Forensic Serology

18

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Abstract

Biological evidences, e.g. blood, semen, vaginal secretion, saliva, urine, and sweat encountered at the crime scene, are the most pervasive in surroundings. Their existence helps in associating the assassin with the victim as well as with the crime scene. Moreover, a lot depends on their collection methods and preservation conditions in identifying the source of such materials. Forensic serology is a sub-discipline of forensic biology concerned with the analytical knowledge of bodily fluid evidences encountered at the crime scene. Identification of such body fluids and their segregation from physically and chemically similar substances is essential for linking the evidences to the crime. Sets of physical observation, preliminary examination and confirmatory examinations have been described for the identification of various bodily fluids. Physical and preliminary tests are essential for excluding or eliminating a stain as a bodily fluid, while the confirmatory examination is the conclusive method of identifying a biological fluid. The chapter focuses on various types of bodily fluids encountered in relation to the crime, physical properties specific to each bodily fluid as well as preliminary and confirmatory tests for their identification. The chapter also provides an insight into the method of crime scene reconstruction methods by means of bloodstain patterns. Different types of blood grouping methods as

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well as techniques of species identification have also been described in the chapter.

Keywords

ELISA · Immunochromatographic assays · Bloodstain pattern analysis (BPA) · Blood grouping · Species identification

18.1 Introduction

Forensic serology is a branch of forensic biology that mainly deals with the examination, identification and classification of bodily fluids such as blood, semen, sweat, saliva, urine, fecal matter, vomit etc. that are majorly associated with violent criminal cases. For example, the identification of blood is crucial in the investigation of the cases involving homicide, sexual assault, burglary etc. Confirming the existence of blood has an empirical value or can support allegations of violent acts and then this information can be used in further investigation. For instance, in crimes related to murder, the presence of the victim's blood on a suspect's weapon can link the victim and the suspect. Same way, the identification of semen, saliva, and vaginal stains becomes important in crimes related to sexual assaults. The stain from these fluids on the victim's and/or perpetrator's body parts, clothing could be helpful in linking the victim with the alleged suspect. This chapter deals with the examination and identification of various biological fluids commonly encountered at crime scene and their evidential value along with various factors and conditions affecting the forensic analysis of such samples (Fig. 18.1).

Analyses of forensic samples encountered at the crime scene are performed at several stages using different techniques. Classification of such examination includes physical examination, preliminary examinations, and confirmatory examinations. Physical examination defines in identifying physical characteristics such as color, density, odour, etc., that are specific to certain kind of bodily fluid. Preliminary examination or assays provides a direction towards establishing the chances of the presence of the expected bodily fluid. Chances of positive reaction are also possible with other substances that have a similar molecular structure. In simpler terms, many substances often give a false positive result with presumptive assays. Therefore, a positive result with preliminary examinations denotes a probability of the presence of expected bodily fluid. However, negative results of such examinations are confirmed indicative of the absence of the bodily fluid being tested. Confirmatory examinations are conclusive and substance-specific method of identifying the bodily fluids.

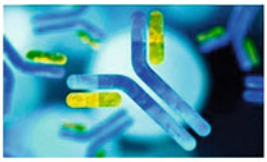
Advantages		Disadvantages
• Detection by emission or absorption of light • Observable Biological Evidences on any type of surfaces • Non destructive • Test Reproducibility	**Light Source**  Blood, Semen, Saliva, Urine (300-700 nm wavelength)	• Non specificity for Biological Evidences • Requires preciseness • Masked reaction in certain surfaces
• Rapid Testing • Ease of handling and cost effective • Can be utilized in crime scene • Identification of Semen in cases of azoospermia • Non destructive	**Chemical**  Blood, Semen (Acid phosphatase), Saliva, Vaginal fluid, Urine	• Non specificity to human • Analyte concentration is essential prerequisite • May infer false positive results with similar substances
• Direct identification of sperm cells • Low Cost • Use of different staining methods	**Microscopy**  Brightfield, Phase-contrast and Fluorescence microscopy	• Sample loss during slide preparation • Requires preciseness • Not suitable for cases of azoospermia or vasectomy • Observation of unstained cells requires contrasts
• Requires less sample • Low cost • Rapid testing • Specificity to Humans • High sensitivity	**Immunological**  Blood, Semen (Prostate Specific Antigen, Semenogelin), Saliva, Vaginal fluid, Urea	• Hook's effect • Destructive • External environment may affect the sensitivity • Chances of false positive results

Fig. 18.1 Advantages and disadvantages of presumptive and confirmatory examinations

18.2 Blood

Blood is one of the most prevalent evidences to be found at a crime scene in various cases like homicide, sexual assault, suicide, accidents etc. Blood is a complex viscous red fluid with a pH of about 7.4, mainly composed of cells and plasma and constitutes about 8% of the human body weight. The liquid part is called plasma and the solids are red cells (erythrocytes), white cells (leukocytes) and thrombocytes (platelets). The cells are also known as corpuscles. When the blood flows out of the body, a part separates out as blood clots consisting of blood cells, discs and fibrin. Fibrin comes out of plasma and is responsible for the clotting of blood. The liquid left is called serum. It is the plasma without fibrinogen which turns into fibrin. Plasma and serum are complex mixtures of proteins, minerals and organic compounds dissolved in water (Hardwick 2008; Basu and Kulkarni 2014).

The presence of blood on evidentiary items is very crucial in arbitrating innocence or guilt during legal proceedings. Interpretation of blood spatter can be relevant in adjudicating how blood was stashed at the crime scene or on an item, helping in the reconstruction of the crime scene. From the collection of samples to examination, every step is crucial in analysis. One of the most important considerations is the collection of the sample with zero or minimal contamination. One must take care of essential precautionary measures during the collection of blood samples such as wearing of surgical mask, latex gloves, and aprons during collection, label all the samples accurately, decontaminate all non-disposable items and dispose-off the disposable items, and clean up the hands with bleach. A sterilized syringe should be used for control sample collection. A note of precaution must be added if biohazards like AIDS, COVID, or hepatitis are suspected. Wet blood-stained objects need to be air-dried after collection. The stains can also be collected on cotton swabs and then air-dried or collected in containers with the help of a syringe. In case of dried blood stains, tape-lifting or scrapping is used (Lee and Ladd 2001; Castro and Coyle 2013). Preservation of blood samples is another important criterion that must be taken care of. The stability of blood is influenced by factors such as the use of stabilizing agents such as heparin or anticoagulant agents such as EDTA used for sample storage, Storage period, storage conditions such as temperature, exposure to ultraviolet radiations, heat, light, humidity and soil contaminations (McNally et al. 1989; Vaught 2006).

18.2.1 Physical Examination

Examination of exhibits in natural light for red, brown or reddish brown stains, crystals or fine powder of reddish brown color, particular areas should be separated for sampling. In cases of washed stains or when stains are not visible, UV light of 230–269 nm frequency should be used for examination. Hemoglobin on treatment with acids, alkalis, reducing agents or oxidizing agents gives a variety of products which have characteristics absorption spectra. They help to identify the blood. The absorption bands are seen in length which is characteristic of blood. Spectroscopic

examination of the blood is very useful as well as convenient. The test is usually carried out microscopically. The blood hemoglobin is changed in two or three forms on the slide itself and characteristic absorption is observed. Usually, alkali hematin and cyanhemochromogen are studied for their characteristic spectra. Ascending paper chromatography using acetic acid, methanol and water solvent system has been employed to study the Rf values. Electrophoresis is used for the separation of various enzyme systems. It is being adopted to study the body's proteins. This technique is becoming important to distinguish between blood samples.

18.2.2 Presumptive Examinations

These tests are the first series of tests that are qualitative in nature and are employed for the identification of a specific substance after a visual study of stains. If a stain gives positive color or luminescent reactions, the stain is possibly a blood stain. If it fails to give a positive reaction, in all probability it is not a bloodstain. However, certain substances similar to blood in molecular structure can give false positive results.

18.2.2.1 Colorimetric Tests

18.2.2.1.1 Guaiacum Test

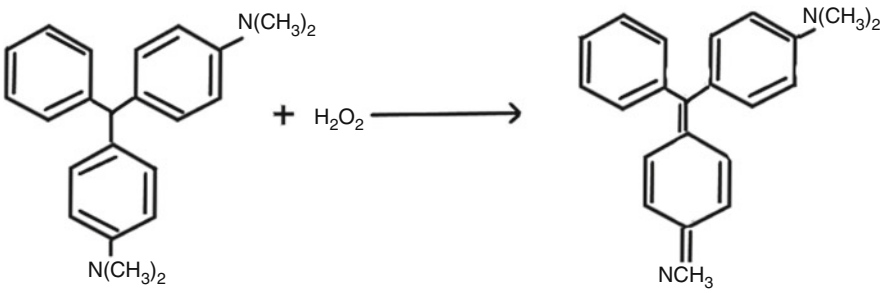
It is the oldest known forensic assay used for the preliminary examination of blood. Guaiacum is a resin isolated from Guaiacum officinale and Guaiacum sanctum and gives deep blue color.

