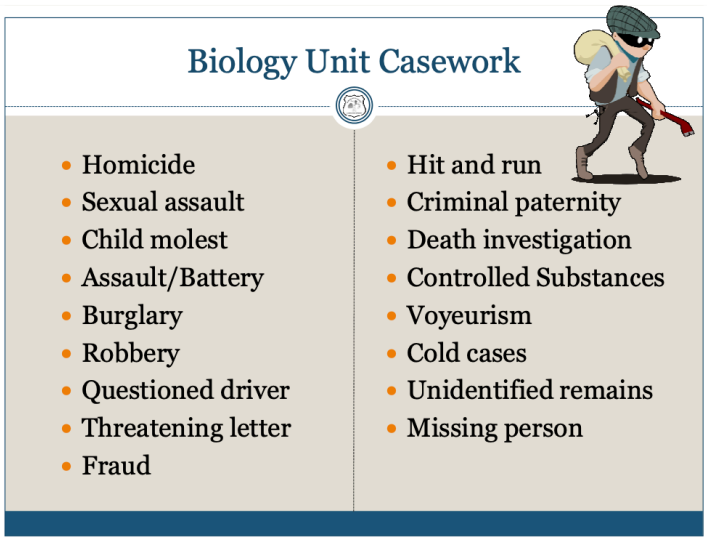
Introduction

Forensic biology and serology is a branch of forensic science which Serology helps in Crime. Serology allows the forensic scientists to segregate these bodily fluids when found at the scene of the crime and then perform a variety of tests on them in order to identify where these fluids originated from - or most importantly - who they came from.



In fact, in many laboratories they are included within the same unit, collectively titled Forensic Biology. In the forensic laboratory, serology analysis refers to the screening of evidence for body fluids while DNA analysis refers to the efforts to individualize body fluids to a specific person.

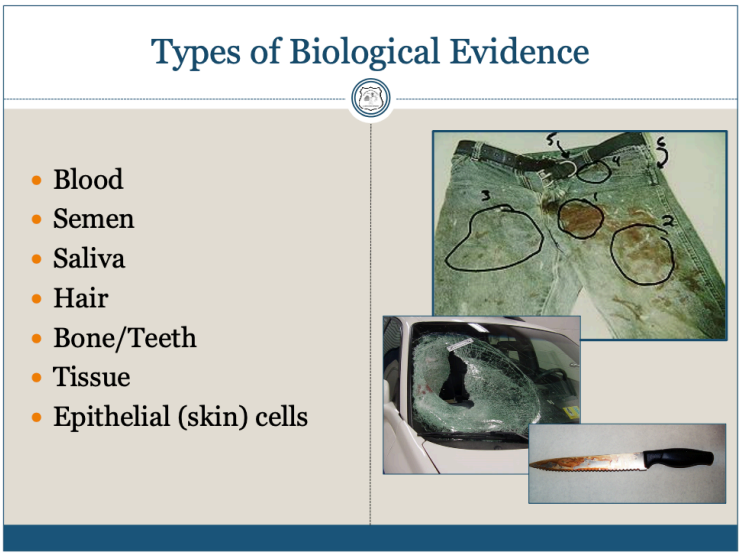
Innocence can be Convicted by Juries, Exonerated by Science.

CHAPTER 1 - Blood

Forensic Biology and serology isa branch of forensic science which deals with biological evidences and their examination. The examination of biological materials play an important role in connecting the criminal with the crime. Such biological specimens may be in the form of body fluids, stains or other materials.

The most important evidences of body fluids are blood and semen personal identification point of view. These materials are ;

1. Blood
2. Semen
3. Saliva
4. Urine
5. Faecal
6. Sweat
7. Nasal Secretions
8. Tears and human Milk
9. Hair



1.1 Composition

Blood is a complex viscous red fluid with a pH of about 7.4. It is mainly composed of two parts: Cells and plasma. 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 clotting of blood. The liquid left is called serum. It is the plasma without fibrinogen which turns into fibrin.

a) Red cells:

There are about five million red cells per micro litre of blood, each about ten micron in diameter. The red cells are continuously being produced and destroyed in the body. The rate of destruction is about ten billion cells per hour in a normal healthy adult.

b) Haemoglobin:

The colouring substance of the red blood cells is haemoglobin. The substance is of great importance to the body functions as it is the carrier of oxygen, injected medicines, salts and food to various body tissues. It is useful in blood examinations.

c) White cells:

The number of white cells is about four to eleven thousand cells per micro litre. They resist the attack of diseases.

d) Platelets:

The number of platelets is about five times that of white cells. They facilitate blood clotting.

e) Serum:

Plasma and serum are complex mixtures of proteins, minerals and organic compounds dissolved in water.

