

## **Distinguishing Blood as Normal or Menstrual Through Instrumental Analysis**

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**ABSTRACT:** Blood is a biological evidence which can be found at any of the crime scene such as murder, rape, burglary, suicide. Blood mainly acts as a vital biological evidence based on which the suspect can be identified. In a human body the blood is mainly considered as oxygenated and deoxygenated but based on location mainly in women they are of two types, the one which flows in blood vessels considered as normal blood and the other which is present in the uterus (endometrial layer) of the women considered as menstrual blood. This review paper, summarizes and focuses on the parameters that the forensic experts use to distinguish the provided blood evidence as normal blood or menstrual blood sample.

**KEY WORDS:** Blood, biological evidence, normal blood, menstrual blood, biological evidence hemoglobin.

### **INTRODUCTION**

Blood is red colored fluid flowing in the human body considered as the largest connective tissue. Blood is mainly composed of water, blood cells (RBCs, WBCs, and platelets) and plasma. The primary function of blood is circulation of oxygen to the body and the lungs and removes the carbon di oxide from the body.<sup>[1]</sup> Blood act as a biological evidence in the field of forensic science and is a crucial evidence for the identification of suspect. Blood can be found in the criminal cases like murder, rape, suicide, accident, dowry death, burglary. The blood found in the rape cases can be normal blood or menstrual blood. The menstrual blood can be the menses blood or blood due to hymen rupture. The basic content of menstrual blood is as of normal blood but also includes endometrial lining, vaginal secretion, mucus, some hormones and may contain the hormones present in birth control pill, if the women consumed it.<sup>[2]</sup> Blood after visualizing through naked eyes cannot be distinguish as menstrual or normal blood. The sample suspected as blood is identified as normal or menstrual to conclude the type of offense that has been happened with the victim. It concludes the offense as normal or sexual assault as there is a lot of chances of menstrual blood as evidence in the sexual offense. After receiving the evidence suspected as blood in the forensic laboratory, the further investigation or the scientific tests are applied by the scientific expert.

### **IDENTIFICATION OF THE EVIDENCE**

The very first step which is carried out in the laboratory is to confirm that the suspected sample is blood only. For that scientific expert visualizes the evidence physically. The color, odor, pattern of the stain (if present on any cloth) is noted down which is followed by the preliminary investigation of the evidence. Preliminary investigation includes

phenolphthaleintest, LMG test, Benzidine test and many other tests which confirms that the evidence sample is blood. After the confirmation that the suspected sample is blood the further investigation of that sample is done to identify that the blood sample is menstrual or normal. <sup>[3]</sup>

## DISTINGUISHING THE BLOOD SAMPLE AS MENSTUAL OR NORMAL

To distinguish whether the confirmed blood evidence is normal blood or the menstrual blood. Various tests are performed to analyze the provided sample. The tests for identification are Hb estimation, number of blood cell estimation and crystal test. <sup>[4]</sup>

### Hemoglobin estimation

Hemoglobin is the protein which is present in the blood that carries the oxygen molecules in the blood. The Hb estimation is done by the hemoglobinometer or by using Sahli's method. This method estimates the amount of hemoglobin in the provided sample. Sahli's hemoglobinometer is used by mixing the blood sample with 1N HCl to form acid hematin followed by adding distilled water into it until the color of the calibration tube matches with the testing tube. The value observed after matching the color of calibration tube and testing tube is the Hb value. <sup>[4]</sup>

The other methods which can be used for hemoglobin estimation are direct cyanmethemoglobin, hemoglobin color scale, Lovibond-Drabkin technique, Tallqvist technique, copper-sulfate technique, HemoCue technique, automated hematology analyzer. All the priorly mentioned methods have their own advantages and disadvantages and are used accordingly. Out of all these technique direct cyanmethemoglobin is widely used because it is simple and less costly. <sup>[14]</sup>

### Blood Cell Count

RBCs and WBCs are expanded as Red Blood Cells and White Blood Cells, respectively. They are the blood cells which have function. Estimating number of blood cells i.e., RBCS and WBCs is done by using the microscope and a slide known as Neubauer's hematocytometer slide. Neubauer's slide has chambers known as Neubauer's chamber which are observed under the microscope and the cells in the chambers are counted and after applying it into the formula, the cell count is estimated. The RBCs and WBCs chambers are different for both the cells. <sup>[4]</sup>

The step of counting the number of cells for differentiating the blood as normal or menstrual is a crucial step but counting the cells manually under the microscope id quite time consuming and chances of occurrence manual error is high so apart from the hemocytometer the automated cell analyzer can be used such as iterative structured circle detection algorithm <sup>[15]</sup>, impedance counters <sup>[16]</sup>, flow cytometry counters. These methods can be used but are not generally used in the forensic laboratory.