18.2.2.1.2 Benzidine Reaction

Benzidine (0.1 g) and dry sodium perborate (0.1 g) are dissolved in glacial acetic acid (10 mL) and sprayed over the stain. A strong blue color indicates blood.

18.2.2.1.3 Leucomalachite Green Reaction

The reagent is prepared by dissolving leucomalachite green (0.1 g), sodium perborate (0.3 g) in 65% glacial acetic acid. The reagent is applied to the stain. Intense green color indicates blood.

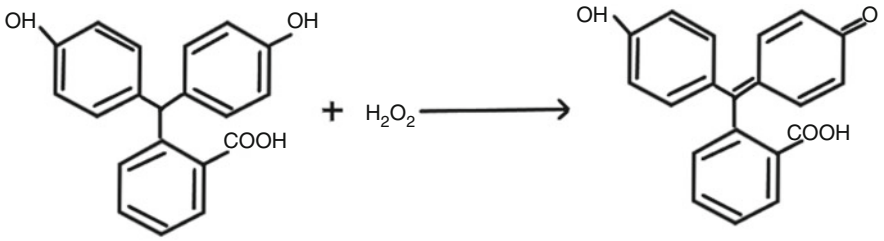


Leucomalachite green
Reduced (Colorless)

Malachite green
Oxidized (Blue-Green)

18.2.2.1.4 Phenolphthalein Reaction

Phenolphthalein (1 g) is reduced and dissolved in acetic acid (100 mL). Sodium perborate (1.4 g) is dissolved in the solution and applied to the blood stain. Pink coloration indicates blood.



Phenophthalin
(Colorless)

Phenophthalein
(Pink)

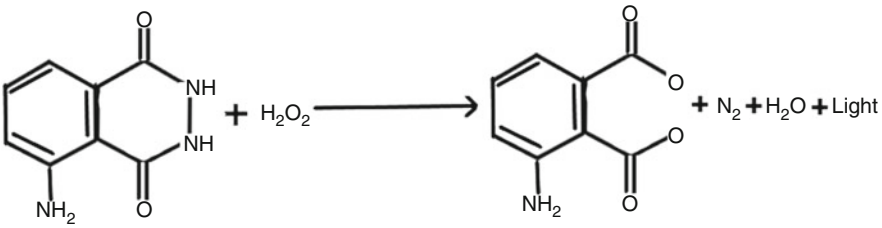
18.2.2.1.5 Aloin Test

Aloin is a mixture of pentosides of aloe plants that gives a bright red color on reacting with blood.

18.2.2.2 Chemiluminescence and Fluorescence Tests

18.2.2.2.1 Luminol Test

Luminol is a chemical which has been used to locate bloodstains. The articles suspected to bear stains are sprayed with luminol. It reacts with haeme of blood to give luminescence thereby visualizing the bloodstains are thus made visible. Even decomposed blood reacts with the reagent. The reagent does not interfere with subsequent blood tests. It is prepared by dissolving sodium perborate (0.7 g) in water (100 mL) and adding 3-arninophthalhydrazide (0.1 g) and sodium carbonate (5.0 g) to the solution. The solution is sprayed upon the article with glass sprayers in a dark room. Blood gives strong luminescence.



Luminol **3-Aminophthalate**

18.2.2.2.2 Fluorescin Reaction

Fluorescin is another reagent that is employed to test for the presence of bloodstains at a crime scene by means of fluorescence. Oxidation and catalysis by heme followed by exposure to light in the range of 425–485 nm using an alternate light source device results in an intense yellowish-green fluorescent light, which indicates the presence of a bloodstain. The light emitted from fluorescin-sprayed stains lasts longer than that of luminol.

18.2.3 Confirmatory Examinations

18.2.3.1 Micro-Crystal Test

Two crystal tests are commonly employed. They are specific for blood but they are not sensitive. They often fail if the conditions are not rigidly controlled or if the blood is disintegrated or contaminated.

18.2.3.1.1 Teichmann Test

The Teichmann test is much older, having been developed or invented in 1853 by Polish anatomist Ludwig Karl Teichmann. Take a dry crust or smear of blood on a slide. Put a drop of potassium iodide, bromide or chloride (0.1 g) solution in 100 mL glacial acetic acid over the blood and cover it with a cover slip. Warm the slide gently till it gives out bubbles. The reagent causes hemoglobin molecules to cleave, producing brownish crystals of pure hemin that have a violet, almost black, sheen (Hemin is the form of heme that contains the Fe^{3+} ion).

18.2.3.1.2 Takayama Test

One of the best-known crystal tests was developed in 1912 by Masaeo Takayama, a Japanese criminologist. A bloodstain is treated with pyridine and glucose (a reducing sugar that is capable of reducing ferric ions) under alkaline conditions to form pink needle-shaped crystals of pyridine ferroprotoporphyrin. Hemochromagens are heme derivatives in which the ferrous iron of the heme forms two bonds with nitrogenous bases (Fig. 18.2).

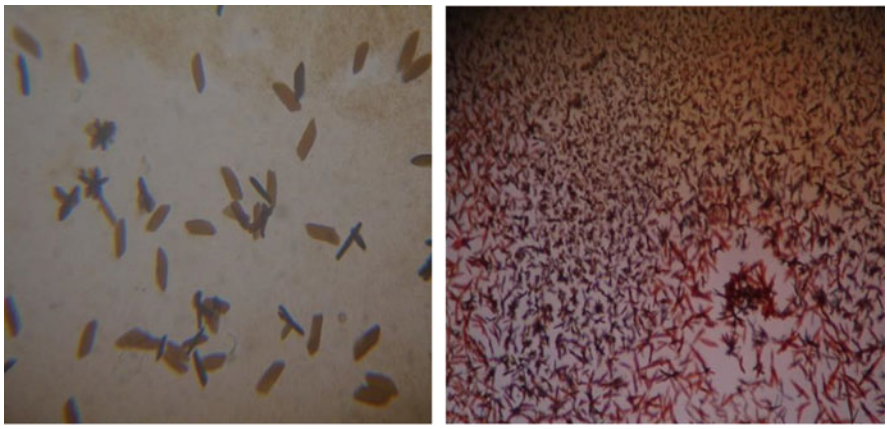


Fig. 18.2 Microscopic examination of blood demonstrating (**a**) hemin crystals in Teichmann test and (**b**) pyridine ferroprotoporphyrin crystals in Takayama test

18.2.3.2 Immunological Tests

Immunological methods utilize antihuman hemoglobin antibodies to detect human hemoglobin and therefore indicate the presence of human blood. These are rapid and sensitive as well as species-specific and can be used in both laboratory and field tests for species identification.

18.2.3.2.1 Tests Based on Haemoglobin Proteins

Bluestar® OBTI (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden) and the ABAcard HemaTraceR (Abacus Diagnostics, California) are commercially available kits that utilize the antibody–antigen–antibody sandwich method by using antibodies that recognize human hemoglobin. These are two-part test that includes a collection tube for the blood sample, and a test bar. The test bar contains a labeled monoclonal antihuman hemoglobin antibody contained in a sample well; a polyclonal antihuman hemoglobin antibody immobilized at a test zone of a nitrocellulose membrane as well as an antiglobulin that recognizes the antibody is immobilized onto a control zone. A sample of the presumed human blood trace is transferred into the tube with a transport medium. This mixture is added drop by drop to the sample well. A positive sample is typically detected within 2–5 min. A single red line at the control zone means the testing liquid is working fine but no human blood has been detected, two red/pink lines, one at the test zone and other at the control zone mean the test has detected human blood.

18.2.3.2.2 Tests Based on Glycophorin-A Protein

Commercial kits like RSID™-Blood (Independent Forensics, Hillside, IL) utilizes antibodies that recognize human Glycophorin-A proteins (GPA). The kit works on the same principle as other immunological assays, making use of monoclonal

antihuman GPA antibody contained in a sample well. A second monoclonal antihuman GPA antibody adhered to a different epitope of GPA, is immobilized onto a test zone of the membrane. An antiglobulin that recognizes the antibody is immobilized in a control zone.

18.3 Menstrual Blood

Menstruation is a periodic biological phenomenon that involves the shedding of the uterus lining of women along with the discharge of blood. Identification of the biological source of any stain can provide important hints in the investigation. Especially in cases of sexual assault, it may be relevant to distinguish the bloodstain of menstrual origin to that of the peripheral origin. The presence of peripheral blood (flowing through arteries and veins) stains indicates the possibility of a traumatic cause while the presence of menstrual blood points to a natural cause. The composition of menstrual blood can be an important criterion to distinguish it from peripheral blood. Menstrual blood comprises vaginal and cervical secretion or mucus, epithelial cells from endometrial linings and blood along with fibrinolytic components. These fibrinolytic components prevent the clotting of blood. On the contrary, the peripheral blood possesses the tendency of clotting due to the presence of thrombocytes and clotting factors or proteins such as fibrinogen, prothrombin, etc. The physical appearance of menstrual bloodstain is another factor useful for its identification. Menstrual blood does not splash instead it is found in the form of trickling descent.