1.2 Functions of Blood

The blood performs following functions:

- It carried oxygen and foo to the body tissues.
- It helps to eliminate waste products of the body tissues through kidneys and other parts of the body.
- It regulates the temperature and acidity of the body.
- It carries administered medicines to the affected parts of the body.

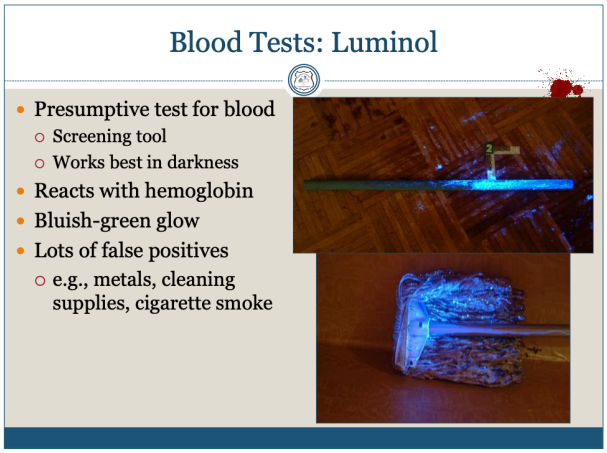
1.3 Examination of Blood Evidences

The identification and evaluation of blood is based in its composition and behaviour under various conditions.

1) Cell structure

Since blood is composed of red and white cells, platelets and plasma. The cells have definite shapes and sizes which in different species. For example, human blood cells have characteristic non-nucleated discs with a diameter of about 0.08mm. The

identification of cell structure is possible when the blood is fresh and moist usually less than 1 day old. When the blood has dried, it is not always possible to completely regenerate the blood cells. The study of cells is made through microscopy.



2) Enzymatic activity

Blood contains heme group in haemoglobin. It behaves like peroxidase. It catalyses the liberation of oxygen from oxygen rich compounds like hydrogen peroxide or sodium perborate. The oxygen is used to carry out certain color reactions. The common color reactions for blood detection are benzidine, phenolphthalein, leucomalachite green and luminol reactions.

Other enzymes have been utilized in electrophoretic techniques. When they are subjected to an electric field due to some polarity, they move towards opposite polarity. The rate of movement varies with their structure, weight and electric charge. The pattern of separation is characteristic of blood.

3) Spectrophotometry

Haemoglobin 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 are characteristic of blood.

4) Visual examination

It is useful to determine whether the given stain is of blood or not. Ordinarily, there is no difficulty in fresh but old stains may not be identifiable. The examination permits:

1. Determination of number and size of stains.
2. Rough estimate of amount of blood shed.
3. The direction from which the blood has fallen is found from the tip of the elongated stain. The tip of the elongated points out the direction.
4. The shape of the stain indicates the height from which the blood has fallen.
    - Bloodstains from the height of upto 50 centimetres are round sharp edges.

- Bloodstains from a height of 50 to 150 centimetres have small spike like projections along the edges.
- Bloodstains fallen from a height over 150 centimetres have corrugated edges.

5. From the positions of the stains, the movement of the victim and the culprit can be determined. If the culprit ran away bleeding, he can be tracked through blood stains.
6. The degree of fluidity, dryness and changes in color permits rough estimation of age of the stains.
7. Gross foreign matter like hair, flesh, bones in the blood may identify the site of injury.

5) UV and IR Examination

Ultraviolet or infrared rays reveal washed or invisible bloodstains from clothes, furniture, earth, doors etc. They also reveal stains on coloured garments or on painted surfaces. They help to reveal minute blood traces.

