BLOCK 3

HUMAN IDENTIFICATION: ESTABLISHING IDENTITY-II

ignou
THE PEOPLE'S
UNIVERSITY

UNIT 8 SEROLOGY*

Contents

8.0 Introduction

8.1 Bloodstains

8.2 Saliva

8.3 Urine

8.4 Semen

8.5 Patterns of Bloodstains

8.6 Summary

8.7 References

8.8 Answers/Hints to Check Your Progress

LEARNING OBJECTIVES

After studying this unit, you shall be able to learn about:

- bloodstain and bloodstain patterns;
- methods of individualisation of bloodstain;
- identification and individualisation through saliva;
- identification and individualisation through urine;
- identification and individualisation through semen; and
- bloodstain pattern analysis and its importance in forensic investigations.

8.0 INTRODUCTION

The goal of forensic science and crime scene reconstruction is to seek the truth. There is no other agenda for the analyst. We revisit what we hope is a not-too-distant past and make an attempt to recreate the incidents that unfolded. The task is anything but simple and the tools employed are all of the forensic disciplines.

Each area of forensic science provides insight and a highlight into this past. Each has its place in evaluating the outcome of crime. "who" of crime related information provided by the majority of the forensic disciplines. Fingerprints, palm prints, serology, fiber evidence, hair evidence and trace evidence allow the investigator to link people or objects with a scene of incident. The "what" of crime has always been the primary link to forensic pathology provides insight into some of the events that occurred during the incident. Bloodstain spatter analysis is a

* ***Contributor:*** *Dr Rajesh Singh, Dy. Director (Biology), State Forensic Science Laboratory, Rajasthan, Jaipur.*

discipline that has reawakened to its role in modern forensic science as an alternative method of revealing the "what" of crime. Simply it links the various events of a violent incident.

In this capacity, bloodstain pattern analysis acts as a critical bridge between classical forensics and crime scene reconstruction.

8.1 BLOOD STAIN

As a forensic scientist we should always be highly alert and attentive towards each and every aspect of the case. Blood is important evidence present in all types of cases like murders, sexual assaults, hit and run, etc., which are highly related to the crime and give important information about the victim and perpetrator.

As you know blood forms 8 % of the body weight, it's likely to be said that the blood forms a major part among the body fluids. So before identifying whose blood it is, its first need is to identify whether the substance is blood or not. If we as an investigator find a dark spot on the floor of a crime scene, the first step is to find out if the spot is blood, mainly human blood. If the spot turns out to be spilled ink or paint, no further testing is likely necessary.

Thus to identify the substance as blood or not, certain tests are performed. These are chemical examinations which are based on specific chemical reaction with the blood.

The chemical detection of blood is usually based on one of four classes of methods which are:

- Preliminary or Presumptive Test
- Crystal Tests
- Catalytic Tests
- Instrumental Methods

8.1.1 Preliminary or Presumptive Test

Preliminary test, also known as presumptive tests are quick, simple tests on evidence left at the crime scene to get a clue as to whether additional testing is needed. A presumptive test either proves that further analysis is not needed or indicates to confirm it thoroughly. Various presumptive tests for blood are as follows:

Adlers' or Benzidine Test: Adlers' test is the earliest test for blood which was developed in 1904 by *Oskar* and *Rudolf Adler*. The Adlers' test made use of benzidine (p-diaminodiphenyl), as it identifies the substance as blood by a characteristic oxidation reduction reaction.

Kastle-Meyer Test: The Kastle-Meyer reagent is made by mixing potassium hydroxide, phenolphthalein, and zinc dust. When hydrogen peroxide and the reagent are added to blood, the hemoglobin in the blood catalyses the conversion of phenolphthalein to its deep pink configuration.

8.1.2 Crystal Tests

The crystal tests, which are now rarely used, are all based on the formation of haemoglobin derivative crystals such as haematin, haemin and haemochromogen. Two common confirmatory tests on bloodstains are the Teichmann and Takayama tests which are as follows:

Teichmann Test: The Teichmann test is much older, having been developed or invented in 1853 by Polish anatomist *Ludwig Karl Teichmann*. In the Teichmann test, the reagent is a mixture of glacial acetic acid and sodium chloride. 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.)

Takayama Test: Probably the best known of the crystal tests was developed by Japanese criminologist *Masaeo Takayama* in 1912. An alkaline solution of pyridine is added to the stain and, if blood is present, salmon-pink color crystals of a complex between pyridine and haem form as the slide is warmed.

8.1.3 Catalytic Tests

These methods depend on the fact that the haem group of haemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide. The oxidizing species formed in this reaction can then react with a variety of substrates to produce a visible colour change. Among substrates in common use are benzidine and various substituted benzidines, ortho-tolidine, leucomalachite green, leucocrystal violet and phenolphthalein. The reaction with 3-aminophthalhydrazide (luminol) to form a luminescent rather than a coloured product is also a catalytic test. The catalytic tests are extremely sensitive (blood can be detected to dilutions of about 1 in 100,000 times), but are subject to a number of interferences and are therefore not totally specific for blood. Substances which can interfere include enzymes such as catalase and peroxidases (which can occur in both plant and animal materials), oxidising chemicals and metals – in particular copper and iron. But it doesn't mean it is useless in forensic field. It helps to identify and emerge the hidden evidence.

8.1.4 Instrumental Tests

The blood obtained from the crime scene is usually not in wet condition, it gets dried up with the passage of time. This dryness of blood results in rupture of red blood cells (RBCs) and it produces difficulties in analysis. However, to overcome the problem, methods are available for use on dried

blood samples.

The two most popular tests are:

- Absorption-inhibition test
- Absorption-elution test

Both tests take advantage of the tendency of antibodies to attach themselves to and agglutinate with antigens.

8.1.5 Individualisation of Blood Stains

The potential for the individualisation of blood is based on typing of proteins and enzymes. Blood proteins and enzymes have the quality polymorphism or of being iso-enzymes, which means they exist in several forms and variants. Generally people are familiar with at least one common polymorphism in blood. There are several methods available for individualisation of bloodstain; we will discuss here the important and economical methods.

8.1.5.1 Serum Protein Polymorphisms

A number of different proteins and enzymes are synthesised in the cells of a single organism. They each have their own distinctive properties and functions and together they define and control complex pattern of metabolic and developmental processes which characterise the species and the individual.

Proteins are composed of one or more polypeptide chains which are made up of the long strings of amino acids linked by polypeptide bonds in a specific linear order. 20 different amino acids may be present and typical polypeptide chains have sequence 100-500 amino acids long, so the number of possible structures is enormous. The sequence of base pair in a given gene determines the sequence of the amino acids in a corresponding polypeptide chain. Thus the structure and proteins of all individual are controlled by the base pair sequence of his genes.

The haptoglobin groups are polymorphic in all human populations; two alleles Hp^1 and Hp^2 accounting for three common phenotypes Hp 1-1, Hp 2-1, Hp 2-2.

8.1.5.2 Haptoglobin (Hp) System

Haptoblobin is a glycoprotein that combines with free haemoglobin from the lysed red cells, thus preventing its excretion by the kidneys. The haptoglobin groups are polymorphic in all human population. The haptoglobin (Hp) molecules consist of four chains $(\alpha\beta)_2$ linked with disulphide bonds. The HPα- chain is polymorphic with three common phenotypes Hp1-1 (HP1), Hp-2-1 (HP2, 1) and Hp 2-2 (HP2) that can be readily detected by conventional starch gel electrophoresis.

Technique:

Electrophoresis is performed using 10%-13% hydrolysed starch gel and following buffers: -

1) ***Tank buffers*** (Borate, pH 8.1)
   a) Boric acid = 18.55 gm
   b) Sodium hydroxide= 2.0 gm
   c) Dissolved in 1 litre distilled water
2) ***Gel buffer*** (Tris/ citrate pH 8.6)
   a) Tris = 9.20 gm
   b) Citric acid = 1.05 gm
   c) Dissolved in 1 litre distilled water
3) ***Sample application*****:** Mix one drop serum or plasma with little haemoglobin to make it pink (about 0.4 ml serum or plasma with 5µl haemoglobin) and load the mixture on Whatman No. 3 filter paper insert. Place inserts in gel 6 cm from cathode.
4) ***Electrophoresis:*** 6 V/cm for 3 to 4 hr.
5) ***Staining*** **:**

i) Benzidine staining method:

Prepare fresh Benzidine staining solution as follows:

a) Benzene =0.2 gm
b) Distilled water = 100 ml
c) Warm to dissolve benzidine, then add glacial acetic acid = 0.5 ml
d) Hydrogen peroxide (30%) = 0.2 ml

Drop the solution by pipette over sliced gel and note the results for haptoglobin (Hp) phenotypes.

ii) Dianisidine staining method:

a) *Staining buffer* (1.5 M Acetate buffer, ph 4.6)

Sodium acetate (anhydrous) 12.3 gm. dissolve in 50 ml distilled water and adjust pH to 4.6 with glacial acetic acid. Adjust final volume to 100 ml.

b) Stain:

O- Dianisidine = 35 mg

Ethanol = 35ml

Staining buffer = 35 ml

Distilled water = 80 ml

Hydrogen peroxide (add just after use) = 1 ml

Pour the stain on the sliced gel placed in a box. Cover the box and rock it in a few times. Leave until the brown bands are intense. Note the results for haptoglobin. The gels may be stored up to few weeks after washing in 2 % acetic acid and placing in 2.5% teepol.

6) ***Reaction Mechanism***:

Haptoglobin has the property of binding the haemoglobin and forming a very stable complex. The separation of haptoglobin types is determined by the size of the molecules of the haemoglobin- haptoglobin complex, which is in turn dependent on the type of the haptoglobin present.

The haeme group of the haemoglobin exhibits a peroxidase like activity which is capable of catalysing the breakdown of hydrogen peroxidase and thus liberating the nascent oxygen. The nascent oxygen thus liberated oxidises the leucobases, such as the benzidine sulphate into a rich blue-coloured benzidine salt. The proposed mechanism of the reaction is given blow:

Figure: 1 Reaction of Benzidine

The other half of the gel may be stained with amino black 10 B solutions for transferrin as follows:

a) Amino acid 10 B = 0.1 gm

b) Acetic acid = 10 ml

c) Methanol = 90 ml

After 1-2 min staining wash gel with methanol (90%) and acetic acid (10%) solution overnight. Note the results for transferrin (Tf) phenotypes.

8.1.5.3 Red Cell Enzyme Polymorphism

A variety of different enzymes and proteins are synthesised in the human body, and the primary amino acid sequence of each of their distinctive polypeptide chains is coded in the DNA of a separate gene locus. Thus there must be a large number of so-called “structural” gene loci in the genetic make-up of every individual. In addition, as a result of mutational events there may occur at any given gene locus a series of different alleles each of which determines a structurally distinct form of the particular polypeptide chain. Therefore the extent to which individual members of a population differ from one another in the structural characteristics of the enzymes and

proteins they synthesise will depend on the numbers of different alleles which are present at these different loci, and on the relative frequencies with which they occur.

Studies on a variety of different enzymes and proteins have led to the discovery of a large number of structurally variant forms which are genetically controlled. The inherited variants of different enzymes and proteins which we find among human populations today must be attributed to specific gene mutations which occurred in single individuals among our ancestors in earlier generations. Thus it appears that at almost all loci coding for enzymes and proteins a large number of different mutant alleles which may be generated by separate mutations; actually exist among living members of our species.

8.1.5.4 Enzyme Polymorphism

The concept of enzyme polymorphism is based on the occurrence of variant forms of an enzyme controlled by two or more alleles in frequencies exceeding 1 percent. Enzyme variants may originate from duplications and deletions of the genetic material, but most mutations leading to amino acid substitution may cause alterations in the electric charge, kinetics properties, and rate of synthesis or stability of the enzyme. Makert and Moller (1959) coined the term isozyme or isoenzyme to describe the existence of different molecular forms of an enzyme which catalysed the same biochemical reaction, but different in their electrophoretic properties.

The technique most commonly used to detect enzyme polymorphism is electrophoresis in starch gels, although agarose and polyacrylamide are also suitable gel media. The amount of material (usually blood) required is small and the technique is convenient for screening a large number of samples in a relatively short time period. Some important techniques are:

a) Adenylate Kinase (AK) System

Adenylate kinase, also called ATP: AMP phosphor transferase occurs in red cells as well as in muscles and other tissues. It catalyses the following reversible reaction.

Adenosine diphosphate $\longleftrightarrow$ Adenosine triphosphate + Adenosine monophosphate (2 ADP $\longleftrightarrow$ ATP + AMP)

Three different groups of isozymes of adenylate kinase – AK1, AK2 (Khoo and Russell, 1972) and AK3 (Wilson et al., 1976) determined by three separate gene loci AK1, AK2 andAK3 have been identified. Genetically determined polymorphism of AK1, isozymes in red cells was first demonstrated by Filders and Harris (1966) and three distinct phenotypes AK 1, AK 2-1, and AK 2 (AK1 1; AK1 2,1 and AK1 2) could be found by starch gel electrophoresis. They showed with the help of family and mother/child studies these to be genetically determined by two alleles AK1 (AK*1) and AK2 (AK*2). These results were confirmed by Harris 1969; Harris et al. and many more.