18.3.1 Microscopic Examination

Along with endometrial cells, vaginal epithelium cells and residual cells are generally present in menstrual bloodstains and microscopical identification of these cells can confirm a bloodstain to be of menstrual origin. Staining tests such as Lugol's iodine test and Dane's staining test are some preliminary tests used for the identification of menstrual blood. Both Lugol's iodine test and Dane's staining test are based on the detection of vaginal epithelial cells in the menstrual blood. Lugol's test is based on iodine-staining and microscopical detection of the glycogens present in the nucleated squamous epithelial cells, which are found in considerably high concentrations in the vaginal cells as compared to buccal cells. However, the test is not specific for vaginal epithelial cells, as epithelial cells present in the buccal area and male urinary tract also consists of traces of glycogen and therefore can give a false positive test. Dane's staining method is considered to be more specific than Lugol's test or Schiff's test. The test is based on the difference in color and morphology of the stained epithelial cells (Bagwe 2018).

18.3.1.1 Lactate Dehydrogenase Immunoassay

Lactate dehydrogenase (LDH) is a tetrameric enzyme, beneficial in glycolysis that catalyzes the reduction reaction of pyruvate into lactate in scarcity of oxygen. LDH

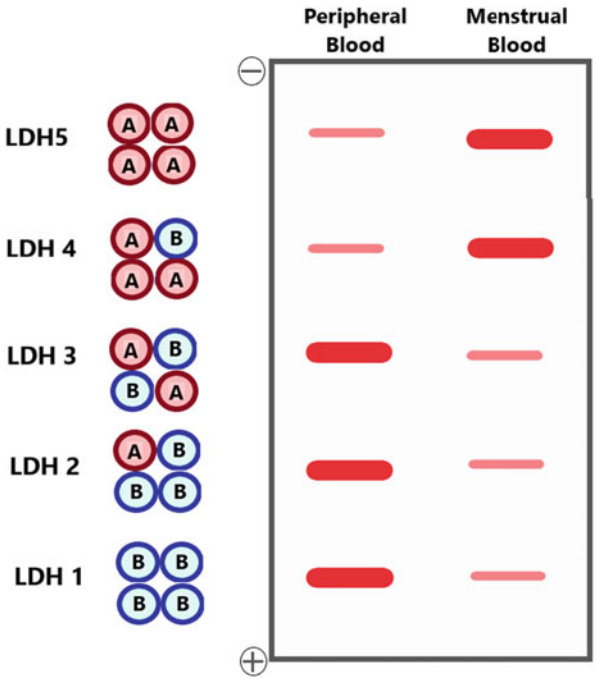


Fig. 18.3 Lactate Dehydrogenase Immunoassay

is present in human blood in the form of five isoenzymes that differ in the structural composition of their subunits. These isoenzymes function in the same catalytic reaction inspite of the difference in their molecular structure and can be separated by means of electrophoretic mobility and colorimetric assay (Fig. 18.3).

LDH1 has the highest electrophoretic mobility and LDH5 has the lowest electrophoretic mobility. Isoenzyme LDH4 and LDH5 are predominant in menstrual blood, while LDH1, LDH2 and LDH3 are predominant in peripheral blood (Li 2015; Holtkötter et al. 2018). This difference in concentration of isoenzymes can distinguish menstrual blood from peripheral blood.

18.3.1.2 D-dimer Identification

Detection of fibrinolysis degradation products (FDPs) that are specific to menstrual blood is another important aspect for distinguishing menstrual blood from peripheral blood. Cleaving of cross-linked fibrin by plasmin during the process of fibrinolysis, results in the formation of these FDPs. D-dimer, a subunit of FDPs is a fibrinolysis-specific fragment of the protein, therefore this assay is also known as D-dimer assay (Holtkötter et al. 2018; Kaur et al. 2018). The application of D-dimer assay for the identification of menstrual blood was suggested by Miyaishi et al. in 1996 (Miyaishi et al. 1996). They reported an extensively higher concentration of D-dimer approximately 200-fold in menstrual blood than that of peripheral blood. Baker et al. later

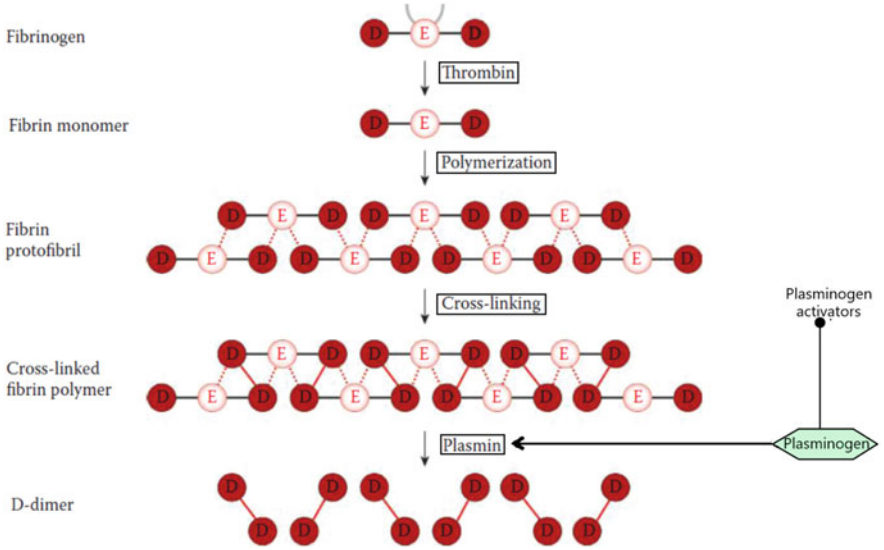


Fig. 18.4 Mechanism of d-dimer formation

identified four assays based on D-dimer detection for the identification of menstrual blood (Fig. 18.4) (Baker et al. 2011).

Immunological assays such as enzyme-linked immunosorbent assay (ELISA), agglutination assays, and immunochromatographic assays make use of D-dimer specific antibodies for the detection of D-dimer in the sample (Bagwe 2018). These techniques are highly sensitive in nature, thereby aid in distinguishing menstrual blood from peripheral blood. However, an adequate quantity of D-dimer subunit is also found in postmortem blood which may pose complications in the interpretation of the result. But at the same time, PM blood is not frequently encountered in cases of sexual assaults; therefore, the assay can be reliably applied in such cases.

18.4 Semen

Majority of cases received in the Forensic Laboratories are sexual assault cases, in which semen is one of the commonly encountered evidences. So, the analysis of semen plays an important role in solving sexual assault crimes. Semen is a viscous, slightly yellowish or grayish, and opalescent secretion having a characteristic odor known as seminal odor. It basically comprises seminal fluid and sperm cells. Seminal fluid is generally made up of water, sugar, protein, vitamins and minerals while the sperm cells comprises spermatozoa (Table 18.1) (Gill et al. 1985; Li 2015).

General exhibits collected for semen examination are swabs, vaginal slides, clothing, bedding items, condoms, etc. Stains from semen are readily visible on

Table 18.1 Percentage contribution of various gland secretions in seminal fluid

Gland	Percentage (approx.)	Description
Testes	2–5%	200–500 million spermatozoa released per ejaculation
Seminal vesicle	65–75%	Amino acids, citrate, enzymes, flavins, fructose, phosphorylcholine, prostaglandins, proteins, vitamin C
Prostate	25–30%	Acid phosphatase, citric acid, fibrinolysin, prostate specific antigen, proteolytic enzymes, zinc
Bulbourethral glands	<1%	Galactose, mucus, pre-ejaculate, sialic acid

fabrics as they exhibit a stiff and crusty appearance. Generally, in many cases, the fabric is been washed or contains a very minute stain, visual examination of the exhibit may help in detecting the stain or some presumptive or confirmatory tests may also be done (Butler 2007; Cotton and Fisher 2015; Li 2015).

18.4.1 Visual Examination

18.4.1.1 Color

Thick, yellowish-white, glary, and opalescent secretion having a characteristic odor known as seminal odor.

18.4.1.2 Texture

On touch, seminal stains are starchy and after drying, they become hard and rough.

18.4.1.3 Smell

The faint smell of fresh or wet seminal stains is characteristic due to a mixture of chemicals.

18.4.1.4 Appearance

In natural light, some stains are reddish colored, while others are brown, yellow or faint grey in color.

18.4.1.5 Peel

Dry stains have a rough feel like dried starch solution and have uneven surface (Stefanidou et al. 2010).

18.4.2 Examination Under UV Rays

The exhibit/article suspected to bear seminal stains gives bluish-white color fluorescence when examined under ultraviolet rays in darkness. It depends a lot upon the quantity and freshness of the semen (Kobus et al. 2002).