6) Microscopy

Microscopic examination of bloodstains is important in many situations:

- Species of origin of fresh bloodstains can be established through microscopic studies and micro measurements sometimes.
- The part of the body from which the blood has come can found out from the nature of extraneous matter. Thus, blood from nose, mouth, vagina or anus can be identified.
- Diseases like leukaemia or syphilis can be detected microscopically.
- Menstrual blood can be identified.
- Blood from an infected site can be identified through the presence of puss.

7) Spectroscopy

Spectroscopic examination of blood is very useful as well as convenient. The test is usually carried out microscopically. The blood haemoglobin is changed in two or three forms on the slide itself and characteristic absorption are observed. Usually alkali hematin and cyanhaemochromogen are studied for their characteristic spectra.

8) Chromatography

Ascending paper chromatography using acetic acid, methanol and water solvent system has been employed to study the Rf values.

9) Electrophoresis

It is used for separation of various enzyme systems. It is being adopted to study the body proteins. This technique is becoming important to distinguish between blood samples.

10) Colour tests

These tests are the first series of tests employed after visual study of stains. If a stain gives positive color reactions in any of the two color tests, the stain is possibly a bloodstain. If it fails to give color reactions in all probability it is not a bloodstain or the same cannot be established.

10.1 Benzidine reaction:

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

10.2 Leucomalachite Green reaction:

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

10.3 Phenolphthalein reaction:

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

10.4 Luminol test:

Luminol is a chemical which has been used to locate the bloodstains. The articles suspected to bear stains is sprayed with luminol. It reacts with blood to give fluorescence. 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 (0.7g) in water (100ml) and adding 3- arninophthalhydrazide (0.1g) and sodium carbonate (5.0g) to the

solution. The solution is sprayed upon the article with glass sprayers in a dark room. Blood gives strong luminescence.

10.5 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.

10.5.1 Teichmann Test-

Take a dry crust or smear of blood on a slide. Put a drop of potassium iodide, bromide or chloride (0.1gm) solution in 100ml glacial acetic acid over the blood and cover it with a cover slip. Warm the slide gently till it gives out bubbles. Typically haemin crystals are observed under microscope. Heating may have to be repeated a number of times.

10.5.2 Takayama Test-

It is also performed similarly. The reagent is prepared from one volume of glucose solution (10%), one volume of potassium hydroxide solution (10%) and two volumes of pyridine which are dissolved in six volumes of water.

11) Precipitin reaction

The reaction is performed to identify the species of the origin of the bloodstains. It is very delicate test and requires only small amounts of blood. A dilute blood solution is used for precipitin reaction. The antisera are not diluted. The blood solution and antisera should be free from turbidity and contamination.

CHAPTER 2 - Semen

2.1 Nature

Semen is found in liquid form, smears or stains or it may be found in vagina, anus or rectum. Fresh semen is a gel like fluid, which liquefies on exposure to atmosphere. A normal ejaculation is about 3.5ml, containing about thirty million sperms. The dry weight is about seven per cent of the liquid weight. The sperm has definite morphological structure. Its identification in a stain establishes the presence of semen.

The shape and size of human spermatozoon is characteristic. But the morphology alone does not permit individualization. Semen of a person does not contain any spermatozoon then it is called as aspermic semen.
This may be due to some disease or it may be due to vasectomy operation. In such cases this criterion for the identification of semen is lost. Immunological test using anti semen sear against seminal plasma are increasingly accepted as reliable test for aspermic semen. Electrophoresis is becoming popular for identification of semen.

2.2 Composition

It is a complex mixture of organic and inorganic compounds. Important constituents of semen from the identification point of view are proteins including enzymes, blood group factors, choline, fructose, citric acid, uric acid and zinc. The composition varies from individual to individual.

Enzyme, acid phosphatase found in the semen is in concentration which are significantly higher than those found in other body fluids. Acid phosphatase offers a very delicate test for the identification and location of semen stains though positive identification of semen are not based upon the acid phosphatase test alone. Choline in semen is used to get crystal tests. Fructose, citric acid and zinc are more or less absent in other body fluids and hence their detection in semen should permit its identification but these substances have not been utilized to any appreciable extent so far.

