

## REVIEW ARTICLE

# Examination of Blood with the help of Chemicals and Scientific Instruments

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**ABSTRACT**

Blood, a vital biological fluid, plays an essential role in sustaining life by transporting oxygen and nutrients, eliminating waste, regulating body temperature, and defending against infections. Blood, which is made up of red blood cells, white blood cells, platelets, and plasma, is extremely important for forensic purposes because of its DNA content and capacity to provide information about crimes. Blood at a crime scene can disclose information about the nature of the crime, identity of individuals involved, weapon used, and the sequence of events. Bloodstain patterns, trails, and smears assist in reconstructing the crime scene and determining positions and movements of both victim and perpetrator. In forensic science, blood detection begins with presumptive tests such as the Benzidine, Phenolphthalein (Kastle-Meyer), Leucomalachite Green, and Fluorescence tests. These rapid and sensitive methods rely on the peroxidase activity of hemoglobin to indicate possible blood presence, although they may yield false positives. The Teichmann and Takayama tests, which react with the heme components in blood to generate characteristic crystals, are used as confirmatory tests to verify the existence of blood. Advanced identification methods include Raman spectroscopy, which offers molecular-level insights due to its non-destructive and highly specific nature. Immunoassays, including ELISA and RSID kits, detect blood through antibody-antigen interactions and are increasingly being adapted for field use. Additionally, spectrographic techniques like FTIR and NIR hyperspectral imaging

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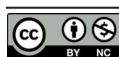
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aid in blood stain detection and differentiation from other substances. While blood evidence may not always hold direct legal weight in court, it plays a crucial role in corroborating or challenging testimonies, establishing crime timelines, and linking suspects to crime scenes. Its scientific reliability and interpretive power make it a “silent witness” that significantly contributes to crime investigation and justice delivery.

## KEYWORD

• Blood • Crime Scene • RBC • WBC • Platelet • Benzidine • Phenolphthalein • Raman Spectroscopy • Spectrographic

## INTRODUCTION

Blood is a vital liquid in the human body, essential for existence and significant for various biological functions. It circulates through the cardiovascular system, delivering oxygen and nutrients to tissues, and removing waste materials, enhancing immune system and protecting against all kinds of infections. Other than this, it also regulates the temperature of the body. Its pH of 7.4 indicates that it is alkaline in nature. Blood appears red because of a complex protein called haemoglobin that contains iron. The globular protein known as hemoglobin is composed of a tetramer, which consists of two alpha and two beta chains. Blood consists of four main components namely, Red blood cells, White blood cells, Platelets, and Plasma.

- **Red blood cells (RBCs):** They are also known as erythrocytes and are responsible for transportation of oxygen throughout the body cells. They make up to 45% of blood. They are biconcave in shape. The lifespan of red blood cell is of 120 days and then they are destroyed and removed while new red blood cells are regenerated simultaneously in the bone marrow.
- **White blood cells (WBCs):** They are also known as leukocytes and are the part of immune system defending the body against foreign substances, infections, and diseases. WBCs are divided into categories including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, with each having particular functions in immune response.

**1. Neutrophils:** Neutrophils are the most common type of white blood cell and make up anywhere from 50% to 80% of all white blood cells. They

act as the first line of defence of our immune system. They typically live up to 6 to 12 hours in bloodstream and once migrated to tissue for defence they can survive for 1 or 2 days.

- 2. Lymphocytes:** T cells, B cells, and natural killer cells are examples of lymphocytes. Their lifespan varies from several days to multiple years, depending on whether they are actively combating infections or contributing to long-term immune memory.
  - 3. Monocytes:** These stay in the bloodstream for about a day or three days before entering tissues and becoming dendritic or macrophage cells, which can survive for a few weeks to months.
  - 4. Eosinophils:** These are the white blood cells, which play a role in fighting off parasitic infections and allergic responses and typically survive for 8–12 hours in the blood and approximately 8–12 days in bodily tissues.
  - 5. Basophils:** These are the least common kind of WBCs, and they typically survive in the bloodstream for around 1–2 days, although they might last longer in tissues.
- **Platelets:** These are also known as Thrombocytes and are small, disc shaped cell fragments, playing vital role in blood clotting by forming clots at the site of injury.
  - **Plasma:** Plasma, the liquid component of blood, makes up around 55% of its volume. About 90% of it is made up of water, along with proteins (such as

albumin, fibrinogen, and globulins), waste products, hormones, electrolytes, and nutrients including glucose and amino acids.