Fig. 18.5 Microscopic examination of sperm cells

18.4.3 Microscopic Examination

It is considered as a confirmatory technique for the presence of semen in the suspected exhibit. By extracting a small portion of a stain with water, followed by gentle vortexing, the cells from a suspected stain on an absorbent material can be transferred to a microscope slide and then heated. Then the slide is observed under microscope. Spermatozoa are slender, elongated structures 50–70 μm long, each with a head and a thin flagellate tail. Microscopic identification of spermatozoa provides the proof of a seminal stain. Histological staining can facilitate microscopic examination. The most common staining technique is the Christmas tree stain. Since spermatozoa holds gram-positive protein, the appearance of purple bodies will confirm the presence of spermatozoa (Fig. 18.5) (Stefanidou et al. 2010; Li 2015).

18.4.4 Phosphatase Method

The Acid phosphate test is one of the best-known and most commonly used techniques for the purpose of identification of semen. Acid phosphatase is an enzyme that is secreted into a seminal fluid by the prostate gland. When it comes in contact with an acidic solution of sodium alpha naphthylphosphate and Fast Blue B dye, its presence can easily be detected. The presence of acid phosphatase in semen helps to search large areas and garments for semen stains.

If a moistened filter paper is rubbed on the suspected area, acid phosphatase may be transferred to the filter paper. Now, on adding alpha naphthylphosphate and Fast Blue B solution, purple color will appear, indicating the presence of acid phosphatase (Romero-Montoya et al. 2011; Li 2015).

18.4.5 Prostate-Specific Antigen

In 1970s, a protein called p30 or prostate-specific antigen (PSA) was discovered which was thought to be prostate-specific. PSA is produced in the prostate

epithelium and secreted into the semen. A more reasonable approach to the unequivocal identification of semen is to use a positive PSA (p30) in combination with an acid phosphatase color test. A more elegant approach to identifying PSA (p30) involves placing an extract of a questioned sample on a porous membrane in the presence of a monoclonal PSA antibody that is linked to a dye. If PSA is present in the extract, a PSA antigen–monoclonal PSA antibody complex forms. This complex then migrates along the membrane, where it interacts with a polyclonal PSA antibody embedded in the membrane. The antibody–antigen–antibody "sandwich" that forms will be apparent by the presence of a colored line. Once the material under examination is proven to be semen, the next task is to attempt to associate the semen as closely as possible with a single individual (Laux 2003; Li 2015).

18.4.6 Biological Tests

Precipitin reaction with anti-human semen serum and specific blood group anti sera are employed. The former determines whether it is human semen and the latter determines the blood group of the secretor. The techniques employed are similar to those applied for blood grouping.

18.4.7 Chemical Tests

The following chemical tests are performed for the detection of semen:

18.4.7.1 Barberio's Test

Barberio's test was discovered by Barberio in the year 1905. A small amount of semen is taken and treated with a saturated aqueous solution of picric acid. Needle-shaped spermine picrate crystals having yellow color with characteristic structure, separate out.

18.4.7.2 Florence Test

This test was discovered by Dr. Florence in the year 1886. A small amount of semen is treated with iodine in a potassium iodide solution (Potassium Iodide + Iodine + Water). Characteristic dark crystals of choline iodide having rhomboidal shape are formed (Table 18.2) (Yudianto and Sispitasari 2016).

Table 18.2 Time duration for the presence of motile and non-motile sperms in the body cavities

	Motile sperms	Non-motile sperms
Vagina	6–28 h	14 h to 10 days
Cervix	3–7.5 days	7.5–19 days
Mouth	–	2–31 h
Rectum	–	4–113 h
Anus	–	2–44 h

18.5 Vaginal Fluid

Vaginal fluid is crucial evidence found in cases of sexual assault as these evidences can support in verifying the allegations of sexual assault. If the stain is originating from the victim, it creates a link between the victim and the perpetrator. But, it may be questioned whether the stain is originated from sweat or vaginal secretion. So, the value of evidence may increase if it is found to be a vaginal stain. A mixture of vaginal secretion and semen stain is generally found and the presence of vaginal secretion confirms the incidence of sexual assault. When these are on clothing, vaginal Secretions appear to be rough and stiff on feeling. If observed under UV light examination, these show fluorescence (Li 2015).

The human vagina is mainly composed of squamous mucosa, submucosa, and muscularis. The vaginal fluid generally is a mixture of miscellaneous proteins from the upper genital tract, cervical mucus, transudate, desquamated cellular debris, leukocytes, epithelial residue, lactic acid and electrolytes (Shrivastava et al. 2020). Cotton swabs can be used for sample collection from a suspect's pubic area or fingers or by cutting or scraping the stain from the suspected item. Stains can be detected by fluorescence of vaginal fluid constituents using the alternate light source (ALS) method. One of the most commonly used methods of vaginal fluid identification is based on immunological assays. Some characteristics used in female hormone markers are 17b-estradiol (E2-17b), estrogen receptors, mucin 4 (MUC4) and human b-defensin 1 (HBD-1) etc.

18.5.1 Lugol's Iodine Test

Lugol's iodine solution is a color test that is utilized for the identification of glycogenated vaginal epithelial cells. The technique is based on the principle that iodine reacts with intracellular glycogen to exhibit a dark brown color. In cases of evidences containing skin, buccal and vaginal epithelial cells, it is necessary to distinguish between these cells. Dane's staining method has been developed to distinguish all three types of cells. Skin cells are stained red and orange; buccal cells are stained predominantly orange-pink with red nuclei; and vaginal cells are stained bright orange with orange nuclei (Rothwell and Harvey 1978).

18.5.2 Vaginal Acid Phosphatase

Very small amounts of acid phosphatase which is produced in normal cervical epithelial cells can be detected in vaginal fluid. But, molecular characteristics of vaginal acid phosphatase are still not known. Earlier, using acid phosphatase catalytic assays vaginal acid phosphatase was used as a biomarker for the identification of vaginal secretions. Using agarose electrophoresis, vaginal and prostate acid phosphatases can also be distinguished on the basis of their electrophoretic mobility. Prostate acid phosphatase has higher electrophoretic mobility toward an anode

(a positively charged electrode) than vaginal acid phosphatase. Thus, the presence of vaginal acid phosphatase can be determined (Adams and Wraxall 1974).

18.6 Saliva

Saliva as an evidence is generally recovered from crime scenes involving oral activities, often in relation with bite marks on the skin as in cases of violent crimes and sexual assaults, on eatables, clothing, cigarette butts, chewing gums, documents, postage stamps etc (Houck and Siegel 2015). Saliva is an extracellular fluid mixture of various secretions from acinar cells of salivary glands, together with secretion from non-salivary sources like nasal secretion, gingival fluid, bronchial mucus, buccal cells, bacterial products and food remains (Tanaka et al. 2017). The colorless and quick-drying nature of saliva stains as well as its presence in trace amounts makes identification and collection of these stains difficult. A double swab technique is preferred for the collection of such samples. In this technique, the surface (skin, clothing etc) should be swabbed twice, firstly, using a wet swab and then followed by second swabbing with a dry swab (Rutty et al. 2003; John et al. 2018).

18.6.1 Characteristics of Saliva

The human salivary glands contribute about 1.0–1.5 L of saliva daily out of which 70% of saliva is released from the submandibular 25% from the parotids, and 5% from the sublingual salivary glands (Li 2015). Besides fluid contents, saliva also contains small amounts of antibodies, electrolytes and enzymes. The enzymes present in the saliva, particularly amylase is the prime factors for the readily digestion of complex carbohydrates or polysaccharides such as starch in the oral cavity. Besides saliva, amylase is also, localized in other body fluids such as blood, urine, sweat, tears, semen, breast milk, feces, and vaginal secretions but is in trace concentration as compared to the salivary fluid. Thus, determining the presence of amylase in the evidences indicates the existence of saliva (Gefrides and Welch 2011; Gunn 2019).

18.6.2 Visual Examination

The colorless nature of dried saliva stains makes them difficult to be identified in normal lights. Application of alternating sources of light in the range of 470 nm excitation wavelength (using red goggles), UV lights, and argon laser lights helps in identifying and locating saliva easily. Microscopic examination with proper histological staining can also be performed to identify the buccal epithelial cells, indicating the presence of a saliva stain.

18.6.3 Preliminary Test

18.6.3.1 Iodine Assay

The enzymatic activity of amylase can be determined by observing the reaction of iodine with the starch. The glucose polymers of starch, namely amylose and amylopectin react with iodine to form dark blue and reddish purple color respectively. In presence of amylase within the sample, the starch breaks down into simpler disaccharides and mono-saccharides, thereby no color appears on adding iodine to the sample.

The iodine assay must be performed on the test sample along with positive and negative control samples. All three samples namely the test sample, positive control, and negative control must be taken in equal quantity or dimension. Two to three drops of 0.5% soluble starch solution are added to each of the three samples and incubated at 37 °C for 1 h. Two drops of Lugol's iodine solution (composed of 1 g Iodine and 2 g Potassium iodide dissolved in 200 mL of distilled water) are added to these samples and observed for notable color change. The appearance of blue color indicates the absence of amylase thereby pointing the absence of saliva in the sample. Discolored reaction or absence of blue color after the reaction indicates a positive amylase reaction and the presence of saliva in the sample.