Technique:

Electrophoresis is performed using 10-12% hydrolysed starch gels and the following buffers:

1) ***Tank Buffer*** (pH 7) :

Citric acid 86.16 g

Dissolve in about ½ 1 distilled water and leave in refrigerator for about 2 hrs. Adjust pH to 7 with strong NaOH solution. Make up the total volume to 1 liter with distilled water.

2) ***Gel Buffer*** (pH 7) :

L-Histidinemonohydrochloride 1.05 gm.

Dissolve in about 50 ml distilled water and adjust pH to 7 with NaOH solution. Make up the total volume to 100 ml with distilled water. Dilute 1:10 before use.

3) ***Sample Application*** :

Apply haemolysates on Whatman No. 3 filter paper inserts at center of the gel.

4) ***Electrophoresis :*** 5 V/cm for 16 to 18 hrs. at 40˚C (till haemoglobin runs towards cathode about 4.5 cm from the origin). After electrophoresis, cut the gel at 4 cm from the origin towards anodic end and 8 cm towards cathodic end.

5) ***Staining :***

(i) **Staining Buffer** (0.1 M, pH 8.0):

Tris- 1.2 gm

Dissolve in about 50 ml distilled water and adjust pH to 8.0 with 1 N HCl. Make up the total volume to 100 ml with distilled water.

(ii) **Agar Gel :**

Dissolve 200 mg agar in 22 ml staining buffer by heating till it is clear and then cool it to about 550˚C.

Staining Mixture

Glucose	40 mg
Magnesium chloride	82 mg
Adenosine-5 – diphosphate	10 mg
ADP (disodium-salt)	
NADP	7 mg
MTT	2.5 mg
PMS	2.5 mg
Glucose-6-phosphate dehydrogenase (G6PD)	10 µl
Hexokinase	2 µl

Dissolve in 5 ml staining buffer, mix with agar gel, and pour it immediately over the sliced starch gel. When overlay is set incubate the gel in darkness at 370˚C for 30 min. Read the result for AK.

6) ***Reaction Mechanism :***

AK converts ADP into ATP and AMP, and since glucose and hexokinase are present in the reaction mixture, glucose-6-phosphate is formed with the regeneration of ADP. In the presence of G6PD and coenzyme NADP, glucose-6-phosphate thus formed is oxidised to 6-phosphogluconate and during this reaction NADP is reduced to NADPH. NADPH in the presence of PMS, in turn reduces yellow MTT to purple formazan which is thus deposited at the site of AK activity. Therefore AK isozymes are seen as purple band against a yellow background.

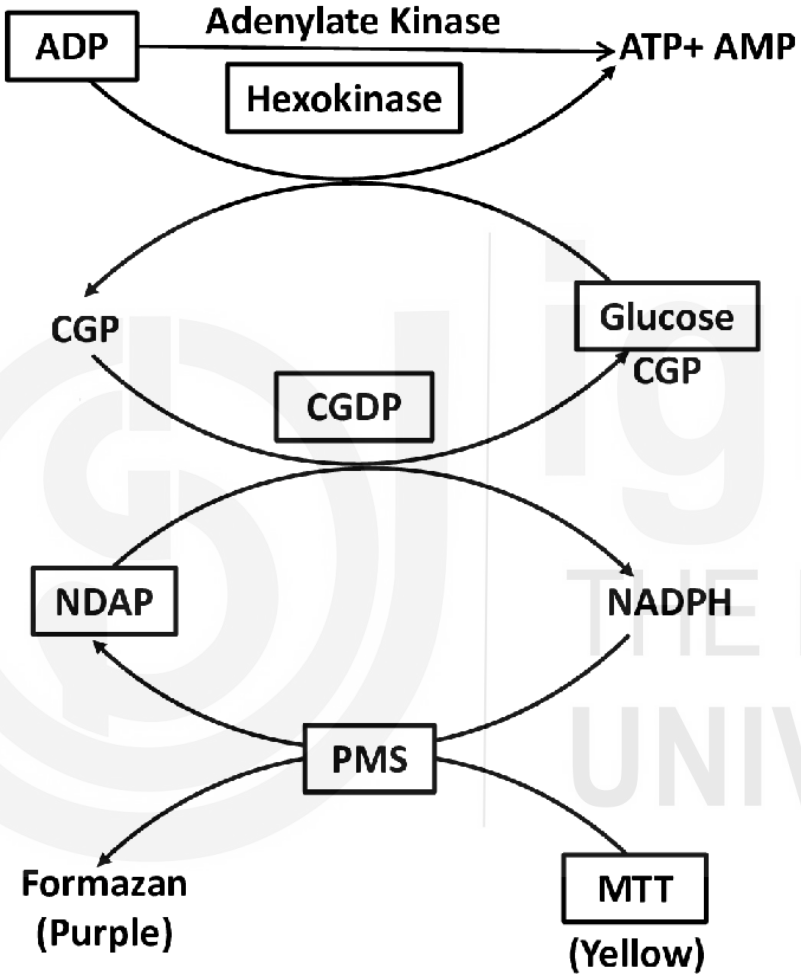


Figure: 2 AK isozymes-sequence of biochemical reactions involved.

b) Adenosine Deaminase (ADA) System

Adenosine deaminase shows an electrophoretic polymorphism genetically determined by the occurrence of two common alleles ADA1 (ADA*1) and ADA2 (ADA*2) at an autosomal locus; correspondingly there are 3 phenotypes: ADA 1, ADA 2 and ADA 2-1 The ADA1 and ADA2 alleles have been observed with frequencies of about 95 per cent and 5 per cent, respectively in most European populations, ADA2 is relatively less frequent (approximately 2 per cent) in Africans (Blacks), but quite frequent (approximately 12 per cent) in Indians (Bhasin and Fuhrmann, 1972a). The

presence of high ADA2 allele frequency in India suggests that perhaps this allele is of local origin proliferating in the region on account of some selective advantage.

The rare ADA alleles encountered in Indian populations include ADA3 among Jats of Punjab (Singh et al., 1974); ADA4 among Muslims of Delhi (Papiha et al., 1976) and Ghanchi and Anavil of Gujarat (Papiha et al., 1981); ADA6 among Vadabalijas of Orissa (Reddy et al., 1989)

Technique:

Electrophoresis is performed using 10-12% hydrolysed starch gels and the following buffers:

1) ***Tank buffer*** (pH 5.0) :

Citric acid 21gm

Dissolve in about ½ l distilled water and adjust pH to 5 with NaOH. Make up to 1l.

2) ***Gel Buffer*** (pH 5.0) :

Use 1:20 dilution of tank buffer.

3) ***Sample Application***: Haemolysates are loaded on Whatman No. 17 filter paper inserts which are placed in the middle of the gel, about 10 cm from cathode end.

4) ***Electrophoresis:*** 5-7 V/cm for 16 to 18 h at 4˚C (till haemoglobin runs towards cathode about 3-4 cm from origin).

5) ***Staining :***

(i) **Staining Buffer** (0.06M Tris, pH 8.0) :

Tris 727 mg

Dissolve in 50 ml distilled water, and adjust pH to 8 with dilute HCl. Adjust total volume to 100 ml with distilled water.

(ii) Agar Gel

Dissolve 200 mg agar in 22 ml staining buffer by heating till it is clear and then let it cool to about 55˚C

Staining Mixture:

Adenosine	10 mg
Sodium arsenate	40 mg
MTT	2.5 mg
PMS	2.5 mg
Xanthine Oxidase (XOD)	10 µl
Nucleoside Phosphorylase (NP)	10 µl

Dissolve stain in 5 ml staining buffer, mix with agar gel and immediately pour it over the sliced starch gel. Keep it in dark and when overlay is set incubate in darkness at 37˚C for 45-60 min. interprets the results for ADA.

6) ***Reaction Mechanism:***

Adenosine is converted to inosine by ADA at the sites of enzyme activity and this in turn is converted to hypoxanthine in the presence of nucleoside phosphorylase and arsenate. Hypoxanthine is then oxidized by the action of xanthine oxidase, and during this reaction MTT in the presence of phenazinemethosulphate (PMS) is reduced to form purple insoluble formazan. The ADA isozymes are therefore seen as purple bands against a yellow background. The sequence of biochemical reactions involved is given below:

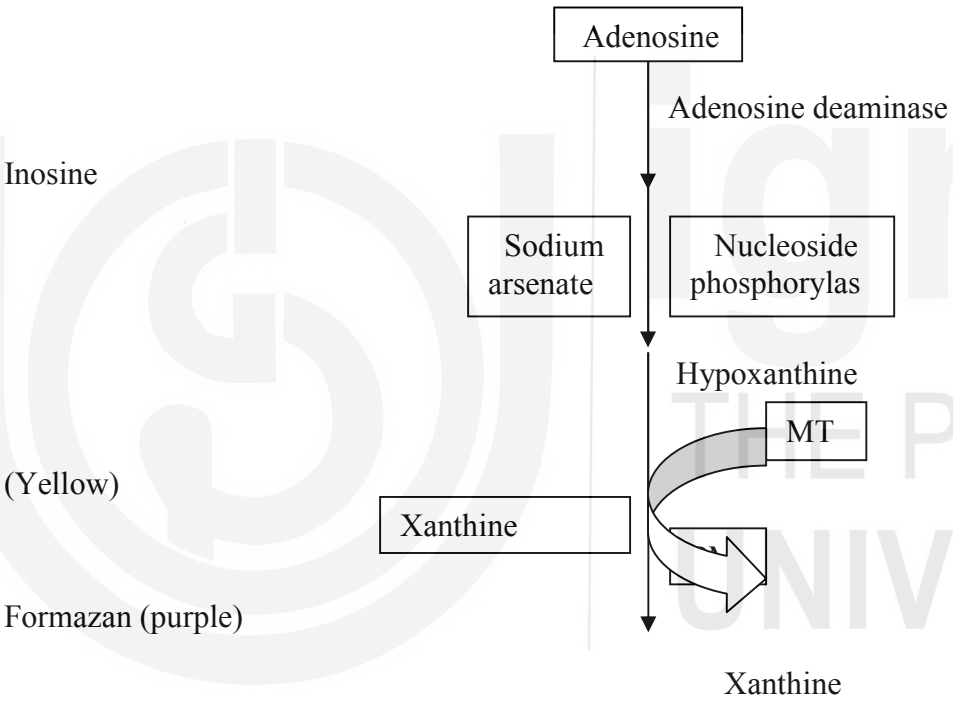


Figure: 3 ADA isozymes show the changes of color band in biochemical reaction.

Check Your Progress 1

1) How will you identify whether the fluid is blood or not?

……

……

……

……

……

……

……

2) Explain common confirmatory tests on bloodstains.

……

……

……

……

……

3) Discuss briefly individualisation of blood stains.

……

……

……

……

……

8.2 SALIVA

Saliva is a viscid, watery fluid, secreted into the mouth by the salivary glands that functions in the tasting, chewing, and swallowing of food, moistens the mouth, and starts the digestion of starches.

8.2.1 Composition

Salivary fluid is an exocrine secretion consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulin's and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health.

There are also glucose and nitrogenous products, such as urea and ammonia. The components interact and are responsible for the various functions attributed to saliva.

Total or whole saliva refers to the complex mixture of fluids from the salivary glands, the gingival fold, oral mucosa transudate, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products. At rest, without exogenous or pharmacological stimulation, there is a small, continuous salivary flow (SF). Whereas, stimulated saliva is produced in the face of some mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around 80% to 90% of daily salivary production. A healthy person's mean daily saliva production ranges from 1 to 1.5L.

8.2.2 Collection of Salivary Stains

A. Questioned Samples

❖ **Dry Stains:**

a) **Swabbing**: This method is best to apply on non-absorbent surfaces. Lightly moisten sterile swab with distilled or sterile water is rubbed over stain while rotating. Swab is then allowed to air dry. Combination of first moistened swabbing followed by second dry swabbing is recommended. Both swabs are examinee in laboratory.

b) **Cutting:** Stained portion should be cut from item.

c) **Scraping:** Stained portion is scrubbed into clean piece of paper using clean blade.

d) **Lifting:** Works for non-absorbent surface; use fingerprint lifting tape that does not interfere with DNA testing to lift stain; lifted stain should be covered with a piece of lifter's cover.

❖ **Wet Stains**

a) **Swab:** Sample should be absorbed onto sterile cotton swabs. Stain should be concentrated on tip and allowed to air dry.

b) FTA paper: Sterile disposable pipetteshould be used to collect liquid saliva. It should be spotted on FTA paper and then allowed to air dry.