2.3 Locating Semen

Semen stains can be located using following techniques:

2.3.1 Visual examination

The suspected place or article is examined visually. A semen stain, if fresh is colourless or pale yellow. It gives its characteristic smell. A freshly dried stain on wetting also gives the characteristic odour. The stain becomes stiff and rough to feel on drying. The stiffness does not wear off unless it is rubbed.

2.3.2 Ultraviolet rays

The surface or the article suspected to bear seminal stains when examined under ultraviolet rays in darkness usually gives fluorescence. Some other substances also give fluorescence while some other mask it. Therefore the technique though useful in most of the cases is not infallible.

The fluorescence of semen depends upon the quantity and freshness of the semen. Therefore, even when a stain shows weak fluorescence, it should be further explored.

2.3.3 Spectromicroscopy

Spectromicroscopic examination of the suspected stain may indicate the characteristic contours and layers like formations in a thick crust of semen stain.

2.3.4. Phosphatase method

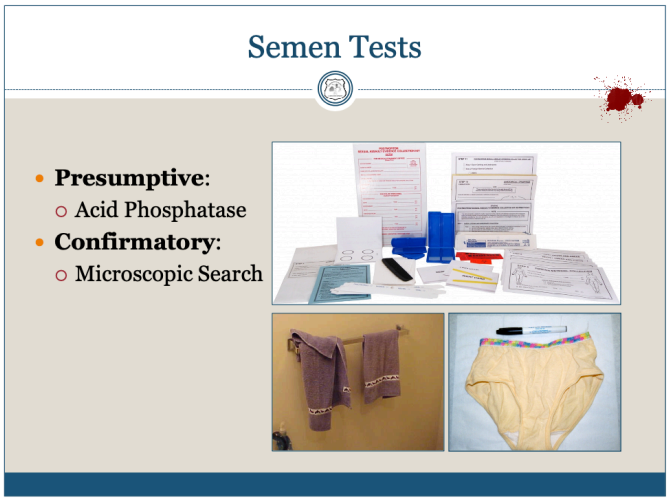
The presence of acid phosphatase in semen helps to search large area and garments for semen stains. The method is:

Large filter paper sheets are dipped in saline. Excess of the solution is removed and the garments to be examined is pressed against the paper or vice versa. The outline of the piece of cloth is marked on the paper to facilitate location of the semen. The filter paper sheet is then sprayed with acid phosphatase reagent. Color spots indicate the location and size of the seminal stains.

2.4 Examination of Semen Stains

Systematic study of seminal stains involves following steps:

- **Smell:** The smell of fresh or wet seminal stains is characteristic.
- **Peel:** Dry stains have a rough feel like dried starch solution and have uneven surface. The contours of the stains are characteristic.
- **Fluorescence:** semen stains give strong characteristic fluorescence. Handling, substrata, the quantity and the age of the stain affect it.



Chemical tests

The following chemical test are performed for detection of semen:

- **Barberio's test**

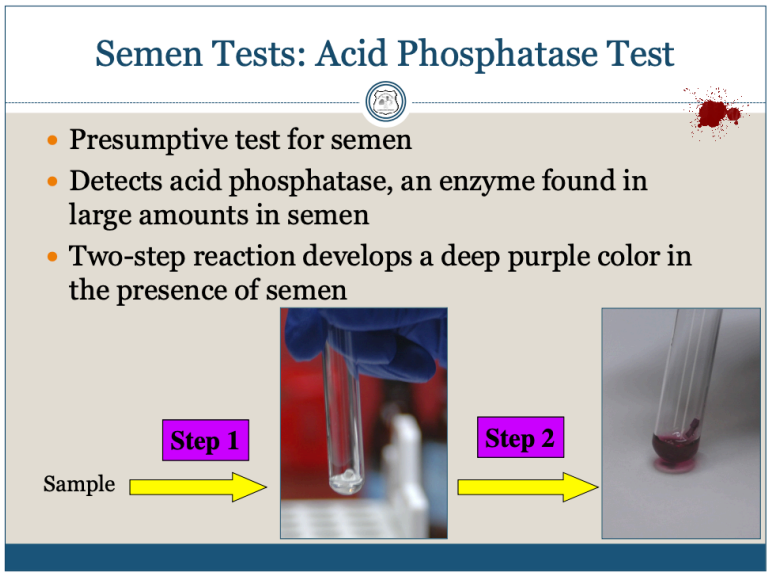
A small amount of semen is taken and treated with a saturated aqueous solution of picric acid. Spermine picrate crystals with characteristic structure, separate out.