18.6.3.2 Phadebas Test

This test is based on the fact that amylase digests starch. Phadebas reagent consists of a dye cross-linked with starch. The presence of saliva digests the starch and releases the dye from the complex. The solution thus becomes blue in color. This indicates the presence of saliva. The test can be used as a quantitative test by measuring the intensity of the developed color at 620 nm wavelength. A standard concentration curve of known concentration of colored dye may be prepared and used for quantitative data.

18.6.3.3 Radial Diffusion Assay

An agar gel containing starch is prepared. A sample well is created by punching a hole in the gel and an extract of the questioned sample is placed into the well. If amylase is present in the sample, it diffuses from the sample well and hydrolyzes the starch in the gel. The gel is then stained using an iodine solution. A clear area in the gel indicates amylase activity, and the size of the clear area is proportional to the amount of amylase in the sample. A linear standard curve can be prepared using a series of standard amylases with known concentrations. The amount of amylase can be quantified by comparing the results with the standard curve.

18.6.4 Confirmatory Test

18.6.4.1 Immuno-Chromatographic Assays

Various commercially produced immuno-chromatographic kits are used for the identification of Human *salivary* α-*amylase* (HSA). RSID®-Saliva kit (Independent

Forensics, Hillside, IL) utilizes a labeled monoclonal anti-HSA antibody is contained in a sample well. A second monoclonal anti-HSA antibody is immobilized onto a test zone of a membrane, and an antiglobulin that recognizes the antibody is immobilized onto a control zone.

18.6.4.2 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) is an immunoenzymatic test used for the quantitation of species-specific antigens or antibodies. For the identification of saliva, ELISA can be employed to quantify the human-specific amylase antigen present in the sample. The method utilizes reporting enzymes that produce colorimetric or fluorometric signals of intensities that are proportional to the amount of binding antigens with the use of an anti-HSA antibody. Antibody-antigen-antibody sandwich is one of the most common configurations in forensic serology. The intensity of the signals can be detected by means of spectrophotometers and the total quantity of HSA can be calculated using a standard of known concentration. ELISA is a species-specific and highly sensitive assay but, it is time-consuming.

18.7 Urine

In cases of violent crimes, hanging, illicit drug screening tests, sexual assault and harassment involving urination, urine may be submitted as important forensic evidence. The location of the stains of urine at the crime scene may provide vital information to determine the site of violence occurred.

The formation of urine takes place in the nephrons in the kidneys. Urine is an aqueous solution, yellow in color, consisting largely of water having urea in the most abundant quantity, which is resulted from the elimination of ammonia, also providing it a unique odor. The average urea concentration in human urine is approximately 9 g/L. Other major components of urine are creatinine, uric acid, and ions such as phosphate, sulfate, chloride, sodium, and potassium. In cases of urine stains, the sample should always be collected from the largest stains that are available. The collection and storage of samples should be properly done in order to avoid degradation. Medicines and foods such as penicillin and red beets, asparagus may affect the odor and color of urine. The ph of urine ranges between 4.6 and 8.0 and a specific gravity between 1.005 and 1.030 (Jones et al. 2012; Li 2015; Joki-Erkkilä et al. 2016; Vyas et al. 2020).

18.7.1 Visual Examination

Urine stains can be located by visual examination based on the characteristic yellow color of urine and the detection of the distinctive odor of urine stains. A suspected urine stain may show fluorescence of pale yellow or pale blue color when viewed under UV light. Medicines, foods and drugs may affect the color of urine.

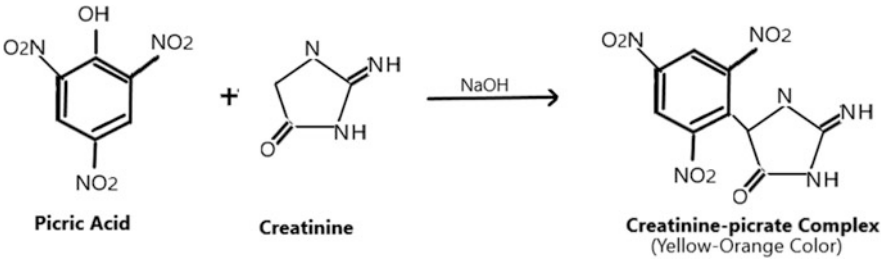
18.7.2 Odor Test

The characteristic odor of urine may be detected by placing a small sample of the stain in a test tube and heating it gently over the flame.

18.7.3 Presumptive Test

18.7.3.1 Creatinine Test

This presumptive test for the detection of creatinine in urine was developed by a German biochemist Jaffe in 1886. Therefore, it is also known as Jaffe's test. To a drop of stain extract on filter paper, add one drop of picric acid followed by one drop of 5% Sodium hydroxide (weak base) to form a deep yellow-orange color.



18.7.4 Confirmatory Test

18.7.4.1 Urea Nitrate Crystal Test

An aqueous extract of stain is made with acetone and then the acetone extract is concentrated by evaporation and then dried, after filtration. The residue, after dissolving in acetone is again evaporated. A thin film is made on a microscopic slide by adding one drop of conc. Nitric acid. In the presence of urea, hexagonal stacked crystals of Urea nitrate can be observed. The crystals are long colorless, four or six-sided rhombic crystals (Desroches et al. 2009).

18.7.4.2 Detection of Indican

In this test, the extracted stain is treated with resorcinol reagent and cupric bromide ($CuBr_2$) solution. Red color crystals are observed, indicating the presence of Indican (Desroches et al. 2009; Li 2015).

18.8 Sweat

Sweat, deposited unconsciously at various surfaces, rarely engages one's attention during the deliberate cleaning of other evidences. Biologically, sweat is a transparent bio-fluid secreted from eccrine and apocrine sweat glands, with low tonicity and a

slightly acidic nature with mean pH of 6.3, that is, more acidic than blood. The majority of sweat evidence that is analyzed in forensic laboratories is sweat stains secreted from eccrine glands. Sweat contains water, minerals, lactate, and urea. Its biochemical composition varies among individuals and their physical activities. Sweat contains low levels of constituents that are also present in other bodily fluids such as urine such as amino acids and urea, potassium, sodium, chloride ions, lactate, pyruvate and xenobiotics. Thus, it has been considered a difficult bodily fluid to identify. Around 650 sweat glands are present in an average square inch of the skin resulting in primary transfer, i.e., deposition of a trace amount of sweat on surfaces that come in contact with the skin (Jadoon et al. 2015).

Sweat as evidence can be analyzed using various presumptive assays. The quality of sweat analysis depends on the efficiency of sample collection and the accuracy and sensitivity of analytical methods. Elemental analysis using scanning electron microscopes coupled with energy-dispersive X-ray spectroscopy in the detection of lactic acid is generally used. Raman micro-spectroscopy is potentially useful for the identification of sweat for forensic purposes. Another technique has been identified as a potential biomarker of human sweat i.e. dermcidin. Dermcidin belongs to a class of human antimicrobial peptides of the innate immune defense system. The detection of dermcidin can be performed through ELISA assays utilizing antibodies specific to human dermcidin. This method of detection is highly sensitive and can detect dermcidin up to a dilution of 10,000-fold (Li 2015).

18.9 Tears

Tears, secreted in very less volume, can be a potential tool to establish the identity. Tears can be found as stains deposited on tissue papers, handkerchiefs, pillows, bedding, etc. Traditionally, investigators have been trained to identify the presence of blood, saliva, semen, urine, etc. but generally, they fail to endorse the possibility of finding tears as evidence. Tears can offer important clues both qualitatively and quantitatively as they may help in personal identification (Aparna and Iyer 2021).

Lacrimation is generally classified into three categories based on their composition—basal tears, biochemical substances such as proteins, lipids, organic molecules, metabolites, and electrolytes. Tears contain a high concentration of proteins, ions and antioxidant compounds such as tear albumin, lysozymes, lactoferrin, transferrin, ceruloplasmin, lipocalin, prealbumin, lipophilin, IgA, IgG, secretory IgA, IgE, Glycoproteins, antiproteinases, Enzymes such as lactate dehydrogenase, Lysosomal enzymes, amylase, peroxidase, plasminogen activators, collagenase, Lipids such as cholesterol, Metabolites as glucose, lactate, urea, catecholamine, histamine, prostaglandins, Electrolytes such as hydrogen ions, potassium, calcium and magnesium.

Cloth or paper material, tissue paper or a handkerchief, and bedding material can be potential sources for finding the presence of tears. The use of Alternate Light Sources (ALS) can help in locating their presence on various surfaces since body fluids are known to fluoresce when exposed to varying wavelengths of light. It is also

possible to perform ABO typing and DNA profiling from tears deposited on various substrates (Shrivastava et al. 2020; Aparna and Iyer 2021).