B. Reference Saliva (Buccal Samples)

a) **Swab:** Swabs should be placed inside of cheek using two swabs. Swabs should be kept on rotating during collection. Swabs are then allowed to air dry.

b) **Filter paper:** Subject's saliva sample should be placed on marked area of filter paper and allowed to air dry.

8.2.3 Examination of Saliva

After collection of saliva sample we perform following test for examination of saliva sample

a) Starch- Iodine Test:

Reagents Preparation:

1) 0.5% soluble starch solution (50 mg soluble starch / 10 ml H_2O).

2) Lugol's iodine solution.

Procedure:

1) Place 3 tubes in a rack and add the following:

a) In the first tube, place a 5 mm x 5 mm piece of sample to be tested.

b) In the second tube, place a similar sized unstained control piece.

c) In the third tube, place a 5 mm x 5 mm piece of a known saliva stain.

2) Add 3 drops of soluble starch solution to each tube.

3) Mix, cork and incubate the tubes for 1 hour at 37°C.

4) Add 2 drops of Lugol's iodine and note the colour formed.

5) A dark blue starch-iodine complex should be observed in the second and fourth tubes. The absence of the dark blue colour indicates that the starch has been hydrolyzed and is no longer available for complexing. Therefore, the lack of blue colour is a positive result for amylase activity, indicative of the presence of saliva. This is not a confirmatory test for saliva.

Notes:

- To dissolve the starch, heat the solution to a boil. Allow solution to cool to room temperature. Always use soluble starch. Do not use hydrolyzed starch.
- Starch solution must be prepared fresh daily. Never use starch solution older than 24 hours.

Check Your Progress 2

4) What is the composition of Saliva?

……

……

……

5) How to collect the Salivary Stains from crime scene?

……

……

……

……

6) Discuss starch- iodine test.

……

……

……

……

……

8.3 URINE

The fluid and dissolved waste substance excreted by the kidneys constitute urine. Urine is a liquid byproduct of the body secreted by the kidneys through process called urination and excreted through urethra.

8.3.1 Physical Properties

Urine is a transparent, yellowish fluid; shade however, depends on its concentration. Its color is due to pigment Urochrome derived from the breakdown of hemoglobin from the worn out RBCs. It is hypertonic to blood plasma. Its specific gravity ranges between 1.003 & 1.04 and pH ranges from 5 to 8.

Urine has a characteristic unpleasant odour. If allowed to stand, urea is degraded by bacteria to ammonia which imparts a strong smell to urine.

8.3.2 Chemical Composition

Urine consists of water, organic and inorganic substances.Water alone forms about 95% of it and other substances form only 5%. The organic substances are mainly nitrogenous compounds but small amounts of non-nitrogenous compounds are also present.

Nitrogenous organic compounds include urea, uric acid, creatinine and hippuric acid. Out of all these, urea is the principle component of human urine. Non-nitrogenous organic compounds include Vitamin C, oxalic acid, phenolic substances and traces of glucose.

The inorganic substances include ammonia & mineral salts such as chlorides, sulphates and phosphates of sodium, potassium, calcium & magnesium. Sodium chloride is the principal mineral salt of the urine.

Urine also contains some other substances like pigments & drugs coupled with some epithelial cells and leucocytes.

8.3.3 Collection of Urine Samples

Urine found at the scene of crime can be removed by dropper or gauge and placed in a sterile test tube or other container. If the sample is present on cloth, entire article should be submitted.

8.3.4 Examination of Urine Stains

1) **Physical Examination-** A suspected urine stain may fluoresce pale yellow or pale blue, when viewed under long and short wave Ultraviolet light. Safety glasses, which absorb UV radiation, must be worn when viewing material for fluorescence.

2) **Odour test-**The characteristic odour of urine may be detected by placing a small sample of the stain in a test tube and heating it gently over flame. Avoid scorching the test material.

3) **Urea Nitrate Crystal test-** An aqueous extract of stain is made and a thin film of it is made on a microscopic slide. Add one drop of concentrated. Nitric Acid (HNO_3) and cover. In presence of urea, hexagonal stacked crystals of urea nitrate are formed.

4) **Gee's method for detection of Urea**- A portion of the suspected stain is extracted with acetone and the acetone extract is concentrated by evaporation. The concentrated extract is filtered and evaporated to dryness. A few drops of acetone is added to the residue and stirred with a narrow glass rod. A drop of the solution thus prepared is taken on a microscopic slide and again allowed to evaporate. At this stage separation of urea crystals (long colorless 4 or 6 sided rhombic prisms) may be observed. A glass rod dipped in concentrated nitric acid is lightly run across the surface of the crystal line area. Formation of characteristic urea nitrate crystals (colorless rhombic or 6 sides tiles) may be observed under microscope indicating the presence of urea in the suspected stain.

5) **Detection of Creatinine-** A concentrate of the stain is placed on a strip of chromatographic paper and it is treated with 2N sodium hydroxide solution and after it one drop of 5% picric acid is added to it. The development of red-orange color indicates the presence of creatinine in the stain.

6) **Indican test-** This test though not always positive, may be undertaken for the identification of urine in addition to the above tests. 1ml of resorcinol reagent (1gm resorcinol in 20ml of ethyl alcohol) is added to a small quantity of aqueous extract of the stain followed by the addition of 1ml of cupric bromide. The mixture is extracted with amylacetate. Red coloration of the extract indicates the presence of indican (a potassium salt).

8.3.5 Determination of Species

Determination of origin of urine stain by precipitin method has not been found satisfactory. However, Popielski, Brzecka and Szczecin (1963) described a method of concentrating urinary proteins and thereafter subjecting that concentrated urine proteins to precipitin test by agar gel double diffusion method for the determination of species.

8.3.6 ABO Blood Grouping

ABO Blood grouping of urine stains can yield useful results when absorption-elution test is employed.

8.3.7 Individualisation from Urine Samples

The use of biological samples and bodily fluids as sources of DNA for identification has been thoroughly investigated and documented. Urine however, has not been heavily studied as a potential source of DNA for forensic identification purposes.

Urine is not considered an ideal source of DNA due to low concentration of nucleated cells present in human urine. The nucleated cells found in urine are typically white blood cells and epithelial cells.

Check Your Progress 3

7) What is the Chemical composition of urine?

……

……

……

……

……

8) Describe the Test methods for urine samples.

……

……

……

……

……

9) How will you determine the Species or origin**?**

……

……

……

……

……

8.4 SEMEN

Seminal fluid is a complex minute of glandular secretion. Seminal fluid accounts for approximately 60% of the ejaculate.

Seminal fluid contains the "flavin" that causes semen to fluorescence under the ultra-violet light.

Prostatic fluid accounts for approximately 30% the ejaculation. This portion of the semen contains high concentration of acid phosphatase (AP) and prostate specific antigen (PSA). Both are very useful to the forensic analyst for detection of semen in case of rape, unnatural offence, hanging, etc. It is an alkaline fluid with pH=7.4.

A vasectomy is the surgical removal of a bilateral segment of the ducts deferens. Aspermia is the condition that causes males to produce no spermatozoa. However, seminal fluid continues to secrete.

8.4.1 Biological Characters

A typical ejaculation releases 2-5 ml of semen that contains seminal fluid and sperm cells i.e. spermatozoa. A normal sperm count ranges from $10^7 – 10^8$ spermatozoa per millilitres of semen.

Each testis is composed of numerous coiled seminiferous tubules in which the spermatozoa are found from spermatogonia. The process of generating spermatogonia is referred to as spermatogenesis.

The spermatozoa are then transported and stored at the tubular network of the epdidymis where they undergo functional maturation. Then through epdidymis it transfers to ejaculatory ducts. Then spermatozoa then follow the ejaculatory duct into the prostatic urethra where they are joined with secretion of prostate.

8.4.2 Structure of Spermatozoa

Human spermatozoa have three distinct regions:

1) Head
2) Middle piece
3) Tail

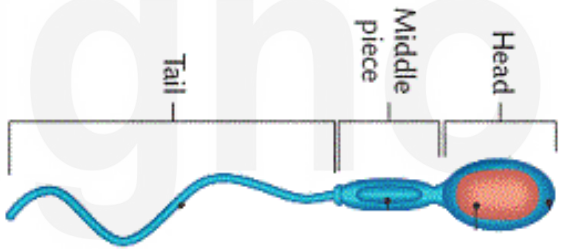


Figure 4: Structure of Spermatozoa
Source: https://brainly.in/question/38704167

The head is shaped like flattened ellipse and contains a nucleus with densely packed chromosomes. At the tip of the head is the acrosomal cap, a membranous compartment containing enzyme essential for fertilization. The neck is also observed in some type of spermatozoans. They consist of centrioles one proximal centriole and other distal centriole.

The middle piece: consists of apical part of the apical filament surrounded by a tightly coiled spiral sheath of elongated mitochondria. The mitochondria contain oxidative enzymes and provide energy for sperm motility. The tail or flagellum consists of a central axial filament, thin layer of cytoplasm and an outer smooth plasma membrane. It is 45-50 μ in length.

8.4.3 Enzymes and Proteins Present in Seminal Fluid

8.4.3.1 Acid Phosphates (AP)

It consists of a group of phosphates with optimal activity is an acidic pH environment. The greatest forensic importance of AP is that the prostate is the most abundant source of AP and contributes most of the activity present in semen. AP levels in semen are not affected by vasectomies.

8.4.3.2 Prostate Specific Antigen (PSA)

PSA is a major protein present in seminal fluid at concentration of 0.5-2.0 mg/ml. PSA is produced in the prostate epithelium and secreted into the semen.

PSA is a protein that has a molecular weight of 30 KDa and is thus also known as P30. It is responsible for hydrolyzingsemenogelin (Sg), which mediates gel formation in semen.

PSA is the member of the tissue kalikrein family and is coded by the KLK3 locus located on chromosome 9. In addition to PSA, outer tissue kalkarines encoded by KLAk 2 and KLK4 loci.

The half-life for PSA in a dried semen stain is about 3 years at 37^0C. Wet conditions may reduce half-life.

8.4.4 Examination of Semen and Seminal Stains

8.4.4.1 Physical Examination:

Colour: Thick, yellowish white, glairy, opalescent, secretion having a characteristic odor known as seminal odor.

Texture: On touch, seminal stains are starchy.

Appearance: Garments sent for forensic examination are usually dirty having variety of stains, in natural light some stains are reddish coloured, while others are brown, yellow or faint grey in colour. These are often mixed with stains of blood vaginal discharge, urine and semen, so as to restrict the investigation to seminal stains only, preliminary examination is done under filtered UV light. The fluorescence of the seminal stains is of a bluish white colour and such stains should be selected for further examination.

8.4.4.2 Presumptive Test:

Acid Phosphate Test: Sodium alpha-naphthyl phosphate method

Reagent Preparations: **Buffer**	
Glacial Acetic acid	1 ml
Sodium acetate anhydrous	2 g
Distilled water	100 ml
Step - 1 Reagent	
Buffer	50 ml
Sodium alpha-naphthyl Phosphate, 0.25% (w/v)	126mg
Step - 2 Reagent	
Buffer	50 ml
Naphthanildiazo blue B, 0.5% (w/v)	250mg

Step 1 Reagent and Step 2 Reagent can be made up in bulk and aliquoted into test tubes and frozen. When needed, one tube of each reagent can be thawed under warm running water for use.

Procedure:

1) Place a small piece (2 x 2 mm) of suspected seminar stain material on Whatman filter paper or other suitable test paper. Use proper standards and controls including positive, negative and unstained controls; see below.

2) Add 1-2 drops of Step 1 Reagent and allow to react for 30 seconds. (No colour should develop at this stage).

3) Add 1 drop of Step 2 Reagent. Record the result after 10 seconds.

4) A positive reaction is recorded upon rapid development of a purple colour, which is indicative of semen. This is not a confirmatory test for semen.

8.4.4.3 Confirmatory Test: Microscopic Examination

Upon obtaining a positive preliminary test for acid phosphates, the suspected stain can be extracted as follows:

1) Cut a small portion (1 cm^2 maximum) of the stain and place in a test tube.

2) Add a few drops of acidulated (slightly acidic) water to cover the stain.

3) Agitate the stain on a vortex, or in an ultrasonic cleaner bath or manually, by flicking the tube. This will aid in freeing the spermatozoa from the dried stain.

4) When the solution is cloudy, withdraw the liquid with a pipette and place into a disposable 400ul plastic centrifuge tube and centrifuge in a microfuge for 30 seconds.

5) Carefully withdraw the supernatant, which contains soluble group-specific substances, enzymes and other solutes from seminal plasma.

6) Collect the button of cellular and other insoluble components from the tube and place on a clean-labeled microscope slide.

7) Fix in dilute H_2SO_4 acid and dry. It is now ready for staining.