- **Florence test:**

A small amount of semen is treated with iodine in potassium iodide solution. Characteristic crystals of choline iodide are formed.

- **Acid phosphatase test:**

The acid phosphatase enzyme present in high concentration in semen liberates phosphate from various compounds attached to them chemically. This property is utilized in liberating Naphthol from calcium naphthyl phosphate. Naphthol reacts with a diazonium compound to give coloured products. Anthraquinon-1-diazonium chloride is often used in the test.



The mixture of various reagents is taken and sprayed over a wet filter paper, previously pressed against the suspected place bearing semen stains. Development of red purple color indicates semen, the technique is extremely sensitive and several years old stains can thus be detected. This method is however not specific. Recently modifications had been made which claim to make it more specific.

■ Morphology of spermatozoon:

The identification of sperms in a stain is considered positive proof of the seminal nature of the stain. Sperm is a unicellular organism with an oval head attached to a comparatively long tail. If head and tail of a spermatozoon are found intact it is not difficult to identify the stain. Intact sperm is often not available in a dried stain.

The sperms may be identified with or without staining. But staining helps:

- Ordinarily double staining with haematoxylin and eosin is done. In this way the nose is stained in pink and the rest of the head is stained purple. This

differential staining distinguishes the sperm from any other organism. Head alone can identify the stain.

- Epithelial cells and leucocytes in excess mask the spermatozoon the difficulty is overcome by staining the spermatozoon with malachite green and eosin. The interference is considerably reduced.

■ Biological test:

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 in are similar to those applied for blood grouping;

- **Individualisation:**
  Semen sample of an individual is unique. With the classical method it was not possible to establish the individuality but DNA profiling has made it possible to pin point the source of origin even from the tiniest speck or even when it is contaminated with vaginal fluid or mixed with semen from other sources.

- **Age of seminal stains:**
  It is difficult to determine the age of seminal stains as the changes with age are largely determined by the environmental conditions.

  The semen is a gel like mass after ejaculation. It liquefies on standing. On drying, it slowly changes to opaque white mass with rough feel. After two or three weeks the color changes to pale yellow and then to brown.

  Sperms in fresh semen are complete with head and tail. On drying and with passage of time it begins to disintegrate, the tail separates out from the head. The head is also affected. The sharp division in differential staining slowly appears.

- **Survival of sperms:**
  The sperms remain motile only for a few hours under normal conditions. The motility seldom continues after twenty-four hours. But if the semen is frozen, the sperms may remain alive for long periods. It shows motility when the sample is brought to normal temperature.

In humid and warm climate, the sperms get destroyed in a short while. In dry and cool climate the dead sperms may be identified even after months.

In vaginal swabs of a living person, sperms may be detected upto about five to ten days, though the number of sperms detected goes on decreasing with passage of time.

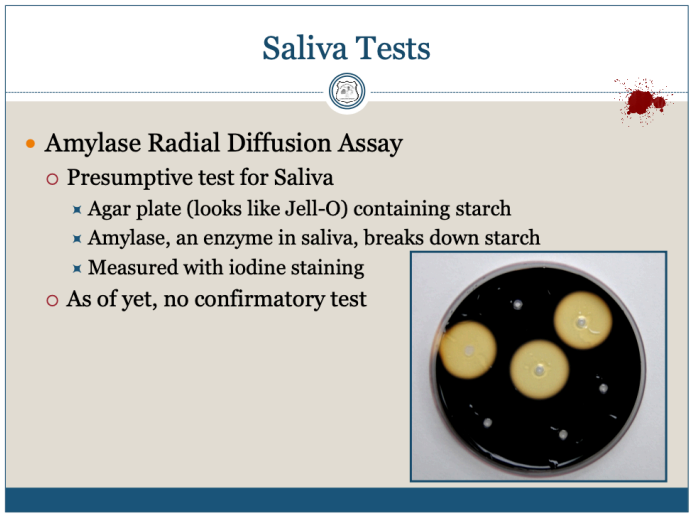
In dead persons, detection of sperms depends upon the condition under which the dead body remained. It may be detected in unputrefied bodies even after a few months. But if the body has putrefied the sperms are also destroyed.