18.10 Bloodstain Pattern Analysis

Bloodstain Pattern Analysis plays a major role in crime scene analysis and reconstruction of crime scene. In violent crimes, it is very usual for the participants to be injured. In general, these injuries are accompanied by blood flow in different blood stain patterns may result which can provide crucial information about the crime. Bloodstain Pattern Analysis (BPA) may be termed as the study of the shapes, sizes, distribution, and locations of bloodstains and may provide information about the events that have taken place during the crime (Wideman 2009). It is principally based on the physical science (cohesion, capillary action and velocity) in addition to chemical, biological (behavior of blood), and medical knowledge. The history of BPA can be traced back to the 1500s as Herbert Leon MacDonnellhas found literature references to bloodshed characteristics. In 1895, an article based on experiments to examine bloodstain patterns resulting from head wounds was published by Dr. Eduard Piotrowski (James et al. 2005). Characteristics of blood in terms of physical laws are essential factors affecting blood stain patterns. This includes:

1. **Surface Tension**: The spherical shape of blood drops is maintained by the phenomenon of surface tension that results in the inward pull of blood drop in horizontal and vertical direction thereby maintaining the shape of blood drop.
2. **Viscosity**: The resisting characteristic of blood towards the flow is called its viscosity
3. **Density**: The mass of blood per unit volume of surface is known as the density of bloodstain.

18.10.1 Categories of Bloodstains on the Basis of Velocity

The abovementioned phenomenon of fluid dynamics plays an important role in retaining the shape of blood stain. Besides, velocity related to the force applied on the blood source also directs the size, shape, and characteristics of blood stain. The diameter of blood stain formed due to a certain force is inversely related to the velocity (Boos et al. 2019; Faiz 2021). Based on this concept, blood stains can be classified into three categories:

18.10.1.1 Low-Velocity Blood Spatter

The blood spatter formed as a result of force with a velocity up to 5 ft/s is said to be a Low-velocity blood spatter. The diameter of such spatter measures more than 4 mm. Free-falling blood drops under gravity, splashing blood, and stepping into the blood pool are examples of low-velocity blood spatter.

18.10.1.2 Medium-Velocity Blood Spatter

The blood spatters with diameter ranging between 1 and 3 mm are produced from the force with a velocity between 5 and 25 ft/s. The wound caused by blunt objects such as fist, hammer, etc and stabbing wound create medium-velocity blood spatter.

18.10.1.3 High-Velocity Blood Spatter

When force of velocity greater than 100 ft/s is applied to a blood source, the spatter formed is called a high-velocity blood spatter. The stains so formed are with a diameter less than 1 mm. High-speed trauma such as gunshot, explosive, or machinery injury causes the formation of high-velocity blood spatter.

18.10.2 Types of Bloodstain Patterns

Bloodstain patterns can be mainly categorized into three types: passive, transfer and projected patterns. Passive patterns are formed due to gravity and include patterns like drip stain, flow stain, blood pool, and serum stain. The blood stain patterns formed due to contact of a blood-bearing surface and non-blood-bearing surface, the pattern is said to be of pattern bloodstains. Projected bloodstain patterns result from excessive pressure or force. Besides the three categories, some of the unusually modified bloodstains can also be observed at the crime scene. In case of physical alteration or change occurs to the bloodstain pattern by means of physical activity, diffusion, dilution, or insects, the pattern is said to be an altered pattern (Fig. 18.6) (Griffin 2006; Houck and Siegel 2015; Li 2015).

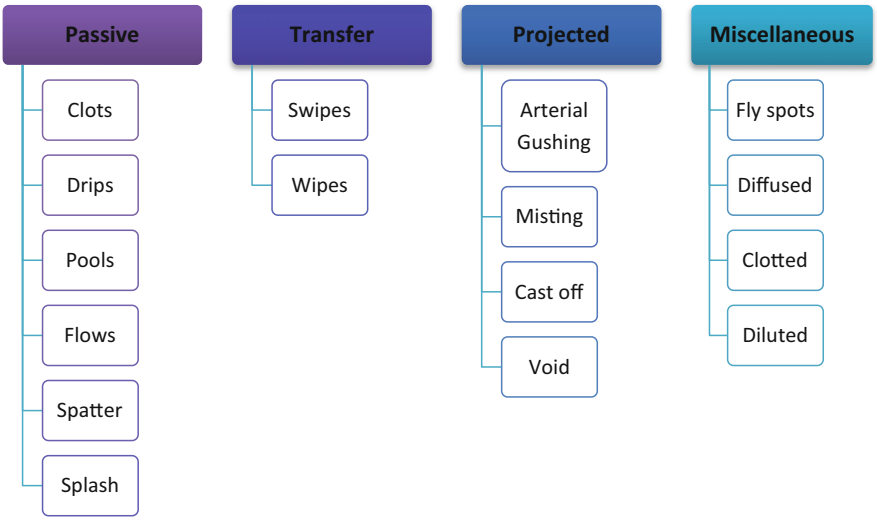


Fig. 18.6 Classification of bloodstain patterns

18.10.2.1 Passive Bloodstain Patterns

Passive bloodstain results from the flow of liquid blood in the form of bleeding generally deposited onto the surface under the influence of gravitational force alone.

18.10.2.1.1 Clots

A clot is can be described as the mass of blood cells trapped with fibrin. The phenomenon of serum separation is often seen in the clotted bloodstain patterns.

18.10.2.1.2 Drips

When drops of blood from any wound or blood source fall on the surface, they are said to be blood drip stains. If the blood source is in motion, the pattern of bloodstain is known as a drip trail. If a liquid falls on other liquid surfaces, with the circumstance of one or both the liquids being blood, a drip pattern is formed. Drip pattern is different from drip stains and results in the formation of secondary spatter.

18.10.2.1.3 Pools

The accumulation of liquid blood on the surface is called a blood pool.

18.10.2.1.4 Flows

Movement or disturbance in the large volume of blood pool on the surface due to some external force, such as gravity or post-mortem disturbance results in the formation of blood flows.

18.10.2.1.5 Spatter

When hard objects are used to strike the blood source, the force applied results in the dispersal of blood drops in the air. The patterns so formed are called spatter.

18.10.2.1.6 Splash

A large volume of blood spilling onto the surface is known as a blood splash.

18.10.2.2 Transfer Bloodstain Patterns

18.10.2.2.1 Swipe

The transfer of blood from any blood-bearing surface to another surface, characterizing the relativity of both surfaces is called a swipe pattern. The impressions probably provide information such as the shape and size of the object.

18.10.2.2.2 Wipe

It is a type of altered or disturbed pattern created by a moving object sliding onto the blood-bearing surface before it is dried and disturbing the patterns of the original stain.

18.10.2.3 Projected Bloodstain Patterns

18.10.2.3.1 Arterial Gushing and Spurts

Expulsion of blood in the form of gush and spurts under pressure fluctuation caused due to any injury to the artery or heart. Arterial gush is large-volume blood stain patterns while spurts refer to lesser-volume bloodstain patterns.

18.10.2.3.2 Misting

Explosive forces such as gunshot spatter result in blood stains formed in the pattern of mist or fine spray. This is known as atomized misting of blood. The blood stain patterns consist of multiple circular stains often microscopic in size. The size of individual stains is inversely proportional to the force implied to the blood source. As the force to the source of blood increases, the size of each stain decreases considerably.

18.10.2.3.3 Cast Off

When a stain is created as a result of blood, being projected from a secondary object in motion or suddenly coming to rest after some motion. The cast-off stain forms a linear pattern that provides information related to the general position of the secondary carrier, the number of blows struck and motions as well as directions.

18.10.2.3.4 Void Pattern

A void pattern is found when an object is placed between the blood source and projection area, it is likely to receive some of the stains, which consequently leads to an absence of stains in an otherwise continuous bloodstain pattern (Fig. 18.7).

18.10.3 Interpretation of Bloodstain Patterns

Bloodstain patterns play an important role in the reconstruction of crime scene. They provide vital information which may be helpful in the investigation and includes:

- Origin or source of bloodstain
- Direction of impact
- Number of blows or strikes
- Relative position and movement of victim, perpetrator or object in the crime scene
- Object or weapon used for the attack that produce particular bloodstain

Physical characteristics such as angle of impact, area of convergence, velocity of strike or spatter, relative distance between object and victim, surface type, and others are often useful parameters that help in the interpretation of bloodstain patterns (Table 18.3) (Damelio and Gardner 2001; Faiz 2021).