TANK BUFFER (pH 9.1)	Tris	0.208 M	25.2 g.
	EDTA (free acid)	0.0086 M	2.5 g.
	Boric acid	0.0307 M	1.9 g.
	Distilled water		1 L
GEL BUFFER (pH 9.1)	Same as tank buffer		

SUPPORT MEDIUM	1% AGAROSE, mr = 0.25, E25 or Sigma Type II.	
AMOUNT REQUIRED	3.5 ml for a 1" x 3" slide / 7 ml for a 2" x 3" slide	
2% STOCK AGAROSE	10 grams of agarose is added to 500 ml distilled water in a large flask. Heat to boiling on a constant stirring hot plate until all the agarose is dissolved. Pour into a plastic sandwich box and store upside down in the refrigerator. Blocks of 2% stock will be weighed out for later use.	
1% AGAROSE	50 grams of 2% stock agarose 50 ml of gel buffer	Melt stock agarose and gel buffer together and pour 3.5 ml quantities into 10 x 75 test tubes. Allow to cool, cork and store at $4^{o}C$.
TEMPERATURE &CONDITIONS	Room Temperature.	
VOLTAGE AND DURATION	**DAY:** 120V for 30 minutes (approximately 30 mA for 1x 3" slide)	
STAIN (0.1% AMIDO BLACK)	Naphthalene Black 10B Methanol Glacial acetic acid Distilled water	1.2g 1 L 200 ml 1 L
DESTAIN SOLUTION	Methanol Glacial acetic acid Distilled water	1 L 200 ml 1 L
CONTROLS	Liquid semen diluted 1:50, 1:100, 1:200 with saline. Extract of unstained control (whenever possible to obtain). Saline black run against anti-P30. Cell free extract of questioned stain run against saline instead of anti-P30.	

Check Your Progress 4

10) What is the forensic significance of semen found in crime scene?

……

……

……

……

11) Explain the test for semen identification.

……

……

……

……

……

12) Describe the Structure of Spermatozoa.

……

……

……

……

8.5 PATTERNS OF BLOODSTAINS

Bloodstain patterns are defined as a deposition of blood on a surface or by a surface contacting blood is known as bloodstain. And a grouping or spreading of bloodstains that indicates through regular or repetitive form, order, or arrangement the manner in which the pattern was deposited is known as bloodstain pattern. Bloodstains are frequently found at various types of crime scene, like homicide, assault, robbery, child abuse, rape, etc. In addition, forensic scientists are often called upon to examine clothing, weapons, vehicles and other physical evidences to identify whether a victim's or a suspect's blood has been transferred or not to those items. We come to know that the bloodstain pattern analysts can examine the blood evidence left behind and draw conclusions as to how the blood may have been shed. From what may appear to be a random bloodstains distribution at the crime scene. The analysts can categorize the stains by gathering information from spatter patterns, transfers, voids and other impressions that can help to investigators in reconstruct the sequence of events that occurred after bloodshed.

Figure 6: Blood spatter patterns appear on a wall

Source: Courtesy of National Forensic Science Technology Center (NFSTC), Largo, Florida

Bloodstain pattern analysis (BPA) is the interpretation of bloodstains at a crime scene in order to recreate the actions that caused the bloodshed. The analysts can examine the size, shape, distribution and location of the bloodstains patterns and can be confirm or refute assumptions concerning events and their sequence.

BPA uses principles of biology (behaviour of blood), physics (cohesion, capillary action and velocity) and mathematics (geometry, distance, and angle) to assist investigators in answering questions such as:

- Where did the blood come from?
- What caused the wounds?
- From what direction was the victim wounded?
- How were the victim(s) and perpetrator(s) positioned?
- What movements were made after the bloodshed?
- How many potential perpetrators were present?
- Does the bloodstain evidence support or refute witness statements?

8.6.1 Types of Blood Stains

The bloodstains and patterns are classified on the basis of their physical features like size, shape, location, concentration and distribution, etc. There is a variety of way to classify bloodstain patterns, but the aim of classification is the overall analysis. Classification sets the steps for the analyst to define more efficiently the origin of any given stain.

As we discussed earlier many texts are classified bloodstain as Low, Medium and High velocity spatter based upon their size distribution and hence the force necessary for their production. Bloodstains are classified based on their appearance and mechanism of transfer onto a surface. It was also classified into three basic types: passive stains, transfer stains and projected or impact stains.

Passive Stains

Passive stains include drops, flow, splashes and pools, was typically resulting from gravity acting on an injured body. These stains are subdivided into:

a) **Drops**– These stains occur when blood drips passively under the influence of gravity. They may occur singly, in clusters or provide a trail and thereby indicate movement and its direction. However, direction may be difficult to determine if the victim moves slowly and therefore the drops fall at 90 degree resulting in the stains being more or less oval. A classic situation is when you cut yourself and leave a drip trail from the place where the accident occurred to the nearest washbasin..

Figure: 6.1 Single drop of blood on a wooden floor

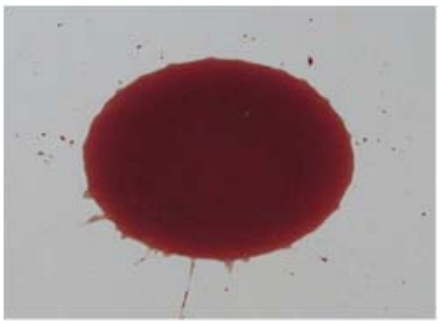


Figure 6.2 Bloodstain from single drop of blood.

Source: Courtesy of John Black, Ron Smith & Associates

b) Flows-These occur when blood flows passively to produce small rivers or streams of blood. These can be useful for determining orientation at the time of attack or movement after death. For example, if a person is stabbed in the chest whilst standing a trickle of blood will head vertically down the body. If, however, the person was lying on the floor the flow will veer to whichever side of the body is angled to the floor.

Figure 6.3: Showing the blood flow on surface

***Source*:** http://www.knoxforensics.com/shoot.php

c) Pooling - A bloodstain resulting from an accumulation of liquid blood on a surface.

Figure: 6.4. Pooling bloodstain from surface

***Source*:** https://www.kindpng.com/imgv/hbJTwwi_blood-clip-art-bloodstain-blood-stain-transparent-png/

Transfer Stains:

Transfer result from objects coming into contact with existing bloodstains and leaving wipes, swipes or pattern transfers behind such as a bloody shoe print or a smear from a body being dragged. These stains are subdivided into:

a) Wipe Pattern -An altered bloodstain pattern resulting from an object moving through a pre-existing wet bloodstain.

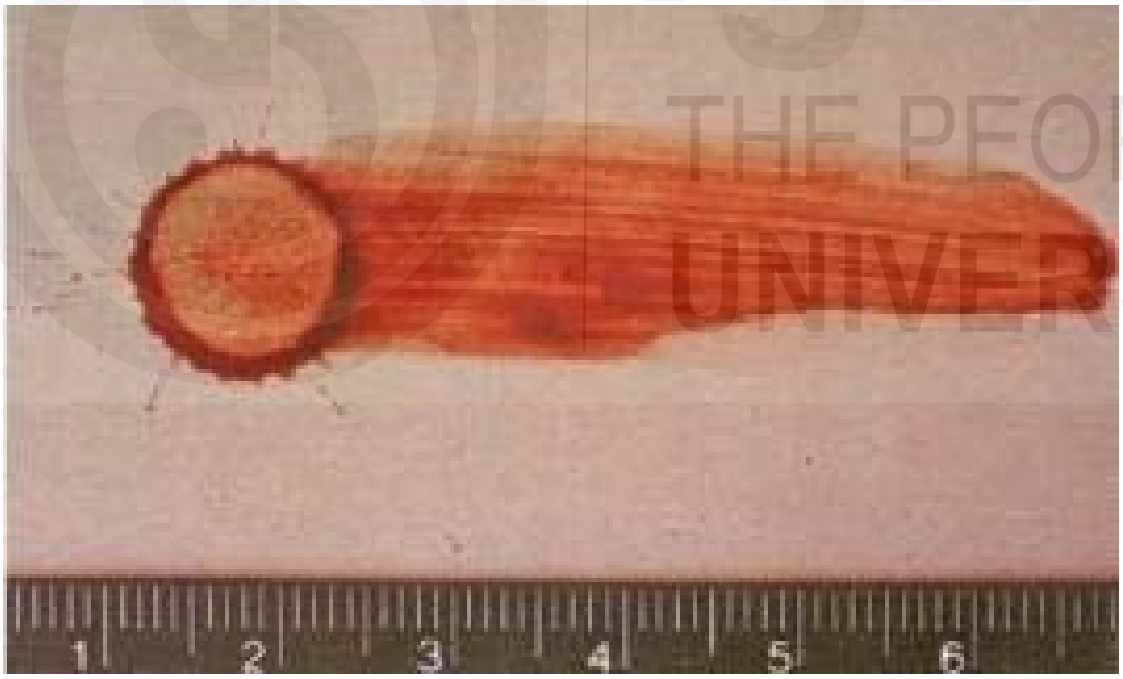


Figure :6.5. Numerous wipe pattern created by the victim feet moving in pre-existing blood pool.

***Source*:**https://www.chegg.com/flashcards/forensics-final-blood-spatter-anaylisis

b) Swipe Pattern - A bloodstain pattern resulting from the transfer of blood from a blood-bearing surface onto another surface, with characteristics that indicate relative motion between the two surfaces.

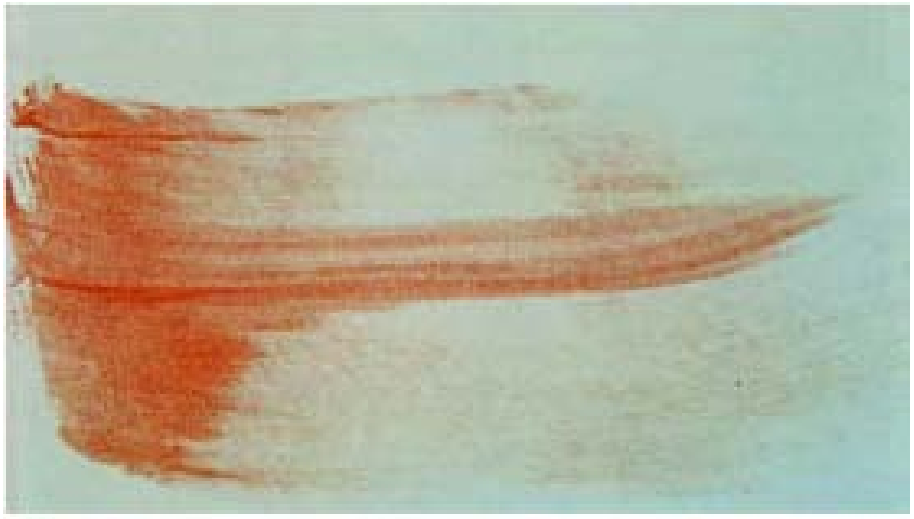


Figure 6.6.Swipe pattern created by bloody hairs.
***Source*:** https://www.chegg.com/flashcards/forensics-final-blood-spatter-anaylisis

c) Transfer Stain - These occur when wet blood is transferred from one object to another. For ex., when a bloody knife is wiped onto clothing, a bloody hand leaves prints on doorknobs or weapons and when a person stepping in blood leaves behind impressions of their footwear.

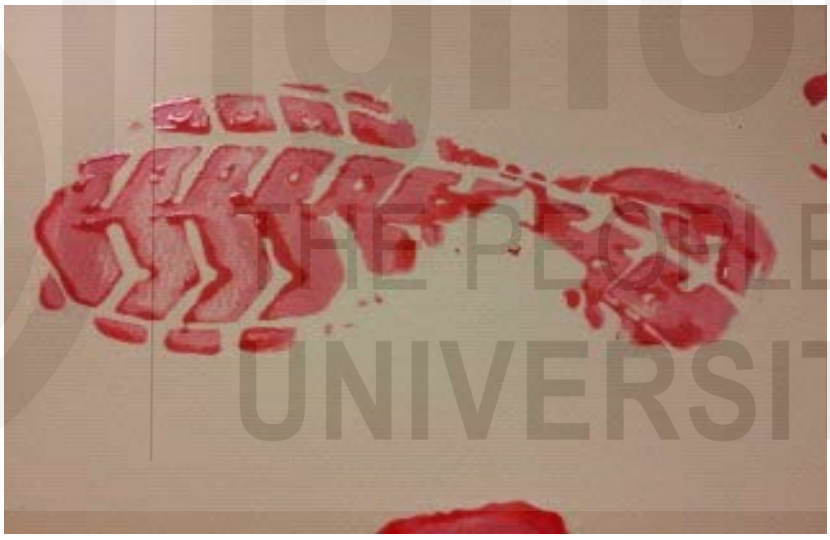


Figure 6.7.Transfer bloodstain made by footwear.
Source: Courtesy of John Black, Ron Smith & Associates

Projected or Impact Stains

These stains are created when a blood source is subjected to an external force greater than gravity. Impact stains result from blood projecting through the air and are usually seen as spatter, but may also include gushes, splashes and arterial spray.