CHAPTER 3 - Saliva

Saliva stains may be found at the scene, on handkerchief, on discarded cigarette stubs, spittal, on cups, tumblers, bottles on postage stamps or envelopes or even tooth picks or they may be found on a piece of cloth used as gag.

Saliva contains an enzyme (ptyalin) which when added to starch, hydrolyses it. Saliva extract, therefore, when added to starch inhibits its color reaction with iodine.

Saliva of secretors contains blood group substances and can be grouped. The saliva on cigarette stubs is often in criminal investigation. DNA profiling of the saliva stains has enhanced the evidential value of the saliva stains and has brought it at par with the other body fluids like that of blood and semen.



Food material mixed in saliva may interfere with blood grouping. Saliva does not give specific precipitin test.

CHAPTER 4 - Urine

Urine is identified from the comparatively large amounts of urea in it. The stain located with ultraviolet rays. It is then extracted with water and tested for urea. Urine stains can now be individualized through DNA profiling.

CHAPTER 5 - Faecal

Faeces are involved in cases of sodomy and bestiality. The stains are found on the undergarments, on the flies of the trousers and on flaps of the shirt of the active agent.

Faeces are identified microscopically. Bacterial studies of the faeces may also lead towards individualization but the same has not been exploited as a routine at present. It is possible to identify the human or animal origin of the faeces.

CHAPTER 6 - Sweat

Sweat stains are involved only in the case when some garments are left at the scene of crime or where the culprit has perspired profusely and left sweat stains. The composition of sweat is similar to urine. However, if the two substances are warmed, the odour is characteristically different. Paper and gas chromatography techniques hold bright future for identification of sweat stains. Sweat stains of secretors can be blood grouped. DNA profiling of the stains is possible.

CHAPTER 7 - Nasal Secretions

Nasal secretions is similar in chemical composition to saliva. They can be blood grouped, if they belong to secretors. They are liable to DNA profiling.

CHAPTER 8 - Tears and human Milk

Tears and human milk stains likewise permit their identification. Blood grouping of the same is possible if they have come from the secretors. DNA profile can be developed for individualization.

CHAPTER 9 - Hair

Hair is one of the common and important physical evidence encountered in a crime scene. Individualization of human hair i.e. whether it is from head or body. Forceful removal of hair may have blood or skin with the root. In such cases DNA typing can be done. There have been advancement in the DNA typing technology. Earlier only hair strand with root attached could be used for typing but now with advanced technology hair without root can also be used if there are enough number of hair strands.

8.1 Identification and Comparison of Hair

Examination of the hair evidence found at crime scene is the first step which should be performed. Hair should be examined to establish the origin of the hair i.e whether it is human hair or animal hair. Comparison of hair with victims and suspects should be done in case of human hair.

Differentiation of human and animal hair can be done with ease. Human hair comparisons should be done with extreme caution. Variable characteristics can exhibited by hair not only from one individual to another but also within a single person.

Morphological features of hair can be observed by careful microscopic examination. These features help in differentiating between human hair and animal hair. Control samples should always be considered before reaching a conclusion. The main characteristics that should be examined are:

- Medullary index
- Scale structure
- Medullary shape

As forensic evidence, hair is used to compare and determine whether the hair recovered at crime is of same origin as that of suspect or not. Scalp or pubic hair are most commonly found at crime scene.

Color, length and diameter of the hair are the most important characteristics that should be matched while comparing hair samples. Presence or absence of medulla, its distribution, shape and color intensity of pigment granules found in the cortex are also important characteristics for comparison of hair. Hair grows at an average of 1 centimetre per month.