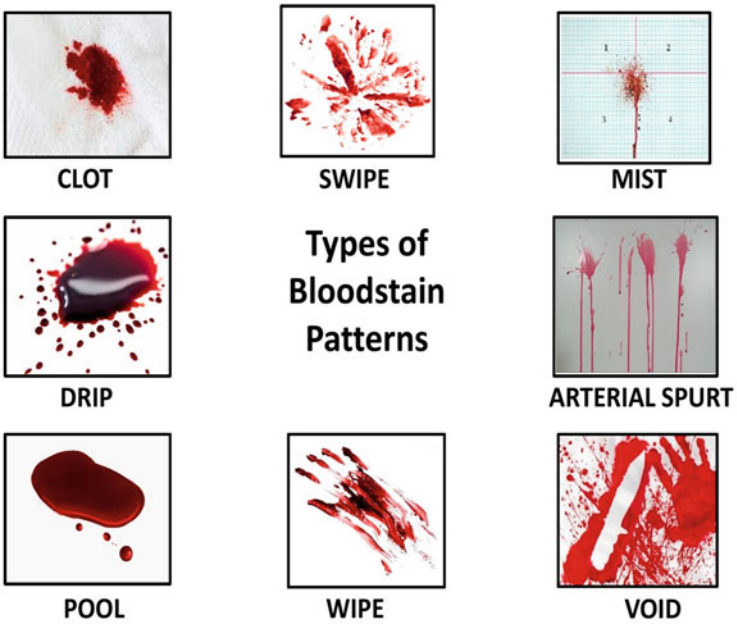


Fig. 18.7 Various types of bloodstain patterns commonly encountered at crime scenes

Table 18.3 Variation in the shape of bloodstains with changes in height

Shape of bloodstains	Height (in cm)
Round sharp edges	Up to 50
Spike like projections along edges	50–150
Corrugated edges	Over 150

18.11 Blood Grouping

The most widespread application of serology is the grouping of whole blood for its A-B-O identity. Blood group refers to the complete vast blood group system which is controlled genetically for specificity while blood type refers to a unique pattern of reaction to testing antisera within a given system. The earlier different blood types were recognized in 1875, but in 1901, Karl Landsteiner named and standardized the groups (Fig. 18.8).

The International Society of Blood Transfusion currently recognizes 43 blood group systems representing over 300 antigen polymorphisms. The ABO system of antigens in human erythrocytes is the most commonly used blood grouping system, which is used for its forensic applications. Other systems like Rh, MNS, Kell, Duffy, and Kidd systems are also used by Forensic laboratories (Rous 1947; Khan et al. 2013; St-Louis 2014; Li 2015; Minari and Mgbada 2017).

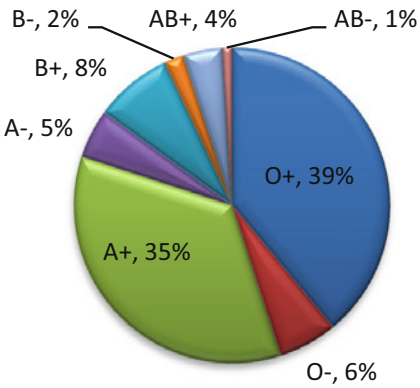


Fig. 18.8 Distribution of different blood-groups among world population

Table 18.4 Antigens and antibodies in different blood types

Blood types	Antigens on red cells	Antibodies in serum
A	A	Anti-B
B	B	Anti-A
AB	AB	Neither anti-A or anti-B
O	Neither A nor B	Both anti-A and anti-B

18.11.1 ABO System

It is of the most important and oldest transfusion and transplantation system. Any person above the age of 6 months possess clinically significant anti-A and/or anti-B antibodies in their serum. Blood group A contains antibody against blood group B in serum and vice-versa and blood group O contains both the antibodies in the serum but no A/B antigen. H-antigen is present in the entire RBC's irrespective of what the blood group system is. The only exception is the rare Bombay phenotype (homozygous for the H gene HH) where antigen A and B are absent. However, iso-antibodies to H-antigen as well as to antigens A and B are produced by these individuals (Table 18.4) (Olsson and Chester 2001; Storry and Olsson 2009; Khan et al. 2013; Landsteiner 2013; Li 2015).

- The "O" type is most common among indigenous people (like Aborigines and Native Americans) and Latin Americans.
- The "A" type is most common among Caucasians and those of European desert.
- The "B" type is most common among African-Americans and certain Asians (eg: Thai).
- The "AB" type is most common among the Japanese and certain Asians (eg: Chinese).

18.11.2 Rhesus System

After the ABO system, Rhesus-system (Rh-system) is the second most important blood group system. Around 50 defined blood group antigens are present in Rh-system out of which only five are important. Landsteiner and Wiener in 1940 discovered the human blood factor. The RBC surface of an individual may or may not have Rh factor or immunogenic D-antigen. Accordingly, the status is indicated as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent) (BOWMAN 1978; Gaensslen et al. 1985; Sinor et al. 1985).

18.11.3 MNS Antigen System

This system was first described by Landsteiner and Levine in 1927. It is generally based on two genes: Glycophorin A and Glycophorin B. Two antibodies called anti-M and anti-N were identified. These two antigens were derived from a single pair of genes which are allomorphic and co-dominant. The frequency for these groups in the human population for Group M is 20%, for Group N is 22% and for Group MN is 50%. Anti-M and anti-N antibodies are usually IgM types and rarely, associated with transfusion reactions (Gaensslen et al. 1985; Reid 2009).

18.11.4 Lutheran System

It basically comprises four pairs of allelic antigens which represent single amino acid substitution at chromosome 19 in Lutheran glycoprotein. The finding of a Lu (a+) child with Lu (a−) mother and putative father can be regarded as evidence of an exclusion based on another blood-group system. Anti-Lub is too rare for regular use and furthermore is of small value as it reacts with over 99% of samples (Gaensslen et al. 1985; Schenkel-Brunner 2000; Daniels 2009).

18.11.5 Kell System

After ABO and Rh system, these erythrocyte antigens are the third most important immunogenic antigen. These are defined by anti-K, an immune antibody. Rarely there is a presence of anti-K and this is sometimes helpful in paternity cases. The finding of a K-positive child with a K-negative mother and putative father would constitute an exclusion of paternity. Around 25 Kell antigens have been discovered so far (Lee et al. 2000; Dean 2005).

18.11.6 Duffy System

First isolated in a patient named Duffy having haemophilia, that's why this system is known as the Duffy system. It is present on the surface of RBCs and is also known as Fy glycoprotein. This system employs the use of two sera anti-Fya and anti-Fyb. Anti-Fya provides evidence of non-paternity when the putative father and mother are Fya− and the child Fya+. Anti-Fyb is a rare serum but if obtainable in sufficient quantity and strength could be usefully used with anti-Fya in the testing of white races (Meny 2010; Howes et al. 2011).

18.11.7 Kidd System

It comprises Kidd antigen (also known as Jk antigen), is a glycoprotein which is present on RBC's membrane and acts as a urea transporter in RBCs and renal endothelial cells. Although, being rare they can still cause severe transfusion reactions. Jka was the first antigen to be discovered by the Kidd blood group system, subsequently, two other antigens Jkb and Jk3 were found (Table 18.5) (Lundevall 1956; Hamilton 2015; Lawicki et al. 2017).

The application and usefulness of blood typing in forensic Science is the identification of individuals. Like, if at a crime scene, the blood found is type A, and the suspect is having type B, then the crime scene sample must have a different origin. And if both are having same blood group, then the sample may have come from the same origin or from a different origin that happened to be type A. Even in dried blood, A and B antigens can be identified after many years. They can also be identified in semen and other bodily fluids (Fig. 18.9).

18.12 Species Identification

After the identification of biological evidence, it becomes necessary to determine whether or not it is of human origin; and if of non-human origin, then to what species it belongs. The specific proteins of species in the bloodstains or any other biological fluid or any tissue of species can be identified with the aid of species-specific antibodies. Before the era of DNA technology, species identification was a common serological method. Currently, most forensic laboratories perform DNA quantitation prior to DNA profile analysis. To eliminate the possibility of nonhuman samples unrelated to an investigation, species identification methods can be vital.