Blood spatter is categorized as impact spatter (created when a force is applied to a liquid blood source) or projection spatter (caused by arterial spurting, expirated spray or spatter cast off an object). The characteristics of blood spatter depend on the speed at which the blood leaves the body and the type of force applied to the blood source.These stains are subdivided into:

a) **Cast-off -** This results when an object swung in an arc flings blood onto nearby surfaces. This occurs when an assailant swings the bloodstained object back before inflicting another blow. Analysts can tell the direction of the impacting object by the shape of the spatter (tails point in the direction of motion).

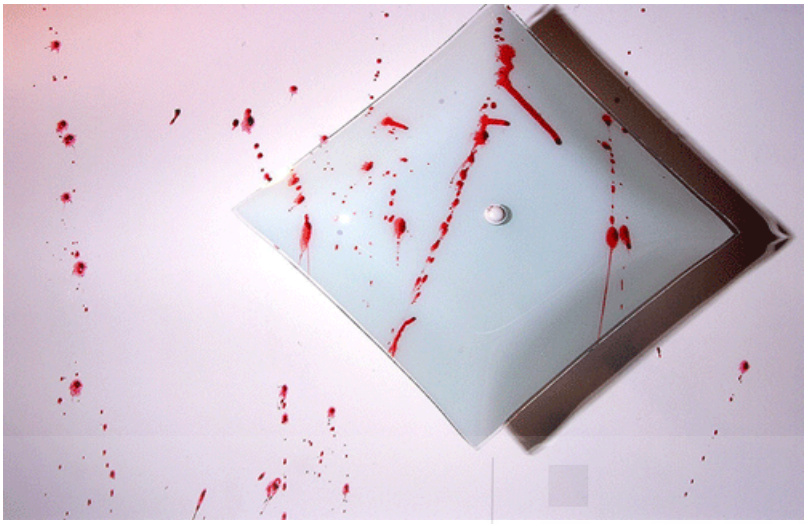


Figure 6.8: Cast-off blood stains on the celling caused by blunt force instrument
Source: https://www.practicalhomicide.com/Research/LOmar2007-2.htm

b) **Expirated spatter** – It is usually caused by blood from an internal injury mixing with air from the lungs being expelled through the nose, mouth or an injury to the airways or lungs. Expirated spatter tends to form a very fine mist due to the pressure exerted by the lungs moving air out of the body. Small air bubbles in the drops of blood are typically found in this type of spatter.

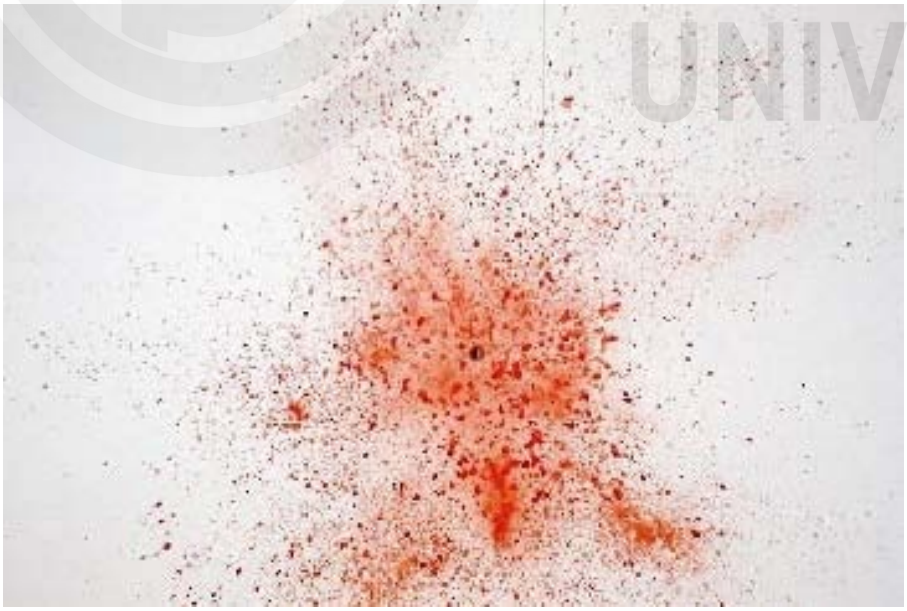


Fig.6.9.Expirated bloodstain caused by mouth injury
Source:https://static1.squarespace.com/static/55dfd21ee4b0718764fb34cc/t/56de10882b8dde6e3795ef39/1457393809358/Bloodstain+Pattern+Analysis.pdf

c) **Arterial spray** – It refers to the spurt of blood released when a major artery is severed. The blood is propelled out of the breached blood vessel

by the pumping of the heart and often forms an arcing pattern consisting of large, individual stains, with a new pattern created for each time the heart pumps.

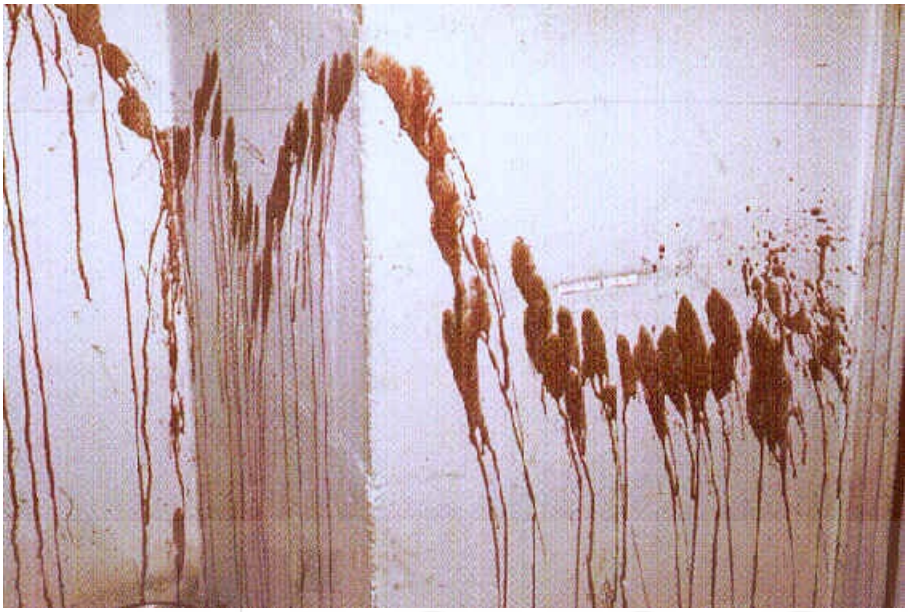


Figure: 6.10.arterial bloodstain on wall surface

Source: https://www.journalijdr.com/sites/default/files/issue-pdf/7128.pdf

d) Impact Pattern -Impact spatter is generated by the application of some force to a blood source resulting in the random dispersion of smaller drops of blood. The blood source may be on the skin's surface, as in the case of a beating, or it may be exposed at the moment of impact, as in the case of a gunshot injury. The resulting impact pattern may consist of thousands of bloodstains.

Figure: 6.11.impact pattern develop in wall surface.

Source: https://www.thinglink.com

e) Splash Pattern - Splash patterns occur when a volume of blood is projected into a scene with minimal force characterised by a large central stain exhibiting minimal distortion. There will be very little satellite

spatter present. They most often appear as large-volume patterns.

Figure 6.12: Splash pattern created on surface.

Source: https://reekoscience.com/science-experiments/miscellaneous/splatter-blood-for-blood-spatter-analysis

Check Your Progress 5

13) Define blood stain and its patterns.

……

……

……

……

……

14) Discuss different type of blood stain.

……

……

……

……

……

15) What information you can get from bloodstain pattern analysis.

……

……

……

……

8.7 SUMMARY

Identification of body fluids is one of the most important components of Forensic Science as it plays a vital role in criminal investigation. Identification and individualisation of a criminal can also be done by the body fluids present on the crime scene such as Blood, semen, saliva, urine, sweat etc. Chemical test can confirm that the blood found is human or not, further it can be analyses the species of origin.

Bloodstain identification can be done by using various sensitive methods. Individualisation of bloodstain can be done by serum protein and Enzyme polymorphisms. Biological evidence can facilitate individualisation of criminals with the help of advanced DNA profiling techniques. Semen or seminal fluid is a body fluid secreted by gonads of male during process of ejaculation. It is one of the major evidence found in the criminal cases related to sexual offences such as rape, sodomy etc. In microscopic examination the identification of spermatozoa is a confirmatory evidence for the presence of semen in a suspected stain.

Saliva, a watery fluid secreted into mouth can be encountered in the investigation of criminal cases like sexual offences etc. Urine is a liquid byproduct of body secreted by the kidneys through the process called urination. It generally consists of water, organic and inorganic substances. Sweat is a transparent bio-fluid with low toxicity and slightly acidic in nature with pH. Examination of sweat stains is usually required in forensic practice in order to determine the blood group of an individual from his wearing apparel. Bloodstain patterns can reveal a great deal about position and movement during the crime. It can link a victim to suspect.

8.8 REFERENCES

Bhasin, M.K., Fuhramann, W., (1972) Geographic and ethnic distribution of some red cell enzymes. *Humangenetik. Vo.. 14*, Pp. 204-223.

Chattergee, C.C. (1951) *Human Physiology, Vol. –II,* Publication- Medical Allied Agency, Calcutta.

Gaensslean, R.E. (1992). *Sourcebook in forensic serology*, *immunology and biochemistry.* (NIJ) 810 Seventh Street NW, Washington, DC 20531, United States.

Gunn, A. (2009). *Essential of forensic biology* (2nd ed.). Liverpool John Moores university, UK: Wiley-Blackwell.

Harris, H. (1969) *Genes and isozymes*. (Review lecture). Proc. Roy. Soc. Lond. B., Vol. 174, Pp. 1-31.

Jackson, A.R.W., & Jackson, J.M. (2011). *Forensic science*. Essex: Pearson Education Limited.

James, S.H., John, J.N., & Bell, S. (Eds.). (2015) *Forensic science: An introduction to scientific and investigative techniques*. CRC Press.

James, S.H., Kish, P.E., & Sutton, P. (Ed.). (2005). *Practical aspects of criminal & forensic investigation.* Boca Raton, Fla: CRC Press. xxv, 542 pp.

Khoo, J.C., Russell, P.J. (1972). Isoenzyme of adenylate kinase in human tissue. *Biochimica et Biophysica Acta (BBA)- Enzymology. Vol. 268,* Issue 1:Pp-98-101.

Mickelsen, O., & Keys, A. (1943). The composition of sweat with special reference to the vitamins. *Journal of Biological Chemistry.*

Modi, J.P. (2016). *Modi's medical Jurisprudence.* New Delhi, Nexis Lexis (Elsevier).

Reddy, K.S.N. (2014) *Forensic medicine and toxicology* (33rd ed.). New Delhi, Jaypee Brothers.

Richard, L. (2014) *Forensic biology.* Wiley. New York, USA.

Scientific working group on bloodstain pattern analysis. [online] Available at: [http://www.swgstain.org]

White, P.C., (2004). *Crime scene to court: The essentials of forensic science.* Cambridge: The Royal Society of Chemistry.

Wilson Jr, D.E., Povey, S., Harris, H. (1976) Adenylate kinases in man: Evidence for a third locus. *Annals of Human Genetics. Vol. 39*, Issue 3, Pp. 305-313.

8.9 ANSWERS/HINTS TO CHECK YOUR PROGRESS

1) Species origin test. Refer to section 8.1.1 for detail.

2) Two common confirmatory tests on bloodstains are the Teichmann and Takayama tests. Refer to section 8.1.2 for detail.

3) The potential for the individualisation of blood is based on typing of proteins and enzymes. Blood proteins and enzymes have the quality polymorphism or of being iso-enzymes, which means they exist in several forms and variants. Refer to section 8.1.5 for detail.

4) Salivary fluid is an exocrine secretion consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulin's and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health.

5) Swabbing, cutting, scraping.

6) After collection of saliva sample we perform starch- iodine test for examination of saliva sample.

7) Urine consists of water, organic and inorganic substances.Water alone forms about 95% of it and other substances form only 5%. The organic substances are mainly nitrogenous compounds but small amounts of non-nitrogenous compounds are also present.

8) Examination of urine stains. Refer to section 8.3.4 for detail.

9) Determination of origin of urine stain by precipitin method has not been found satisfactory. However, Popielski, Brzecka and Szczecin (1963) described a method of concentrating urinary proteins and thereafter subjecting that concentrated urine proteins to precipitin test by agar gel double diffusion method for the determination of species.

10) Seminal fluid contains the "flavin" that causes semen to fluorescence under the ultra-violet light. Prostatic fluid accounts for approximately 30% the ejaculation. This portion of the semen contains high concentration of acid phosphatase (AP) and prostate specific antigen (PSA). Both are very useful to the forensic analyst for detection of semen in case of rape, unnatural offence, hanging, etc. It is an alkaline fluid with pH=7.4.

11) Examination of semen. Refer to section 8.4.4 for detail.