Blood found at the crime scene reveals a lot of viable information about the crime because of its ability to unmask the critical inputs about the perpetrator and simultaneously providing inputs on the victim. It also has the property to retain DNA which can help to identify the people involved in the scenario. In addition to this, other useful information such as nature of the crime, cause of injury or death, type of weapon used and whether struggle is involved or not. These things make blood as the most significant evidence found at the scene. The pattern which blood spatter makes which is found at the crime scene provides a lot of information like the positions of victim and the perpetrator at the time of crime, the direction and force of attack, the motion that has taken place. The presence of such things can to recreate the scenario for introspection. The other evidences such blood trails, smears or drag marks puts up more information about movements happened on the crime scene. Other than this the drying time of blood can help to identify the timeline for the crime. Blood as evidence facilitates the connection between the incident and the culprit. For instance, the presence of blood on a suspect's clothing, shoes, or body can be verified, and the combination of DNA profiles discovered in the blood stain can demonstrate the victim's and the offender's physical resistance. Even after the cleaning attempts like trying to wash or wipe the blood will leave behind traces which can be detected through luminol testing. It will also help to reveal the hidden information which wasn't visible to bare eyes. The patterns left by cleaned or smeared blood can also help to identify where the crime occurred or what was moved after the ordeal. Although blood evidence holds no importance in front of court, still it can be used to confirm or reject testimonies; it can also support as well as challenge both the claims of self-defence or accidental harm. There are several examples which prove this, like the location and distribution of blood can help differentiate between a deliberate attack or self defence. Furthermore, the amount of blood spilled can reveal the intensity of the violence, highlighting once more the difference between a crime of passion and self-defense. Blood trails also help

to provide secondary places which shed even more light on the crime. As blood evidence has a scientific credibility as well as psychological stand, it has a role of silent witness which offers critical clues to recreate the crime while linking individuals to it, ultimately helping in the delivery of justice. Blood on any crime scene can be identified via running different chemical tests such as colour changing test luminal test, fluorescence test, when they come in contact with blood.<sup>1-3</sup>

## METHOD AND METHODOLOGY

The procedure begins with presumptive tests, which are swift and sensitive methods to evaluate if a substance might be blood. Luminol, which combines with hemoglobin to generate a visible glow, and the Kastle-Meyer test, which uses phenolphthalein, are examples of common assays. While presumptive tests are beneficial for preliminary identification, they are not definitive, as they may respond to substances like rust or specific botanical materials. For this we need a more specific kind of tests that would confirm that there is presence of blood. It also is crucial as it determines the origin of blood, whether it is of animal or human. There are two commonly used confirmatory tests: the Teichmann test and the Takayama test. Let us now look at the test in more detail.

### Presumptive Tests for blood:

The presumptive tests are based on oxidation reduction reaction principle and it can detect very minute amount of blood if present. The following are a few tests that are performed to ascertain the same:

**Benzidine test:** It is a presumptive test that indicates the presence of blood but cannot offer definitive confirmation; it is also referred to as the Adler test. This test is based on hemoglobin's peroxidase-like activity in red blood cells, which catalyzes the reaction of hydrogen peroxide with benzidine and results in a color shift.

**Procedure:** The method for the benzidine test entails creating a solution of benzidine, usually dissolved in water or alcohol, to which hydrogen peroxide is introduced as an oxidizing agent. The blood sample that is suspected is obtained using a swab or tested directly on the stained area. A small quantity of the benzidine solution is then added to the

sample, followed by two to three drops of hydrogen peroxide. If hemoglobin is present in the sample, it speeds up the reaction and causes benzidine to oxidize. This chemical reaction results in a distinguishable blue-green colour, suggesting a potential presence of

blood. The size of the color change indicates the amount of blood present in the sample. The test is rapid and sensitive, but it is crucial to remember that the procedure must be conducted with care due to the cancer-causing properties of benzidine.