The most common primary binding assays are immune-chromatographic assays. The most commonly used secondary binding assays are precipitation-based assays that rely on the binding of an antigen to an antibody, causing the formation of visible precipitation. These precipitation-based assays include the precipitin tube method, double diffusion (ring assays, Ouchterlony assays) and crossed-over immune-electrophoresis. These assays utilize antihuman and anti-animal antibodies to identify human and animal species, respectively. An antihuman antibody can be made by

Table 18.5 Globally recognized blood group systems

S. No.	System name	System symbol	Gene name(s)	Chromosomal location
1.	ABO	ABO	*ABO*	9q34.2
2.	MNS	MNS	*GYPA*, *GYPB*, (*GYPE*)	4q31.21
3.	P	P1PK	*A4GALT*	22q13.2
4.	Rh	RH	*RHD*, *RHCE*	1p36.11
5.	Lutheran	LU	*LU*	19q13.32
6.	Kell	KEL	*KEL*	7q34
7.	Lewis	LE	*FUT3*	19p13.3
8.	Duffy	FY	*DARC*	1q23.2
9.	Kidd	JK	*SLC14A1*	18q12.3
10.	Diego	DI	*SLC4A1*	17q21.31
11.	Yt	YT	*ACHE*	7q22.1
12.	Xg	XG	*XG*, *MIC2*	Xp22.33
13.	Scianna	SC	*ERMAP*	1p34.2
14.	Dombrock	DO	*ART4*	12p12.3
15.	Colton	CO	*AQP1*	7p14.3
16.	Landsteiner-Weiner	LW	*ICAM4*	19p13.2
17.	Chido/Rodgers	CH/RG	*C4A*, *C4B*	6p21.3
18.	Hh	H	*FUT1*	19q13.33
19.	Xk	XK	*XK*	Xp21.1
20.	Gerbich	GE	*GYPC*	2q14.3
21.	Cromer	CROM	*CD55*	1q32.2
22.	Knops	KN	*CR1*	1q32.2
23.	Indian	IN	*CD44*	11p13
24.	Ok	OK	*BSG*	19p13.3
25.	Raph	RAPH	*CD151*	11p15.5
26.	John Milton Hagen	JMH	*SEMA7A*	15q24.1
27.	I	I	*GCNT2*	6p24.2
28.	Globoside	GLOB	*B3GALT3*	3q26.1
29.	Gill	GIL	*AQP3*	9p13.3
30.	Rh-associated glycoprotein	RHAG	*RHAG*	6p21-qter
31.	Forssman	FORS	*GBGT1*	9q34.13
32.	Junior	JR	*ABCG2*	4q22
33.	Langereis	LAN	*ABCB6*	2q36
34.	VEL	VEL	*SMIM1*	1p36.32
35.	CD59	CD59	*CD59*	11p13
36.	Augustine	AUG	*SLC29A1*	6p21.1
37.	KANNO	PRNP	*PRNP*	20p13
38.	SID	SID	*B4GALNT2*	17q21.32
39.	CTL2	CTL2	*SLC44A2*	19p13.2
40.	PEL	PEL	*MRP4/ABCC4*	13q32.1

(continued)

Table 18.5 (continued)

S. No.	System name	System symbol	Gene name(s)	Chromosomal location
41.	MAM	MAM	*EMP3*	19q13.33
42.	EMM	EMM	*PIGG*	4p16.3
43.	ABCC1	ABCC1	*MRP1/ABCC1*	16p13.11

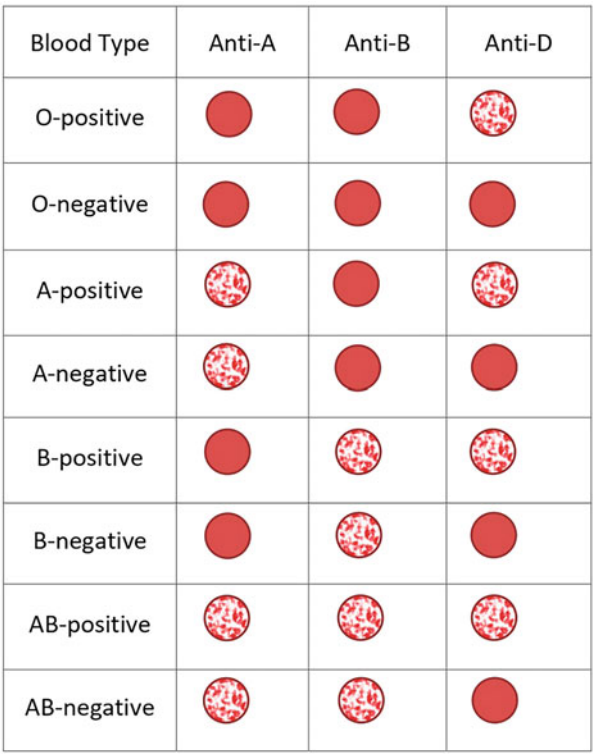


Fig. 18.9 Commonly employed kit for Human Blood Grouping and its agglutination reaction with different types of blood groups

introducing human serum into a host animal, for which specific antibodies are produced against the human serum proteins. The antihuman antibody reacts highly with human albumin. Then the serum part is collected from the blood drawn from the host and this serum is a polyclonal antihuman antiserum. Commercially produced immune-chromatographic kits such as the Hexagon OBTI and the ABAcard HemaTrace® are available to utilize the antibody–antigen–antibody sandwich method by using antibodies that recognize human Hb. Commercially produced immunochromatographic kits such as RSID™-Blood (Independent Forensics, Hillside, IL) use antibodies that recognize human GPA.

18.12.1 Precipitin Tube Method

Take six precipitin tubes (the number can vary on the number of anti-sera used) and place them vertically in a precipitin tube stand and label them. Put a drop of the bloodstain/tissue extract in the tubes. Carefully add one drop of antiserum for species origin (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.) along the walls of the tube. Leave undisturbed for 30 min at room temperature. Carefully examine the white ring at the interface of the two solutions. If a precipitate is formed, it belongs to that specific anti-serum.

18.12.2 Double Diffusion Methods

In this method, both of the reactants, antigen and antibody diffuse towards each other in an agar gel plate, and when an antigen combines with its specific antibody at optimum proportions, precipitin is formed. Fill the central well with tissue extract and peripheral wells with different anti-sera for species origin (anti-Human serum, anti-Fowl serum, anti-Dog serum, anti-Cow Serum, anti-Goat serum, etc.). Cover the petri dish and keep the gel in a moist chamber overnight. Examine the gel for the presence of a precipitin band formed.

18.12.3 Crossed-Over Electrophoresis

Crossed-over electrophoretic technique is a fusion of immunodiffusion assay and electrophoresis. Two sets of wells are created in the agarose gel by punching holes arranged opposite to one another, the one proximal to anode is used for loading antibody and other proximal to the cathode is filled with samples. Electrophoresis is operated by the application of current to the gel plate that drives antigen (or samples) and antibody toward one another. Antigens being negatively charged migrate toward anode while antibodies being positively charged migrated due to the process of endo-osmosis or migrate under fluid flow. Observation of a sharp precipitate band indicates a positive result.

18.13 Polymorphic Enzyme Typing

Forensic Serologist uses multiple enzymes PGM, GLO I, EsD, EAP, AK, ADA, etc., for individualization purposes. The choice of markers employed completely depends upon the degree of polymorphism of the marker in the given population and its stability in aged bloodstains and tissues. Polymorphism in enzymes may be defined as the variant or allele of an enzyme encoded by one locus. Due to limitations in the blood grouping system, genetic protein polymorphic markers have been used to minimize the chance of matching among two unrelated individuals. The sequences of amino acids in proteins vary in the human population. Around 20–30% of human

proteins are polymorphic and individuals can be divided on the basis of this polymorphism. For parentage testing and in analysis of criminal cases, a combination of blood grouping system and enzyme typing can be employed in order to decrease the possibility of match between two unrelated persons. Protein polymorphism identification process is based on electrophoretic separation which depends on molecular weight of proteins and charge on its variants.

18.13.1 Matrices Supporting Protein Electrophoresis

To separate various macromolecules, Electrophoresis of proteins is generally carried out in a support material called the matrix. Conventionally, two types of matrices for protein profiling were used: papers such as cellulose acetate; and gels composed of starch, agar, agarose, or polyacrylamide. The oldest polymorphic protein marker was phosphor-glucomutase which was used on starch-gel electrophoresis. But, agarose and polyacrylamide became more popular due to better reproducibility and reliability.

18.13.2 Separation by Molecular Weight

This separation is completely based on the molecular weights of the proteins. Non-denaturing electrophoresis (Native electrophoresis) was used to isolate proteins for studying their functions.

18.13.3 Separation by Isoelectric Point

This separation method, generally known as isoelectric focusing (IEF) technique is based on the isoelectric points (pI) of the proteins. The pI is the pH value when the net electric charge of an amino acid is zero and each amino acid has its own characteristic pI and is unable to migrate under electric charge.

18.13.4 Erythrocyte Protein Polymorphisms

Isoenzymes are multiple forms of an enzyme which vary in their amino acid sequences but catalyze the same reaction. The individuals can also be divided into units or groups based on different isoenzymes present in their erythrocytes according to Mendelian principles. Around 200 Hb variants have been identified of which Adult human consists of two α chains and two β chains. In cases of infanticide and concealed birth, the detection of fetal Hb in a bloodstain via electrophoresis process can provide important evidence. The ethnic origin of a perpetrator can be identified using a protein polymorphic marker e.g. Hb S polymorphism.

18.13.5 Serum Protein Polymorphisms

One of the most widely used polymorphic serum proteins in the forensic scenario was Haptoglobin (Hp). This method is based on the net charge, size, and shape of the protein and can be determined by electrophoresis or serological methods.

18.14 Conclusion

Advanced technologies and methods have revolutionized the use of biological evidences even collected decades ago in the hopes of linking criminals with the victim and the crime scene. Collection and preservation methods must be taken into consideration while collecting these evidences (serological evidences) as the condition of the exhibit during examination endures to be a critical element for its successful detection and analysis. Hence, pertinent handling standards during identifying, collecting, packaging, storing, and transporting samples are the elemental prerequisites.

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