nm (green filter). Drabkin's reagent is used in this method along with Cyanmethemoglobin standard solution. It is a type of "Spectrophotometric Method". Some other Spectrophotometric methods for haemoglobin estimation includes Oxyhemoglobin method and Alkaline hematin method [10].

Some other methods of Haemoglobin estimation are Specific gravity method, Haemoglobin estimation in autoanalyzer, Portable hemoglobinometer (hemocue system), Lovibond-Drabkin method, Tallquist method and copper-sulfate method [11].

C. Blood Cell Count

Red blood cells and white blood cells, or RBCs and WBCs, respectively, are enlarged. These are the blood cells that provide a purpose. RBC and WBC numbers are estimated using a microscope and a slide known as Neubauer's haematocytometer slide [12]. The chambers on Neubauer's slide, known as Neubauer's chambers, are counted and added to the formula to estimate the cell count. Neubauer's chambers are chambers that can be seen under a microscope. A vital step in determining whether blood is menstrual or normal is measuring the number of cells, but doing so manually under a microscope takes a lot of time and increases the likelihood of human mistake [13]. As an alternative to the hemacytometer, an automated cell analyser can be employed. Although these techniques are available, they are not frequently used in forensic laboratories.

The following criteria are used to differentiate between menstruation and regular blood:

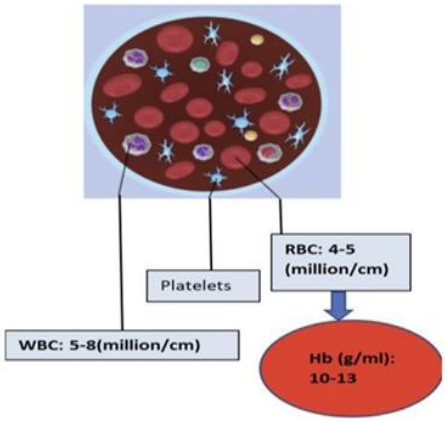


Fig.3 Peripheral Blood

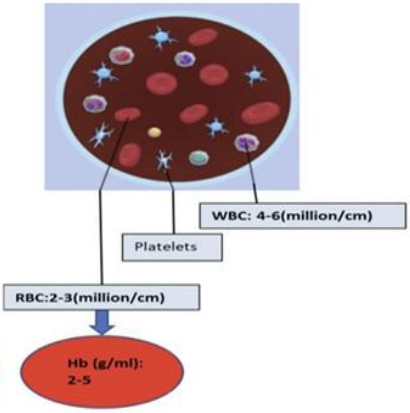


Fig.4 Menstrual Blood

D. mRNA Biomarkers

mRNA biomarkers are molecules that are transcribed from DNA and carry the genetic information necessary for protein synthesis. mRNA biomarkers are specific to different types of cells and tissues and can be used to identify the source of a biological sample. Real-time RT-PCR, an appropriate method to evaluate the mRNA expression, might be used to distinguish between menstrual and peripheral blood stains using HBA, MMP7, and MMP11 as helpful indicators. The scientist uses biomarkers that express genes to identify menstrual blood. The biomarkers to be taken into consideration are MMP7 (Matrix Metalloproteinases), MMP11, and HBA (Haemoglobin alpha). Blood from menstruating women and peripheral blood both include the biomarker HBA gene expression. Only menstrual blood has the biomarkers for MMP7 and MMP11 gene expression. Comparatively more MMP7 than MMP11 is present in the body. Because MMP7 and MMP11 are present in the uterine endometrial layer and are involved in its shedding, they are exclusively expressed in menstrual blood [14][15]. Overall, mRNA biomarkers are a promising method for discriminating between peripheral blood and menstrual blood due to their specificity and sensitivity. However, more research is needed to validate the use of mRNA biomarkers in forensic analysis and medical diagnosis.