12) Human spermatozoa have three distinct regions: Head, Middle piece, Tail. Figure 4, section 8.4.2 for detail.

13) Bloodstain patterns are defined as a deposition of blood on a surface or by a surface contacting blood is known as bloodstain.

14) The bloodstains and patterns are classified on the basis of their physical features like size, shape, location, concentration and distribution, etc. There is a variety of way to classify bloodstain patterns, but the aim of classification is the overall analysis. Refer to section 8.6.1.

15) Bloodstain pattern analysis (BPA) is the interpretation of bloodstains at a crime scene in order to recreate the actions that caused the bloodshed. The analysts can examine the size, shape, distribution and location of the bloodstains patterns and can be confirm or refute assumptions concerning events and their sequence.

UNIT 9 PERSONAL IDENTIFICATION FROM TEETH*

Contents

9.0 Introduction

9.1 Human Teeth

- 9.1.1 Dentition
- 9.1.2 Structure of Tooth
- 9.1.3 Anatomy of Tooth
- 9.1.4 Calcification and Eruption of Teeth
- 9.1.5 Tooth Development
- 9.1.6 Dental Lamina
- 9.1.7 Stages of Tooth Development

9.2 Forensic Odontology

- 9.2.1 Historical Background
- 9.2.2 Use of Teeth in Personal Identification
- 9.2.3 Age Estimation from Teeth
- 9.2.4 Sex Determination from Tooth
- 9.2.5 Race, Habit and Culture Determination
- 9.2.6 Role of Teeth in Disaster Victim Identification (DVI)
- 9.2.7 Bite Marks

9.3 Summary

9.4 References

9.5 Answers/Hints to Check Your Progress

LEARNING OBJECTIVES

After studying this unit, you shall be able to learn about:

- forensic odontology;
- determination of age, sex from teeth;
- importance of teeth in Disaster Victim Identification (DVI);
- bite marks and its individual characteristics;
- teeth and its type; and
- tooth structure and its development.

9.0 INTRODUCTION

The word "Personal Identification" has great significance in the field of forensic science. We have been utilising various methods for the purpose of

* ***Contributor:*** *Dr Vijay Yadav, Assistant Professor, Dr APJ Abdul Kalam Institute of Forensic Science & Criminology, Bundelkhand University, Jhansi.*

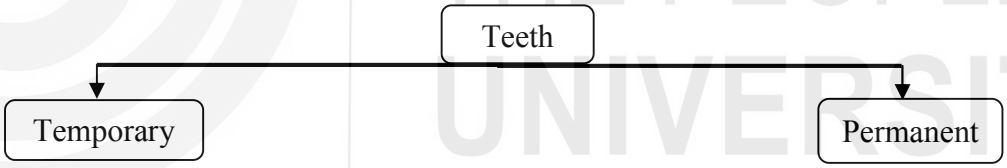
identification since ancient times like facial features, fingerprint, voice characteristics, etc. With the introduction of latest technologies, new methods for personal identification have also been introduced. Each method of personal identification has its advantages and some limitations also.

> *In the field of forensic science many cases have encountered in which traditional methods of identification did not play a very significant role. Cases of mass disaster, burial of body, burning of body have decomposed and unidentified bodies. Alternative method of personal identification is required for handling these types of cases. Teeth are harder and more resistant to environmental conditions than any other body fluid. So forensic dental examination plays very crucial role in personal identification especially in cases where no ante-mortem information is available.*

Apart from this dental information is also useful in age estimation of person where no documentary evidences are available. Human dental evidence is mainly used for the purpose of age estimation because it shows age related changes.

9.1 HUMAN TEETH

Teeth are bony structure set in the sockets of jaw of most vertebrates used for chewing and biting. Study of teeth is known as 'Odontology' while the Forensic Odontology is considered as use of teeth for legal purpose.



- Temporary teeth are also called deciduous teeth or Milk teeth.
- These are 20 in number (4 incisors, 2 canines & 4 molars in each jaw).
- Smaller, lighter, narrower.
- Roots of molars are smaller and more divergent than permanent th are

- Permanent teeth are 32 in number (4 incisors, 2 canines, 4 premolars & 6 molars in each jaw.
- Heavier, stronger and broader.
- Roots of molars are larger and less divergent than temporary teeth.

Permanent teeth are further classified into two types: -

i) **Superadded Permanent:** These teeth do not have deciduous predecessors. All permanent molars are superadded permanent teeth. These are 6 in each jaw.

ii) **Succession Permanent:** These erupt in place of deciduous teeth. Succession teeth are 10 in each jaw.

Dental Formula for Temporary teeth is $\frac{2102}{2102} \times 2 = 20$

Dental Formula for Permanent Teeth is $\frac{2123}{2123} \times 2 = 32$

9.1.1 Dentition

Human milk teeth are 20 in numbers while permanent teeth are 32 in numbers. Milk teeth are replaced with permanent teeth. At the age of 11 years there are 20 permanent teeth in oral cavity. Mixed dentition is seen up to 12 years and at the age of 14 years there are 28 permanent teeth and no deciduous teeth.

i) **Incisors:** Root is single and neck is slightly constricted. These are front teeth used for cutting purpose.

ii) **Canines:** These are larger than incisors and also have larger and conical crown. Canines have single root and are used for tearing purpose.

iii) **Premolars:** These have flat biting surface which have two cusps. So sometimes these are also known as Bicuspids. Root is usually single but may be double. These are used for crushing the food.

iv) **Molars:** These are the largest teeth with three or more cusps having largest and broad crown. The function of molars is to chew, crush and grind the food materials. Also known as Tricuspids.

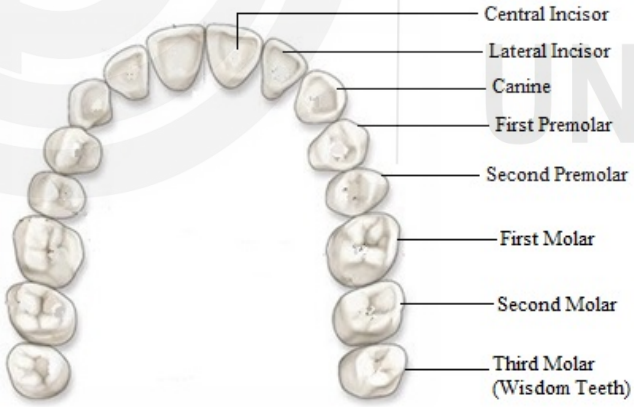


Figure 1: Human Dentition

***Source*:**https://ciroccodentalcenterpa.com/dental-hygiene/the-many-different-types-of-teeth

9.1.2 Structure of Tooth

Each tooth has a crown, neck and root embedded in jaw bone. Crown is covered by enamel which is the hardest substance of the human body. Root of the tooth is covered by cementum. The undivided part of the root is known as 'Trunk'.

Teeth may have single, double or triple root.

i) Single Root: All the anterior teeth and the premolar teeth, except the upper first premolar, are single rooted teeth.

ii) Double Root: This is present in lower molars and upper first premolars. Root bifurcates and forms two arms.

iii) Triple Root: There is trifurcation in root present in upper molars.

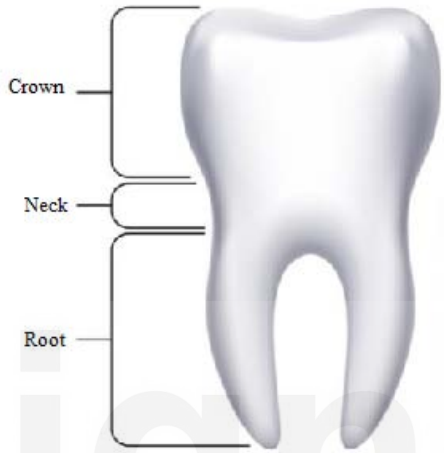


Figure 2: Structure of Human Tooth

Source:https://www.drbarryapplegate.com/a-few-facts-about-root-canals

9.1.3 Anatomy of Tooth

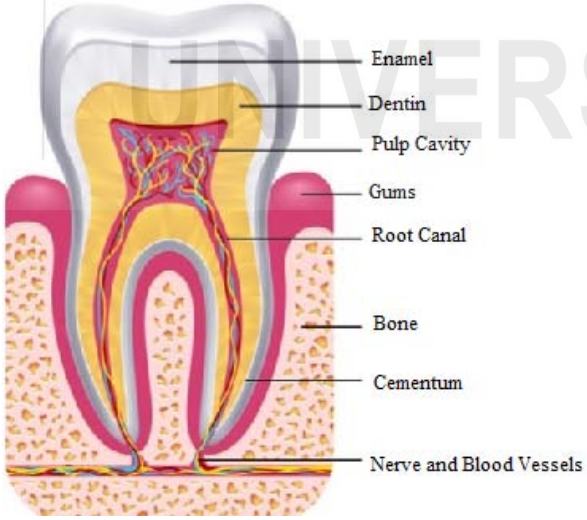


Figure 3: Anatomy of Human Tooth

Source:https://www.drbarryapplegate.com/a-few-facts-about-root-canals

Teeth are the one of most important organ of human body. Teeth are helpful in cutting, chewing, grinding and speech. Following are components of the teeth:

i) **Enamel:** Enamel is the hardest substance of the human body. This is the outer most part of tooth which is generally made up of calcium

phosphate. Dense mineralisation of the enamel gives the ability to resist the wear and tear that the tooth is subjected to.

ii) **Dentin:** Dentin is hard dense calcified tissue which is softer than enamel and harder than cementum and bone. The junction between dentin and enamel is called 'Dentino-Enamel Junction'. Dentin contains microscopic tubes which are responsible for sensitivity or pain in case when enamel is damaged and heat or cold enters into the teeth by the tubes. Dentin is present under the enamel layer.

iii) **Pulp Cavity:** This is the softer part of the teeth that contains blood vessels and nerve cells.

iv) **Cementum:** Cementum covers the root of the tooth. This is the layer of connective tissue which binds the roots of the tooth from the gums. Cementum is less dense and less hard than dentin and enamel but denser and harder than bone.

9.1.4 Calcification and Eruption of Teeth

The appearance of the superior part of the crown of the tooth appears at level with the surface of alveolar bone in deciduous and permanent teeth. The eruption and number of milk teeth is more regular than permanent teeth. Generally, the dental and skeletal ages, correspond closely in the male but in female the skeletal age is generally one year ahead of the dental age. It is generally accepted that eruption of the teeth occurs earlier in urban areas and warmer climate. For age estimation in children, teeth give better result than skeleton. There are many factors which play a role in eruption and calcification of teeth like environment, heredity, endocrine reactions, nutrition, etc.

Table 1: Calcification and Eruption of Deciduous Teeth

Tooth	Eruption	Calcification Begins	Calcification of Root Completed
Central Incisor Lower	6 to 8 months	5 to 6 months	$1^{1/2}$ to 2 years
Central Incisor Upper	7 to 9 months	,,	,,
Lateral Incisor Upper	7 to 9 months	,,	,,
Lateral Incisor Lower	10 to 12 months	,,	,,
First Molar	12 to 14 months	,,	2 to $2^{1/2}$ Years
Canine	17 to 18 months	,,	$2^{1/2}$ to 3 years
Second Molar	20 to 30 months	,,	3 years

Source: The Essentials of Forensic Medicine and Toxicology, Dr. K.S. Narayan Reddy and

O.P. Murty, 33rd Edition, Jaypee Brothers Medical Publishers (P) Ltd. Pg- 71

Table 2: Calcification and Eruption of Permanent Teeth

Tooth	Eruption	Calcification Begins	Calcification Complete
First Molar	6 to 7 years	At birth	9 to 10 years
Central incisor	6 to 8 years	3 to 4 months	10 years
Lateral incisor	7 to 9 years	1 year	11 years
First bicuspid	9 to 11 years	$1^{1/2}$ years	12 to 13 years
Second bicuspid	10 to 12 years	2 years	12 to 14 years
Canine	11 to 12 years	4 to 5 months	12 to 13 years
Second Molar	12 to 14 years	$2^{1/2}$ to 3 years	18 to 25 years

Source: The Essentials of Forensic Medicine and Toxicology, Dr. K.S. Narayan Reddy and O.P. Murty, 33rd Edition, Jaypee Brothers Medical Publishers (P) Ltd. Pg- 71

9.1.5 Tooth Development

Tooth development is a sequential development process of reciprocal epithelial mesenchymal interactions, morphogenesis and mineralisation. Morphologically, tooth formation commences fromstructure of dental epithelial thickening, dental lamina which proliferates and invaginates into underlying mesenchyma. At the same time, signals from thickened epithelium induce condensation of mesenchymal cells, which is known as tooth germ. Condensed mesenchyme then further guides epithelial invagination and convolution to progress the enamel organ through bud, cap and bell stages of tooth morphogenesis.