Following features are observed and compared for identification through hair:

- Color of hair
- Area of origin
- Presence of micro residue material
- Unusual appearance of hair
- Pigments

- **Color of Hair**

The general color of human hair varies from black, blackish brown or reddish brown. In absence of pigmentation they turn grey and then eventually white. Dyed or bleached hair can be predicted by examining under microscope. Time passed since colouring can also be estimated if growth of hair since hair was coloured or bleached is present. The cortex is coloured throughout when dyed or coloured. Yellowish tint is observed on bleaching hair due to removal of pigments from hair. Granules are present in naturally coloured hair. The texture of naturally coloured hair is similar to a picture coloured by a crayon whereas smooth uniform color is displayed in case of artificially dyes or coloured hair.

- **Area of origin**

The hair of one part of the body differ from other part of the body. Scalp hair are usually longer than hair on any other part of the body. Scalp hairs usually have flat tips as they are most often cut. They are soft with tapering ends. Constant combing causes tapering end to fray out into a brush like form. Periodical cropping shows sharp cut tips which become blunt and round about a week later. On cross section, the hair appear oval or circular in outline. Pubic hair are curly, short and stout. Beard and moustache hair are coarse. The tips are mostly cut. On cross section, they are oval and more flattened as compared to scalp hair. Hair from nose, eyebrow, eyelids or ear are short and stubby with wide medulla. They taper rapidly to form a fine tip.

- **Presence of Macro residual material**

Sometimes, due to external circumstances, the residual material can be found on hair. Materials like blood, grease, paint, powder, soil, dust etc. can be found on the hair which can further lead to important clues when examined.

- **Unusual appearance of Hair**

Due to clipping, pulling, burning or crushing of hair an unusual change in the structure of hair is observed. Normally the tip of the hair is pointed but when it is cut it becomes flat. Due to pulling the diameter of shaft reduces and the length of the hair increases. It the hair is pulled beyond its threshold limit of elasticity then it breaks and the ends become cone shaped.

- **Pigments**

Various pigments are present in hair. One of the pigment is melanin which is responsible for color of the hair. Presence of large amount of eumelanin (one of the types of melanin) results in dark black hair. In case of red hair, pigment called pheomelanin is present. According to the types of pigments present and its distribution, the comparison between two specimens can be done. The pigment distribution and internal structure can be helpful in the identification between species.

- **Identification of Race**

Through the gross characteristics of hair, a person's identification race can be predicted. A cross section of it can show the structure, diameter, the thickness of cuticle, pigmentation, hardness of hair etc. which can be helpful in identification. For example, In Negroid race, the pigmentation is more and their distribution is also disturbed. Hair are hard, small, curly and stiff in nature. In Mongoloid race, hair are usually straight and have more pigments, their cuticle is thick and cross section is circular in shape.

- **Microscopic Examination of hair**

Light microscopy is used for examination of human hairs in forensic laboratory. Two steps are followed in this examination: identification of questioned hairs and comparison of questioned and known hair. Microscopic examination is conducted to analyse whether two or more individuals came in contact or a person came in contact with an object. The value of hair evidence is related to the variability of

hair characteristics between individuals in the population, which can be visualized through the use of comparison microscopy.

Various factors affect the reliability of hair association which includes experience training, adequacy of equipment and suitability of known hair standards. Although hair evidence is a valuable tool in human identification, it is difficult to establish a statistical probability for a particular association due in part to the lack of reliable quantitative assessments of the microscopic characteristics present in hairs.