E. Immunochromatographic assay (SERATEC PMB test)

It has been demonstrated that immunochromatographic tests for bodily fluid detection are extremely specific and simple to operate. Fibrinolysis, the endogenous breakdown of fibrin after blood coagulation, is a crucial step that enables menstrual fluid to easily pour out during menstruation. Recent initial work successfully introduced immunochromatographic assays that detect degradation products of fibrinolysis (FDPs) as novel methods for the identification of menstrual fluid in forensic samples. [16] The most important subtype of FDPs is D-dimer, a tiny protein fragment that is unique to the fibrinolysis process. The PMB test was created for the identification of blood and menstrual fluid, both at the crime scene and in the lab. It is a duplex test combining human haemoglobin and D-dimer detection. D-dimer is typically 200 times more abundant in menstrual blood than peripheral blood. The test cassettes were simple to use, and the results' interpretation was simple and clear. To successfully use the test, no specialized training was required. In cases of alleged sexual assault, biological fluids such vaginal fluid, semen, saliva, and urine may be included in a mixed sample of evidence. Because all combinations including menstrual blood produced a positive signal for the presence of D-dimer and no false-positive findings were obtained for other examined body fluids, confirming its excellent specificity [17]. A study published in the Journal of Forensic Sciences in 2015 found that the SERATEC PMB test had a sensitivity of 100% and a specificity of 97% for detecting menstrual blood. However, it should be noted that the SERATEC PMB test is not a confirmatory test and should be used in conjunction with other methods of discrimination, such as DNA analysis or microscopic examination. Overall, the SERATEC PMB test is a useful tool for discriminating between peripheral blood and menstrual blood in forensic analysis or medical diagnosis. However, it is important to interpret the results of the test in the context of other available evidence and to use other methods of discrimination to confirm the results [18].

F. Examination via Spectroscopy

1) *Raman Spectroscopy:* Raman spectroscopy is a spectroscopic method that can be used to examine the chemical makeup of materials by detecting vibrational, rotational, and other states in a molecular system. Based on the Raman Infrared Spectroscopy, blood and other bodily fluids can be distinguished from one another. Similar to this, using the Raman spectra, it is possible to distinguish between menstrual blood and peripheral blood. Both the menstrual and peripheral blood spectra are distinct, 100% sensitive, and 100% specific [19]. The method outperforms other damaging and chemical methods. This technique measures the inelastic scattering of light by molecules and provides information about the molecular structure and chemical composition of the sample. In this method, a laser is used to excite the molecules in the sample, and the scattered light is collected and analysed. To use Raman spectroscopy for discriminating between peripheral blood and menstrual blood, a small sample of each type of blood would be collected and placed onto a Raman spectroscopy sample holder. The sample would then be irradiated with a laser, and the resulting Raman spectrum would be collected and analysed [12]. Overall, Raman spectroscopy is a promising method for discriminating between peripheral blood and menstrual blood due to its high sensitivity and specificity.
2) *ATR-FTIR Spectroscopy:* ATR-FTIR stands for Attenuated Total Reflectance- Fourier transform Infrared spectroscopy. ATR-FTIR spectroscopy works by shining an infrared light onto a sample and measuring the absorption and transmission of the light as it interacts with the sample [14]. The resulting spectrum is unique to the chemical composition of the sample, allowing for the identification and discrimination of different types of biological fluids. In the case of discriminating peripheral blood and menstrual blood, ATR-FTIR spectroscopy can detect differences in the molecular composition of the two types of blood. For example, menstrual blood contains higher levels of lipids, proteins, and carbohydrates compared to peripheral blood. These differences in the molecular composition can be detected using this spectroscopy. To use this method for discriminating peripheral blood and menstrual blood, samples of each type of blood would be prepared and analysed using an ATR-FTIR spectrometer. The resulting spectra would be compared, and any differences in the spectra would be used to discriminate between the two types of blood [20]. Overall, ATR-FTIR spectroscopy is a promising technique for discriminating between peripheral blood and menstrual blood due to its high sensitivity and ability to detect molecular differences between the two types of blood.