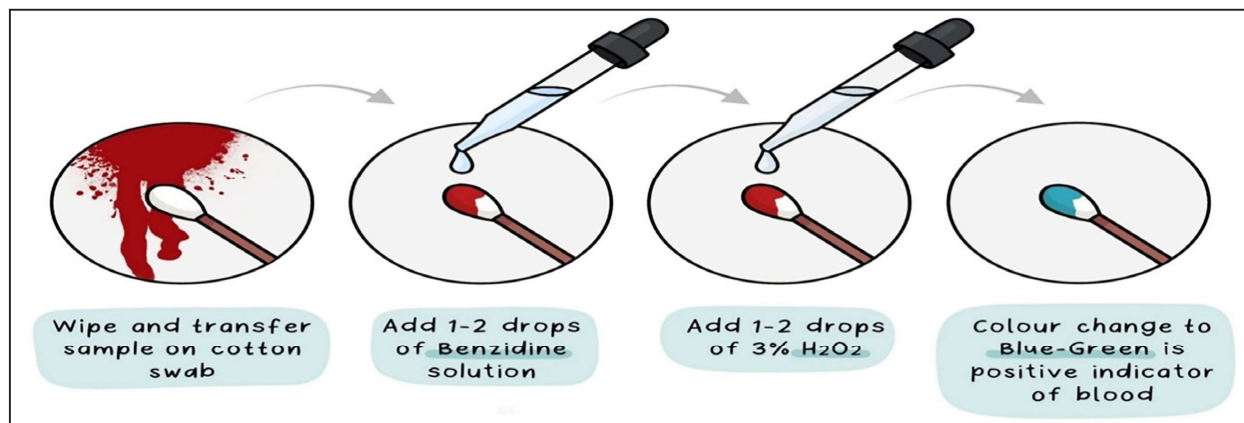


Figure 1: Showing Examination of Blood with the help of Benzidine Regent

**Phenolphthalein test:** It is commonly known by the name Kastle-Meyer test, is again a presumptive test that works on the principle that haemoglobin in blood has peroxidase-like activity, which catalyses the reaction between hydrogen peroxide and phenolphthalein.

#### Procedure:

To carry out the test, a small sample of the suspected stain is gathered with a cotton swab or is tested directly on the surface. Ethanol is initially applied to the sample to reveal haemoglobin and improve the reaction. A few drops of the reduced, colorless phenolphthalein reagent are then added, followed by hydrogen peroxide. When haemoglobin is present, it

decomposes the hydrogen peroxide into water and oxygen. The oxygen then oxidizes the phenolphthalein, resulting in a bright pink coloration, which indicates a positive test for blood. This test is extremely sensitive, rapid, and safe to use, making it a vital tool in forensic examinations. However, it is not exclusive to human blood and may yield false positives with substances such as vegetable peroxidases or chemical oxidizing agents. Confirmatory testing is therefore required to verify the findings. The phenolphthalein test has predominantly superseded older techniques like the benzidine test because of its non-carcinogenic properties and dependability.