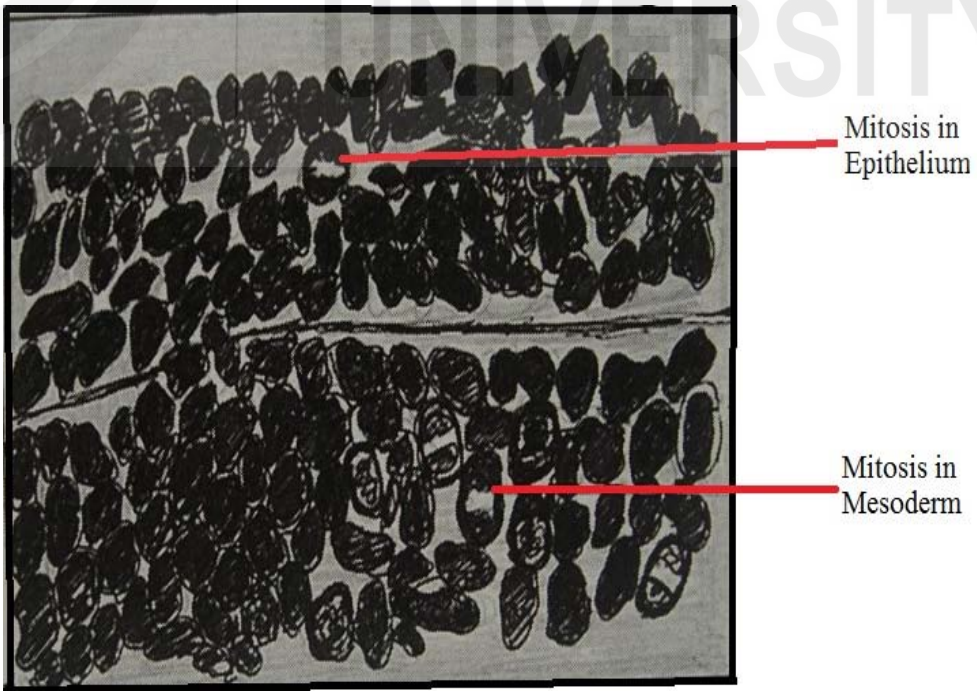


Figure 4: Epithelial Thickening by Mitosis

Source: Textbook of Dental and Oral Histology with Embryology by *Satish C., et al.*

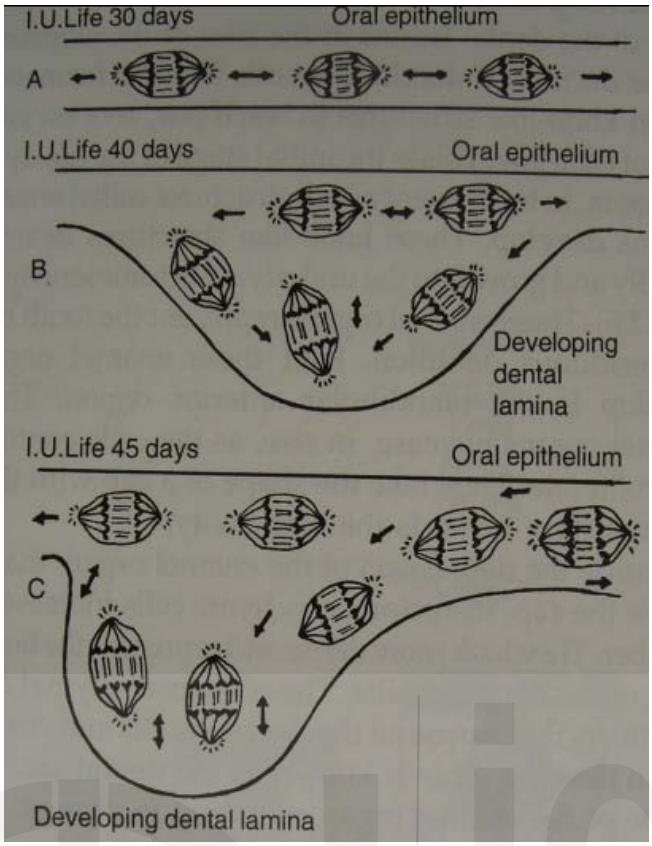


Figure 5: Changes in the Plane of Cleavage of Cells

Source: Textbook of dental and oral histology with embryology by *Satish C., et al.*

After about 45 days of uterine life, dental lamina begins to take shape due to condensation of the epithelial cells.

9.1.6 Dental Lamina

Dental lamina is the first sign of tooth development. This is the band of epithelium cells that has invaded the ectomesenchyme along each of the horse shoe shaped arches.

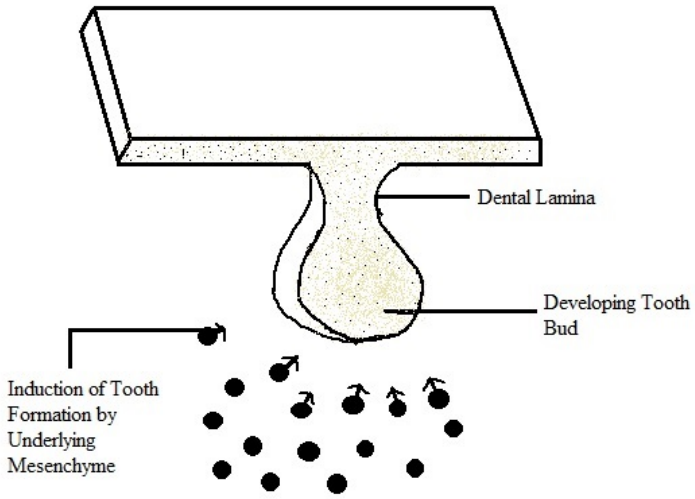


Figure 6: Dental Lamina

Source: Textbook of dental and oral histology with embryology by ***Satish C., et., all***.

The primary epithelial band divides into two processes, first one is known as dental lamina and second one is known as vestibular lamina. Vestibular lamina is also known as lip furrow band.

9.1.7 Stages of Tooth Development

There are four stages of tooth development i.e. bud stage, cap stage, bell stage and advanced bell stage. These stages may be related to the following incidents of tooth development.

Bud stage- Initiation

Cap Stage- Proliferation

Bell Stage- Histo Differentiation

Advanced Bell Stage- Morpho Differentiation

i) **Bud Stage:** This stage appears when fetus is approx 6 weeks old. This stage can be characterized by the appearance of tooth buds without any systematic arrangement of cells. Tooth buds itself are the group of cells which are generally present at the periphery of the dental lamina. Each bud is separated by ectomesenchyme by a basement membrane. Dental papilla and dental sac are not well defined in the bud stage, they are well defined during bell and cap stages.

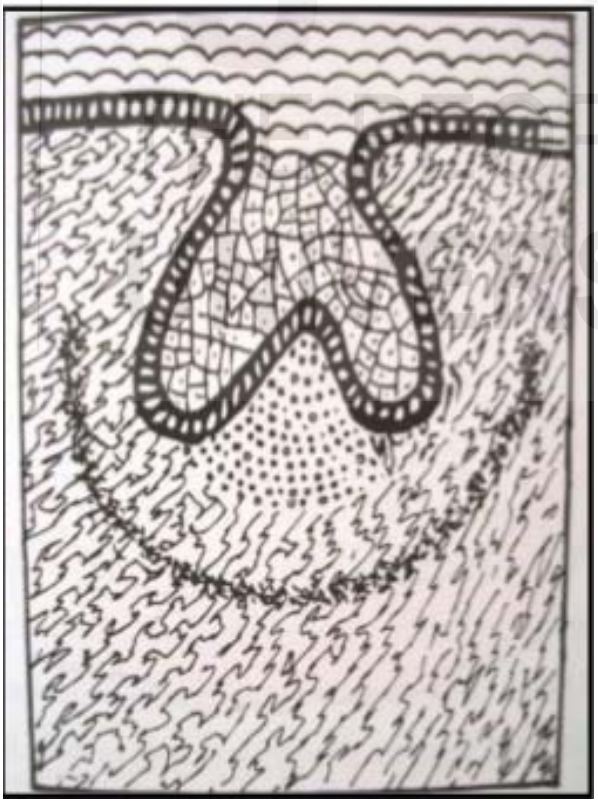


Figure 7: Bud Stage of Tooth Development

Source: *Orban's oral histology and embryology, G.S. kumar, 24-46 13th edition2013*

ii) **Cap Stage:** In cap stage, the cells of tooth bud begin to arrange systematically. When tooth bud continue to proliferate, it does not expand uniformly into a large sphere. This unequal growth in tooth bud

leads to cap stage of tooth development. Tooth buds growaround the ectomesenchymal region which forms cap like structure. Ectomesenchymal cells condense and these cells are known as dental sac which surround the enamel organ covering dental papilla. Enamel organ produces enamel, dental sac producessupporting structure of tooth and dental papilla produces dentin and pulp. The peripheral cells of cap stage are cuboidal.

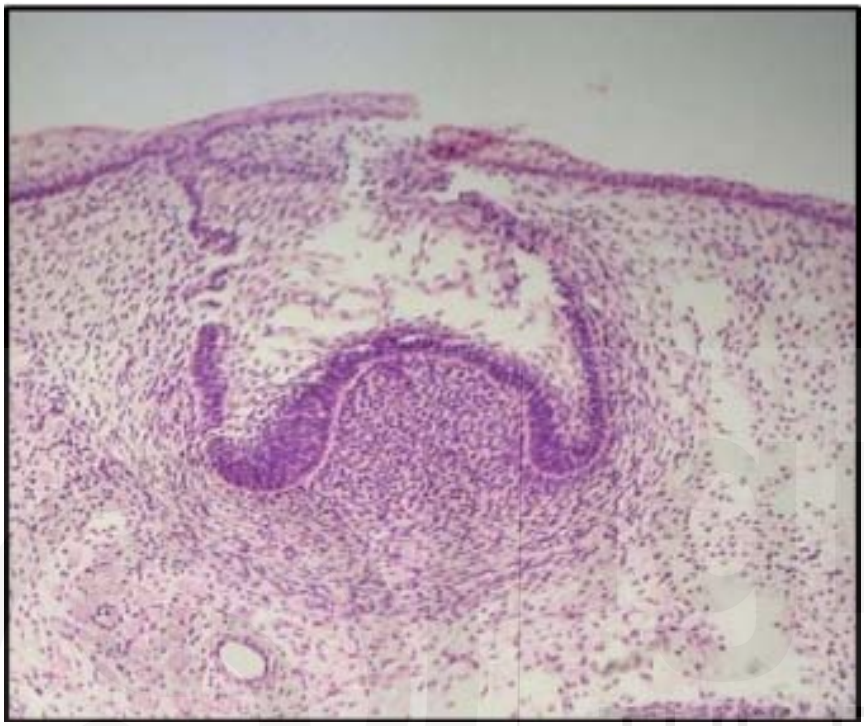


Figure 8: Cap Stage of Tooth Development

Source: *Orban's oral histology and embryology, G.S. kumar, 24-46 13th edition2013*

iii) Bell Stage: Bell stage is known for histo-differentiation. Dental organ takes the shape of bell and due to star shaped appearance of the cells, the cells are known as stellate reticulum. Peripheral cells of enamel organ separates into four layers i.e. OEE, IEE, stratum intermedium and cervical loop.

Cuboidal cells which are present on the peripheral region of the dental organ known as Outer Enamel Epithelium (OEE). The columner cells of enamel organ which are adjascent to enamel pappila are known as Internal Enamel Epithelium. The layer between these two cells is known as stratum intermedium and the rim where internal and external epithelium joins, is called a cervical loop. Other incidents which happens during the bell stage are: dental lamina disintegrates leaving behind developing teeth separated from epithelium. Inner enamel epithelium influences the crown of tooth which also begin to take shape during this stage.

All the teeth go through the same process.

Figure 9: Bell Stage of Tooth Development

Source: *Orban's oral histology and embryology, G.S. kumar, 24-46, 13th edition2013*

iv) **Advanced Bell Stage:** Advanced bell stage is known for morpho-differentiation of the tooth. In this stage, hard tissues like enamel and dentin develop. Sometimes this stage is also called Crown Stage or Maturation stage. During this stage, many cellular changes occur and formation of final mineralised hard tissue takes place. This time Internal Enamel Epithelium Cells changes into preameloblasts cells. The cells of dental pappila increases in size and differentiates into odontoblasts. Odontoblast forms the dentin. Odontoblast secretes an organic substance around itself which forms the dentin. After beginning the dentin formation, internal enamel epithelium secretes a matrix. This matrix immediately mineralises and becomes initial layer of enamel of the tooth.

v) **Dentin Formation:** Dentinogenesis is the process of dentin formation. Initiation of dentin formation is the first significant character of crown stage of tooth development. The formation of dentin takes place before than enamel formation. Odontoblast are the dentin formation cells which secrete an organic substance. This organic substance contains collagen fibers. Odontoblast cells moves towards the center of the tooth and forms an extension which is known as odontoblast extension or process. The odontoblast extension secrets the hydroxyapatite crystals. The area where mineralisation occurs is called mantle dentin.