The parameters used to distinguish between menstrual and normal blood are as under:-

PARAMETERS	MENSTRUAL BLOOD	NORMAL BLOOD
Hemoglobin (gm/ml)	2-5	10-13
RBCs Count (million/cm)	2-3	4-5
WBCs Count (million/cm)	4-6	5-8

### Crystal test

Crystal test is considered as the confirmatory test for the blood. The two crystal which are mainly for Takayama and Teichmann test. The composition of both the reagents are different. The crystal test is performed by treating sample with the reagent placing it on a slide and giving it the heat shock and observing it under the microscope for crystals. The crystal in case of Takayama test are pink in color and are needle shaped (pyridine ferroprotoporphyrin/hemochromogen crystals) whereas in the case of Teichmann test, the crystals are brown in color and are rhomboid shaped (ferriprotoporphyrin chloride).<sup>[4]</sup>

The crystal test is positive for the normal blood, but it comes out to be negative for the menstrual blood means the crystals are not observed in the cases of menstrual but are formed in the case of normal blood.

### SERATEC PMB Test

SERATEC PMB Test is an immunochromatographic test is specifically for identifying the detection of D dimer that is the product of fibrin degradation which is present in the blood clots. This test identifies the D dimer/ hemoglobin which gives the positive result for menstrual blood. SERATEC test is performed with the help of immunochromatographic test kit, onto which the blood sample is poured/placed on the sample well and the colored lines are observed, it gives positive response for menstrual blood as it contains the blood clots.<sup>[5],[6],[7]</sup>

### mRNA BIOMARKERS

Gene expressing biomarkers are used by the scientist for the identification of menstrual blood. The biomarkers to be considered are HBA (Hemoglobin alpha), MMP7 and MMP 11 (Matrix Metalloproteinases). HBA gene expressing biomarker is present in both peripheral and menstrual blood. MMP7 and MMP11 gene expressing biomarkers are only present in the menstrual blood. the quantity of MMP7 is comparatively higher than that of MMP11. MMP7 and MMP 11 gene expressing biomarkers are only found in menstrual blood because they are present in the endometrial layer of uterus and responsible for its shedding.<sup>[8],[9],[10]</sup>

### SPECTROSCOPY ANALYSIS

#### ATR-FTIR Spectroscopy

Menstrual blood and normal blood can be distinguished by ATR-FTIR (Attenuated Total Reflectance- Fourier transform Infrared) spectroscopy combined with the chemometric tools such as LDA, PLSR, PCA, and PLSDA. The results of ATR-FTIR combined with several chemometric tools gives different percent of accuracy. The results of the ATR-FTIR combined with PCA is 93.3% accurate and the result of ATR-FTIR combined with LDA, PLSDA and PLSR are 100% accurate. ATR- FTIR spectroscopy is one of the rapid, reliable and the non- destructive technique to differentiate between the menstrual blood and peripheral blood.<sup>[11],[12]</sup>

### RAMAN SPECTROSCOPY

Blood and other body fluids can be differentiated based on the Raman Infrared Spectroscopy. Similarly, menstrual blood and peripheral blood can also be distinguished based on the Raman spectra. The spectra for both menstrual and peripheral blood is different and is 100%

sensitive and specific. The technique is better than other chemical and destructive technique.<sup>[13]</sup>

## DISCUSSION

The menstrual blood differs a lot from the normal blood. The hemoglobin level of menstrual blood is comparatively very low from the normal blood. The blood content of menstrual blood and normal blood is different because menstrual blood contains reproductive hormones, vaginal secretions, mucus which makes it different from the normal blood. The cell count of menstrual blood is comparatively low as compared to normal blood. The crystal test which comes out to be positive for normal blood is negative for menstrual blood. The above parameters are used to distinguish the menstrual blood from the normal blood. The immediate analysis of menstrual or normal blood could be done by SERATEC PMB test that gives the immediate result and the suspected blood sample can be distinguished as menstrual or normal blood. The gene expressing biomarkers MMP7 and MMP11 are only present in menstrual blood as they are present in the human endometrium and is specifically responsible for the extracellular matrix breakdown. Spectroscopy techniques are the non-destructive techniques with almost 100% accurate result.

## CONCLUSION

Based on the amount of hemoglobin, number of RBCs, WBCs and crystal test are used to distinguish between the menstrual blood or normal blood. Other than hematology study, blood can be distinguished as normal or menstrual based on the chromatographic assay. The menstrual blood and the normal blood also vary in the protein content and other secretions. The spectrometers such as ATR-FTIR and Raman Spectroscopy are the non-destructive techniques which can differentiate the blood as normal or menstrual. These techniques are almost 100% accurate and specific.

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