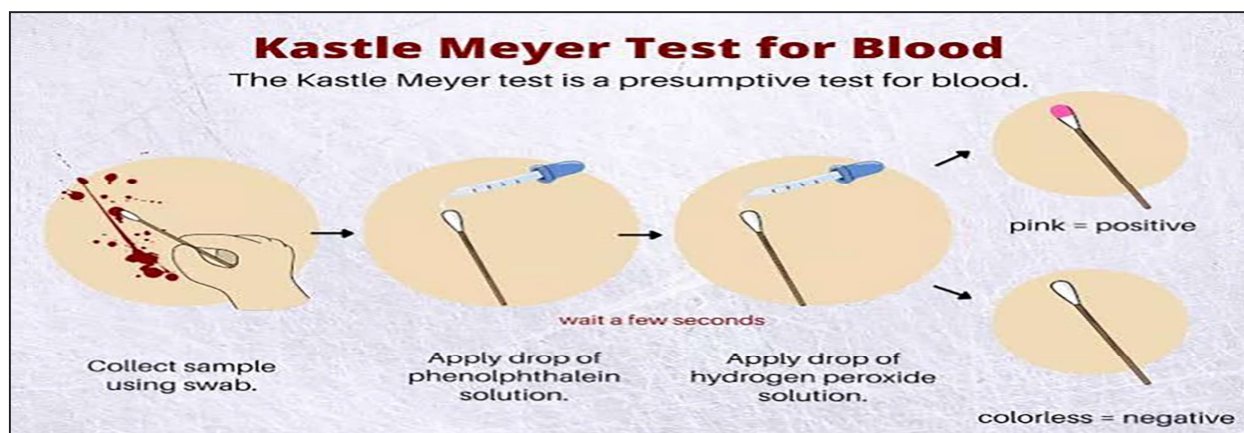


Figure 2: Showing Examination of Blood with the help of Phenolphthalein Regent

**Leucomalachite Green Test:** The dye known as malachite green is a triphenylmethane compound. Leuco base in malachite green renders it a colourless substance. Place one to two drops of leucomalachite green on the suspected blood stain and then add one to two drops of hydrogen peroxide. Observe any prompt shift to a blue-green. A negative outcome is indicated if there is no change in color.<sup>4</sup>



**Figure 3:** Showing Examination of Blood with the help of Leucomalachite Green Reagent

**Fluorescence Test:** In fluorescence testing, fluorescein serves as a reagent to detect the presence of blood and its stain patterns at the crime scene. When fluorescein is applied to the suspected area, it gets catalyzed and oxidized by heme, resulting in fluorescence. Using an alternate light source, a fluorescent area can be detected when illuminated with light between 425-485 nm. If blood is present, fluorescein emits a bright yellow-green fluorescence light.<sup>5</sup>

#### Advantages of Presumptive tests

These tests are highly sensitive making them detect even little amount of blood and also give results within seconds which helps the investigator to make quick decisions at the crime scene. Apart from this, these tests are very simple to perform making them practical to perform on site of crime. These tests are not only cost effective but also are easily available.

#### Disadvantages of Presumptive tests

These tests sometimes also show false positive which makes them non reliable. Further these tests also are inconclusive, that means it can detect blood but cannot confirm whether it is human blood or animal blood. This can mislead the process of investigation at times.

#### Confirmatory test for blood

These tests are crucial for determining whether blood comes from humans or animals. These

form crystals when come in contact with blood thereby eliminating the chances of error or confusion and give accurate results.

#### Teichmann test:

**Reagent Preparation:** Potassium Chloride - 0.1 g, Potassium Bromide - 0.1 g, Potassium Iodine - 0.1 g, Glacial Acetic Acid - 100 ml. Combine and keep in a bottle with a stopper.

#### Procedure:

1. Place the sample to be examined on a small slide and cover it with a cover slip.
2. Let the Reagent run beneath the cover slip.
3. Slide should be gently warmed for 10-20 seconds at 65°C on a hot plate.
4. Let cool and examine at 100X magnification.
5. It is a favorable reaction for heme if brown rhombohedron shaped ferroprotoporphyrin chloride crystals occur.

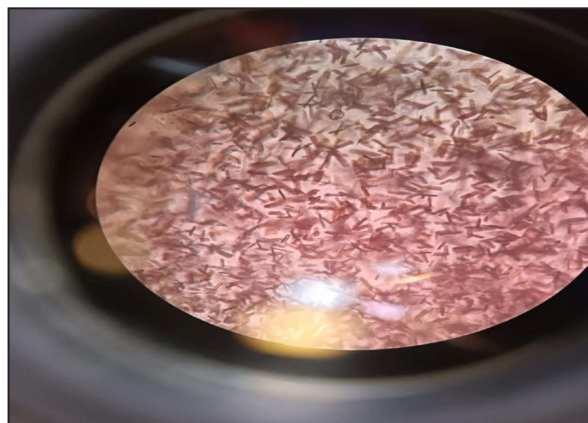


**Figure 4:** Showing confirmatory test (Brown Rhombohedron shaped crystals) of Blood with the help of Teichmann Reagent

#### Takayama test:

**Reagent preparation:** Standard Glucose Solution (100g/100ml) - 3 ml, 10% Sodium Hydroxide - 3 ml, Pyridine - 3 ml, Distilled Water - 7 ml. Reagents should be made fresh daily.