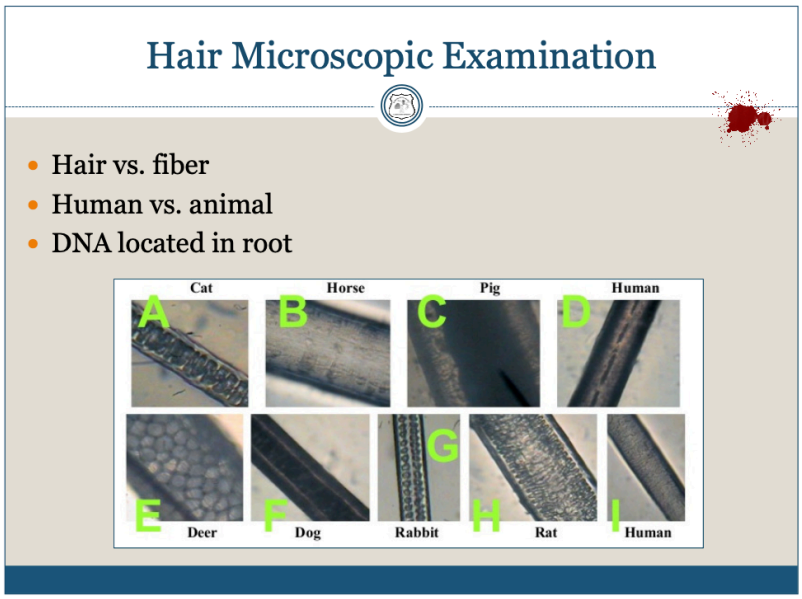
Various microscopes used for hair examination are:

a) Stereomicroscope

For initial examination of mounted or unmounted hairs stereomicroscopes are used with magnification range of upto 100X.

b) Transmitted Light Microscope

For identification and examination of hair high quality transmitted light microscope is required. The objectives and eyepieces should permit observations in the range of approximately 40X to 400X. Eamie ability to observe certain features is enhanced by using a polarized light microscope.



c) Comparison Microscope

While comparing microscopic characteristics of hairs, high quality transmitted light microscope is necessary. High quality objectives are important. The objectives and eyepieces selected should permit observations in the range of approximately 40X to 400X. A high-intensity tungsten light source, suitable for photomicrography and equipped with a daylight correction filter which is

present for providing adequate lighting. Both sides of a comparison microscope should be balanced for light intensity and color. A comparison microscope may be equipped with one of several types of stages.

- **Microtomy:**

The cross sections of hairs are obtained with the help of an instrument called microtome, clean hair is embedded in hard wax, plastics or flesh (hardened by special treatment) and sliced. The cross sections of hairs, obtained, are placed on a microscope slide treated with albumen. The embedding material is removed with a suitable solvent and the sections are fixed in Canada Balsam. Microscopy reveals the cross sectional structure of hair.

Microtomy is helpful to determine pigment distribution, medullary shape and medullary index of the hair. In human hair the pigment is found concentrated near the periphery of the cortex close to cuticles while in animals, pigment concentration are near the medulla and in the cortex. It also permits proper study of the shape of cross sectional area.

- **Density**

It is one of the most important physical properties of hairs. It is best studied and compared by density gradient tube method. The question and sample hair are placed in the columns of two similarly prepared gradient tubes and allowed to settle. They settle at the levels where the densities of the hairs correspond to the liquid mixtures. If the hairs settle at the same height in two columns, a common source is indicated.

- **Refractive Index**

Refractive index of hair is determined by Beckline method. There are minor variations in the refractive indices of hairs of the same individual, the variations are statistically ignored.

Refractive index of hair also gives rough idea about the age and sex of the source of origin. The phenomenon of deviation of light passing through some transparent materials, differently at the different angles is called birefringence. The deviation is characteristic of the materials. The hairs also show the phenomenon but due to inconsistency in the deviations of different hairs of the same person, it has not been used to any appreciable extent to identify or individualize hairs.

CHAPTER 10 - Conclusion

Forensic biology and serology is a branch of forensic science which deals with biological evidences and their examination.

Forensic biology is the application of biology to associate a person(s), whether suspect or victim, to a location, an item (or collection of items), another person (victim or suspect, respectively). It can be utilized to further investigations for both criminal and civil cases.

Forensic serology is the detection, identification, classification, and study of various bodily fluids such as blood, semen, saliva, urine, breast milk, vomit, fecal matter and perspiration, and their relationship to a crime scene.

A forensic serologist may also be involved in DNA analysis and bloodstain pattern analysis. Serology testing begins with presumptive tests which gives the analyst an indication that a specific bodily fluid may be present, but cannot completely confirm its presence.