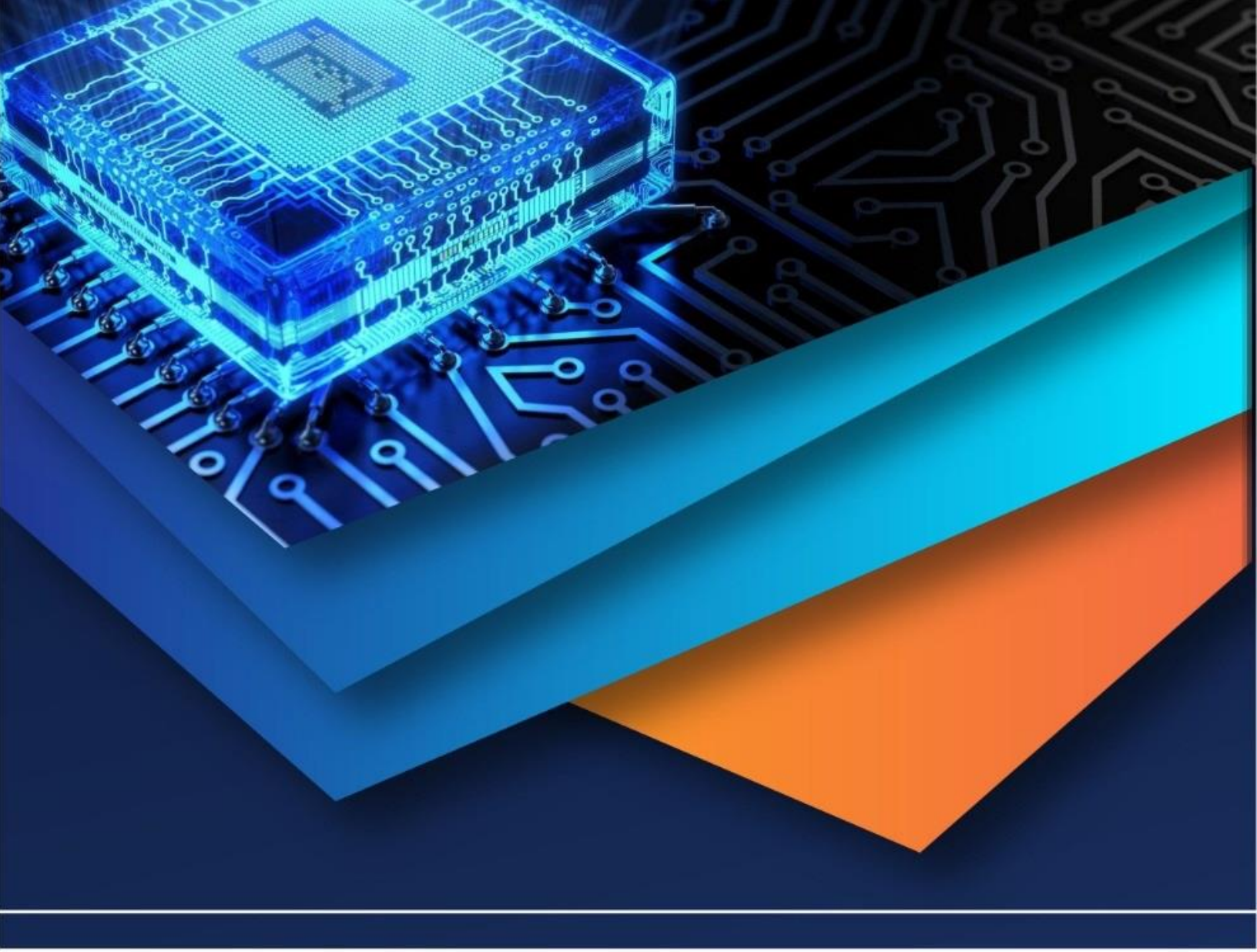
IV. CONCLUSIONS

In conclusion, the differentiation of menstrual blood from peripheral blood can be achieved using a combination of different techniques, including crystal test, hemoglobin estimation, blood cell count, mRNA biomarkers, immunochromatographic assay (SERATEC PMB test), and examination via spectroscopy. The crystal test is a simple and inexpensive method that can be used to detect the presence of characteristic menstrual blood crystals. Hemoglobin estimation and blood cell count can also provide useful information, as menstrual blood typically has a lower hemoglobin concentration and a higher white blood cell count than peripheral blood.

The mRNA biomarkers, which are specific to endometrial tissue, can also be used to differentiate menstrual blood from peripheral blood. Additionally, immunochromatographic assays such as the SERATEC PMB test can provide rapid and accurate identification of menstrual blood. Examination via spectroscopy can also be used to detect the presence of specific compounds that are unique to menstrual blood, such as prostaglandins and iron. It is important to note that while each of these techniques can provide valuable information, a combination of methods may be necessary to achieve the most accurate and reliable results. Additionally, careful consideration of the timing of sample collection and any relevant medical history or physical examination findings can help to further support the differentiation between menstrual and peripheral blood.

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