#### Procedure:

1. Place the sample to be examined on a small slide and cover it with a cover slip
2. Put a drop of Takayama reagent behind the cover slip and let it to run.
3. Slide should be gently warmed for 10-20 seconds at 65°C on a hot plate.

4. Let cool and examine at 100X magnification.
5. Pyridine Hemochromogen (Pyridine ferroprotoporphyrin) forms pink needle-shaped crystals, indicating a favorable reaction for heme.

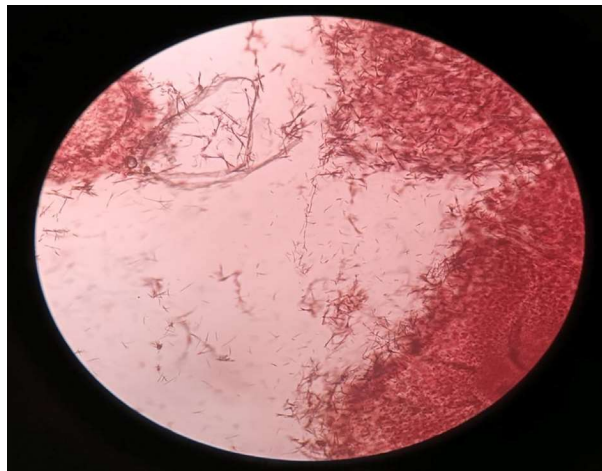


**Figure 5:** Showing confirmatory test (Pink Needle shaped crystals) of Blood with the help of Takayama Reagent

#### Identification of blood using scientific instruments:

**Raman spectroscopy:** It is an effective analytical tool to identify and characterize blood because of its capability to deliver molecular level insights regarding its composition. It works on the principle that monochromatic light can be scattered with the help of molecules. It is very helpful in blood detection because to its non-destructive nature, high specificity, speedy detection, and low sample usage. Blood from paint and stains, human blood from feline blood, and other bodily fluids can all be distinguished using Raman spectroscopy.<sup>6-9</sup>

**Immunoassays:** Immunoassays are bio molecular instruments for identifying or quantitatively assessing the concentration of biomarkers in a fluid specimen through an antibody antigen interaction. Wet-lab immunoassays, including enzyme-linked immune sorbent assay (ELISA), are well recognised and have extremely high sensitivity, and in recent years, there has been an initiative to use this technology beyond the laboratory.<sup>10</sup> There are several immunoassays available for forensic purpose such as rapid stain identification (RSID™) kits. RSID kits are designed for quick on-site blood detection.<sup>11-13</sup>

**RSID-Blood:** Numerous presumptive tests exist for the purpose of identification of blood,

yet these are unable to offer any insights regarding humans' source of stain identified because of which numerous verification tests are to be conducted. There has been a suggestion made lately for the same purpose known as Rapid stain detection blood, also known as RSID blood. Since RSID blood test is less sensitive than any other immuno chromatographic confirmatory tests therefore sufficient biological material needs to be present in order to provide an STR profile.<sup>14</sup>

**Spectrographic techniques:** Spectroscopy is currently being utilized for the preliminary identification and positioning of bodily fluids using forensic light sources that operate within the visible and ultraviolet (UV-Vis) spectrum. Furthermore, the utilization of more specific methods, such as near infrared NIR hyperspectral imaging (HSI), infrared Fourier transform (FTIR) and Raman spectroscopy is under exploration. Portable hyperspectral cameras working along NIR range have been successfully tested for the detection of blood stains.<sup>15-18</sup>

#### Result

Blood is a vital fluid in the human body, performing key functions such as transporting oxygen and nutrients, removing waste, regulating temperature, and supporting immunity. Its slightly alkaline nature (pH 7.4) and red color are due to haemoglobin a globular protein with iron-containing alpha and beta chains. Blood comprises four main components red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma. RBCs transport oxygen, have a lifespan of 120 days, and are produced in the bone marrow. WBCs, part of the immune system, include neutrophils, lymphocytes, monocytes, eosinophils, and basophils each with specific defensive roles. Platelets aid in clotting, and plasma, which is mostly water, carries proteins, hormones, nutrients, and waste. At crime scenes, blood provides critical forensic evidence. It retains DNA, indicating identity, and offers clues about the nature of the crime, such as weapon type, force used, and struggle. Patterns of blood spatter indicate the locations and motions of those involved. Blood trails and drying times assist in reconstructing timelines and locating secondary crime scenes. Even cleaned or hidden blood can be detected using chemical tests like luminol, which reacts with haemoglobin to produce luminescence. While

not direct legal proof, blood evidence can validate or challenge testimonies and support conclusions about self-defence or intentional harm. Presumptive Blood tests, these tests quickly indicate potential presence of blood using oxidation-reduction reactions, although they may yield false positives. Benzidine Test: Produces a blue-green color if haemoglobin is present, but benzidine is carcinogenic, Phenolphthalein (Kastle-Meyer) test produces a pink color and is safer and more commonly used today, Leucomalachite Green test, a colorless dye that turns blue-green in presence of blood, fluorescence test uses fluoresce in which emits bright yellow-green under specific light if blood is present. Confirmatory blood test, these tests form characteristic crystals to definitively confirm blood, Teichmann test Forms brown rhombohedral crystals of haematin chloride, Takayama test Forms pink needle-shaped crystals of pyridine ferroprotoporphyrin. Instrumental Techniques like Raman Spectroscopy A non-destructive method offering molecular-level identification of blood, even distinguishing it from similar substances. Immunoassays (e.g., ELISA, RSID kits) Detect specific blood proteins and can differentiate human blood. Although it takes a large sample for DNA profiling, the RSID-Blood test is a more recent confirming technique for identifying human blood. Spectroscopic Techniques (FTIR, NIR-HSI): Portable systems help in on-site detection and mapping of blood stains using various light spectrums.

## DISCUSSION AND CONCLUSION

Blood serves as a crucial biological fluid that plays a vital role not only in sustaining life but also in forensic investigations. Its unique properties such as the presence of hemoglobin, ability to retain DNA, and characteristic patterns upon impact render it an essential form of evidence at crime scenes. Because blood is made up of red and white blood cells, plasma, and platelets, forensic specialists can obtain a variety of data, such as biological origin, immunological response, and trace DNA for identification. White blood cells, in particular, contribute to DNA analysis, aiding in pinpointing individuals involved in a crime. Presumptive tests such as Benzidine, Kastle-Meyer (phenolphthalein), Leucomalachite Green, and fluorescence tests provide a preliminary indication of the presence

of blood. These tests are valued for their simplicity, cost-effectiveness, and sensitivity, allowing investigators to make quick decisions during initial crime scene analysis. However, their limitations such as false positives and inability to distinguish human blood from that of animals necessitate the use of confirmatory tests for reliable outcomes. Confirmatory tests like the Teichmann and Takayama tests provide a higher level of specificity by identifying crystal formations characteristic of hemoglobin derivatives. These tests are definitive, ruling out false positives and affirming the presence of blood through microscopic examination. Although they require controlled lab conditions and technical precision, their reliability makes them indispensable in forensic science. In recent years, the integration of advanced scientific instruments has significantly enhanced the accuracy and sensitivity of blood detection and analysis. Techniques such as Raman spectroscopy offer non-destructive, high-specificity detection, enabling the differentiation of human blood from other substances. Immunoassays, particularly ELISA and RSID kits, have revolutionized blood testing by enabling rapid, on-site detection with high specificity toward human blood. These techniques are especially valuable in cases where immediate and reliable differentiation is necessary. Moreover, modern spectrographic techniques, including FTIR and hyperspectral imaging, allow for the identification and mapping of bloodstains even on complex surfaces or after cleaning attempts. These technologies are crucial in reconstructing crime scenes, identifying points of contact, and verifying suspect involvement. Even when blood is cleaned or concealed, trace residues can be visualized using luminol and other reagents, further reinforcing the concept of blood as a silent witness.

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## REFERENCES

1. S.H. James, J.J. Nordby, Forensic Science: An Introduction to Scientific and Investigative Techniques, CRC Press, 2002 exhibit.
2. B. Budowle, J.L. Leggitt, D.A. Defenbaugh, K.M. Keys, S.F. Malkiewicz, The presumptive reagent fluorescein for detection of dilute bloodstains

- and subsequent STR typing of recovered DNA, *J. Forens. Sci.* 45 (5) (2000) 1090–1092.
3. F. Barni, S.W. Lewis, A. Berti, G.M. Miskelly, G. Lago, Forensic application of the luminol reaction as a presumptive test for latent blood detection, *Talanta* 72 (2007) 896–913.
4. Li, R., 2015. *Forensic Biology*. Edn 2, CRC Press Taylor & Francis Group, Boca Raton, pp: 6, 232-240.
5. Gaensslen, R.E., Camp, F.R., 1983. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, National Institute of Justice, Washington, DC, pp:45- 53.
6. K. Virkler, I.K. Lednev, Raman spectroscopy offers great potential for the non-destructive confirmatory identification of body fluids, *Forensic Sci. Int.* (2008).
7. K. Virkler, I.K. Lednev, Raman spectroscopic signature of semen and its potential application to forensic body fluid identification, *Forensic Sci. Int.* 193 (2009) 56–62.
8. K. Virkler, I.K. Lednev, Blood species identification for forensic purposes using Raman spectroscopy combined with advanced statistical analysis, *Anal. Chem.* 81(2009) 7773–7777.
9. K. De Wael, L. Lepot, F. Gason, B. Gilbert, In search of blood – detection of minute particles using spectroscopic methods, *Forensic Sci. Int.* 180 (2008) 37–42.
10. Wu G., Zaman M.H. (2012). Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. *Bulletion of the World Health Organisation*. Vol. 90, pp. 914-920.
11. Turrina S., Filippini G., Atzei R., Zaglia E., De Leo D. (2008). Validation studies of rapid stain identification blood (RSID-blood) kit in forensic caseworks. *Forensic Science International: Genetics Supplement Series*. Vol. 1, pp. 74-75
12. Casey D.G., Price J. (2010). The sensitivity and specificity of the RSID™-saliva kit for the detection of human salivary amylase in the Forensic Science Laboratory, Dublin, Ireland. *Forensic Science International*. Vol. 194, pp. 67-71
13. Old J., Schweers B.A., Boonlayangoor P.W., Fischer B., Miller K.W.P., Reich K. (2011). Development validation of RSID™-Semen: A Lateral Flow Immunochromatographic Strip test for the Forensic Detection of Semen. *Journal of Forensic Sciences*. Vol. 57, pp. 489-499.
14. *Rapid Stain Identification of Human Blood*, Technical information sheet, Independent Forensics, Hillside, IL.
15. Lin A.C., Hsieh H.M., Tsai L.C., Linacre A, Lee J.C. (2007) Forensic applications of infrared imaging for the detection and recording of latent evidence. *J Forensic Sci* 52: 1148-1150.
16. De Forest P.R., Bucht R., Kammerman F., Weinger B., Gunderson L. (2009) Blood on Black-Enhanced Visualization of Bloodstains on Dark Surfaces. Report to the U.S. Department of Justice.
17. Schuler R.L., Kish P.E., Plese C.A. (2012) Preliminary observations on the ability of hyperspectral imaging to provide detection and visualization of bloodstain patterns on black fabrics. *J Forensic Sci* 57: 1562-1569.
18. Finnis J., Lewis J., Davidson A (2013) Comparison of methods for visualizing blood on dark surfaces. *Sci Justice* 53: 178-186.