The process of formation of primary dentin is quite different. Odontoblast increases in size, addittionaly collages and secretes in smaller amounts which results in them being more tightly arranged. Thus the formation of primary dentin takes place.

As root formation completes, secondary dentin forms from a very slower rate. The development of the secondary dentin occurs throughout life.

vi) **Enamel Formation:** Amelogenesis is the process of enamel formation. Amelogenesis occurs during the maturation stage of tooth development. There are two stages of enamel development: Secretory stage and maturation stage. Ameloblasts are the cells that are responsible and in secretory stage, ameloblast secretes enamel protein. Enamel protein contribute to enamel matrix. This matrix is mineralized by the alkaline phosphatase enzyme.

In maturation stage, ameloblast transport some substance out of enamel which was used in enamel production. This substance is generally proteins which is used for the mineralization of the enamel.

vii) **Cementum Formation:** The process of cementum formation is called cementogenesis. Cementoblasts cells are responsible for cementum formation. There is two types of cementum is formed during the process of cementum formation: cellular and acellular. Formation of acellular cementum takes place before cellular cementum. After completion of the tooth formation, cellular cementum begin to form.

viii) **Tooth Eruption:** It occurs when tooth becomes visible in oral cavity. There are various theories regarding tooth eruption. According to widely accepted theory, periodontal ligaments promote eruption by shrinking and cross linking of their collgen fibers. The eruption sequence of decidous and permanent teeth has already been discussed before in the starting of this unit. On the basis of eruption sequence of the teeth, age estimation can be performed. In starting primary dentition starts at mandibular central incisors.

Check Your Progress 1

1) Explain dental formula for temporary and permanent teeth.

……

……

……

……

……

2) What do you mean by lip furrow band?

……

……

……

……

……

3) Describe the bell stage of tooth development.

……

……

……

……

……

9.2 FORENSIC ODONTOLOGY

Forensic Odontology is defined as the proper handling, examination, and evaluation of dental evidence for the purpose of Justice.

or

Forensic Odontology is the application of dentistry in legal proceedings deriving from any evidence that pertains to teeth.

It is the branch of Forensic Medicine where the Odontologists use teeth as evidence for personal identification during bite mark, sexual assault, child abuse and in all phases of disaster victim identification. According to the Disaster Victim Identification Guide, if a positive match is found using dental identification it can be trusted as a standalone identifier.

Identification by dental means, gains more importance because the dental tissues are often preserved even if the deceased person is skeletonised, decomposed, burnt or dismembered. Dental tissues are often used to determine the age, sex, and ethnicity of the person who can either be a victim or a suspect. Dr. Oscar Amoedo was considered as the father of the Forensic Odontology. The thesis done by him entitled 'L' Art Dentaire en Medicine Leagale' to the Faculty of Medicine earned him a doctorate. This was the first comprehensive text on Forensic Odontology.

9.2.1 Historical Background

The evolution of Forensic Odontology started right back in the Garden of Eden. But well-documented evidence to the use of teeth for identification began during 66 AD with Agrippina and Lollia Pauline case. Agrippina after her marriage with Claudius, emperor of Rome, tries to secure her position. She feared that the rich divorcee, Lollia Paulina may still be a rival for her husband. She decided that it would be safer if Lollia Paulina was dead. She instructed her soldier to kill Lollia Paulina and further instructed to bring the head back. She was satisfied by Lollia Paulin death by the identification of dental alignments and certain distinctive characteristics. It was the first use of dental identification where there is a record.

The first forensic identification in India started in 1193 when Jai Chand, great Indian monarch was destroyed by Muhammad's army and Jai Chand, Raja of Kanauji was murdered and he was identified by his false teeth.

Peter Halket's was killed in 1758 during Indo-French wars in a battle near Fort Duquesne. Halket's son identified his father's skeleton by an artificial tooth.

At the battle for Breed's Hill in Boston, Dr. Joseph Warren was killed in the year 1776. His face was not identifiable as he suffered from a fatal head wound. A dentist, Paul Revere, identified Dr. Warren's, dead body by a small denture that he had fabricated for him. The identification made by Paul Revere made it possible to bury Dr. Warren on April 8th, 1776 with full military honor.

A professor and a physician of Harvard University, Dr. George Parkman who was also a real estate speculator and money lender, failed to return from dinner on November 23, 1849. A suspicion was laid onJohn White Webster as it was known that he owed some amount of money to Dr. Parkman. When his laboratory was searched, the remains of the human body were found. Dr. Parkman's dentist, Dr. Nathen Cooley Keep identified Dr. Parkman's body, by his teeth as a part of upper and lower denture which he had made for Dr Parkman three years back. Dr. Keep showed the evidence to court, and he fitted the portions of the lower denture to the models and also showed the grinding adjustment of the lower denture that he had made for Dr. Parkman. Dr. Webster was found guilty and later hanged to death. This was the first case of a dentist giving expert testimony in courts of the United States.

9.2.2 Use of Teeth in Personal Identification

Teeth are the hard and protected structure of the human body. Enamel, the outer layer of teeth is the hardest part of the human body. There are many cases encountered by the forensic scientists and investigators that need human identification. Teeth are hard, durable, resistant to decomposition and temperature and remain unchanged even after death. So teeth are the choice of many scientists for the identification purpose. In disaster incidents where highly mutilated and unidentifiable bodies are recovered, teeth evidence plays a major role in identification purpose. The dentition pattern of each individual is unique. No two person share the same dentition pattern. Dentition pattern is unique because of dental features like tooth morphology, missing tooth, shape and size variation, number and position of teeth, wearing pattern, the spacing between the teeth, color, restoration, etc. Teeth can provide important information about gender, age, race, occupation, etc.

9.2.3 Age Estimation from Teeth

We all know that each tooth has fixed timing and sequence of its eruption. There are various radiographic techniques that provide an accurate stage of mineralisation of the tooth that further help in better estimation of age. For age estimation in persons who have attained adulthood, the best method is to look for the number and eruption sequence of teeth. describes the eruption sequence of the teeth that provide the age group of an individual.

Gustafson(1950) developed a technique for age estimation from a single tooth. In the present time, Gustafson's method is widely used by forensic scientists for age estimation from the tooth.

Gustafson's Method: This method consists of the microscopic examination of the longitudinal section of the central part of the tooth. The changes in the tooth are observed as a result of wear and tear with the age. This was the first scientific study for age estimation from tooth, developed by Gosta Gustafson in 1950. Following changes are observed in the cross section of the tooth:

a) **Attrition or Gradual Wearing Out of Teeth (A):** Continuous wear and tear of the teeth leads to attrition first involving enamel then dentin and the last is dental pulp which is exposed in extreme old ages.

b) **Secondary Dentin Deposition or Infilling of the Normal Root Cavity (S):** Secondary dentin may be developed within the walls of the pulp cavity which decrease the size of the tooth cavity. The deposition is caused by aging effect but some pathological conditions like paradentosis also influence the secondary dentin deposition.

c) **Periodontosis or Loosening of Teeth (P):** With the age, tissues and gums surrounding teeth becomes loose and exposes the neck and other parts of the root. As the result teeth become loose.

d) **Cementum Apposition or Increase in the Tissue Holding the Root in Place (C):** Incremental lines are formed by the continuous apposition of the secondary cementum. This process occurs throughout life. Due to cementum apposition, incremental lines appear as cross striations. These cross striation can be seen by the histological section.

e) **Root Resorption (R):** This generally occurs in late age. Root resorption starts towards upwards. Some pathological conditions are also responsible for the early root resorption condition.

f) **Dentine transluency (D) :** Among all, this is the most reliable criteria. Generally, this character is seen after 30 years of age. At first, root canals becomes wide and filled by minerals and canals becomes invisible. With age, dentin becomes transparent.

For each changesa different score ranging between0-3 wasassigned

0 = No change indication

1 = Iniatiation of the change

2 = Obvious change

3 = Change at its maximum limit

The X value is generated by doing the sum of scores of the changes.

$$A+S+P+C+R+D = X$$

Now age estimation canbe done by applying following formula:

Age (in years) = 11.43+4.56X

Generally, the value of X is directly proportional to the age. Value of X increases as the age increases. The error rate in Gustafson's method is said to be between 3 to 5 years.

Besides Gustafson's method, there are several methods for age estimation from teeth. According to Boyde's method, the development of many cross striations takes place on the enamel. In infants, neonatal lines are formed very soon after birth and it can be observed by electron microscopy. Generally, this is beneficial in estimating the age of the dead infant.

Another method of age estimation from teeth is Stack's method. A scientist named Stack invented the method for age determination of infants by weight and height of erupting teeth. This method is useful for both deciduous and permanent teeth in their erupting phase.

9.2.4 Sex Determination from Tooth

Although sex determination from the tooth is not conclusive it can be used as supportive evidence. In cases where other evidence for sex determination are not available, the tooth can be used as an organ for determining the gender of an individual. Previously several attempts have been made to determine the sex from teeth on the basis of measurements. Dental indices like mandibular canine index, incisor index, crown index, etc., are found to be helpful in sexual dimorphism. Mandibular canine teeth show greater variation in measurement than maxillary canine. Measurement-based conclusion for sex determination from teeth is not always authentic. The error rate is always high in these type of conclusions.

Nowadays many other techniques are employed for determining sex from teeth for higher accuracy of the result. The presence or absence of Barr bodies in the dental pulp is helpful in sex determination. Barr body is the condensed inactive X chromosome which is present in the somatic cells of females. The cells of dental pulp are observed microscopically and the presence of Barr bodies indicates the female tooth and absence indicates the dental pulp of the male tooth.

Amelogenin is the protein used for sex determination. Amelogenin is widely used for gender determination by using various DNA techniques.

9.2.5 Race, Habitat and Culture Determination

There are some morphological features which are used to determine the race or ethnicity of an individual. Race determination from these morphological features is still debatable. Some characters, occupational marks can give about the idea of habits and occupation. Some dental features like taurodontism, Carabelli's cusp, shoveling of upper incisor, etc., can be used to determine the ethnicity of the person.

Sometimes restoration marks on teeth are also helpful in estimation of habits and social class of the person. Method and type of restoration indicate the

region or country which may be unique to that place. Expensive tooth restoration indicates the economic standard of the person. Wear and tear marks on tooth indicates the occupational habits of the person. For example, tailors keep the needle between their teeth which sometimes creates needle marks on the teeth. Other habits like cigarette smoking, chewing paan masala, etc., also leave marks on the teeth which help in the determination of habits of a person. There are some tribal groups which create artificial deformities on their teeth. Generally, this type of artificial deformation includes central incisors because these are easily visible. These types of deformation are unique to each tribal community. So these types of characters are very helpful in determining the race, community and occupational habits of the person.

9.2.6 Role of Teeth in Disaster Victim Identification

In mass disaster incidents, it is very important to make an identification from human remains. Post mortem dental remains can be compared with the dental record of the person. Teeth are the very first choice for disaster victim identification. There are several advantages of teeth for using it as an identification parameter such as teeth is hard as it is resistant to temperature and other environmental factors. Individuals who have complex and unique restorations, it is easier to identify them.

The American Board of Forensic Odontologists (2017) recommends four conclusions while reporting dental identifications.

1) Positive identification: Post mortem and antemortem dental record match without any discrepancies.

2) Possible Identification: The identification cannot be positively established. Both antemortem and postmortem have some relevant features due to poor quality of data.

3) Insufficient Evidence: Evidences are not sufficient to make a conclusion and

4) Exclusion: Data provided is completely inconsistent.

During the time of mass fatality, teeth also play an important role in age determination of infants and children.

The success rate depends on the degree of the injury to the tooth. If a complete dental record is found, success rate becomes higher. There are five grades of the severity of the injury.

Grade 0: No injury

Grade 1: Injury to anterior teeth

Grade 2: Injury to anterior and posterior teeth unilaterally

Grade 3: Injury to anterior and posterior teeth bilaterally

Grade 4: Fragments of both jaws including tooth and root

Grade 5: No dental remains

Besides morphometric identification, DNA profiling from the tooth can be performed. As we havediscussed before that teeth are resistant to temperature and other environmental factors, so DNA material does not degrade easily.

9.2.7 Bite Marks

Bite marks are the impression of teeth found on skin or items left at crime scenes. It usually outlines teeth placement but the dentition pattern of every individual is unique. Even a single tooth shows great variation. So the marks inflicted by teeth are highly considerable. There are many cases where bite marks play an important role and are most likely used for exclusion. Bite marks may be present on the body parts of the victim and sometimes the culprit. These marks are commonly found in rape incidents, sexual assault cases, etc. Besides body parts, bite marks are commonly found on fruits, hard cheese, etc. Bite marks also contain saliva which may be used as DNA source of the assailant. Bite marks may also be self-inflicted. Common sites of the bite marks are breasts, arms, legs, neck, back, shoulder, etc. For standardisation of bite mark terminology, Pretty (2006 and 2007) developed Bite mark severity Index. Generally, human bite marks are oval or circular in shape.

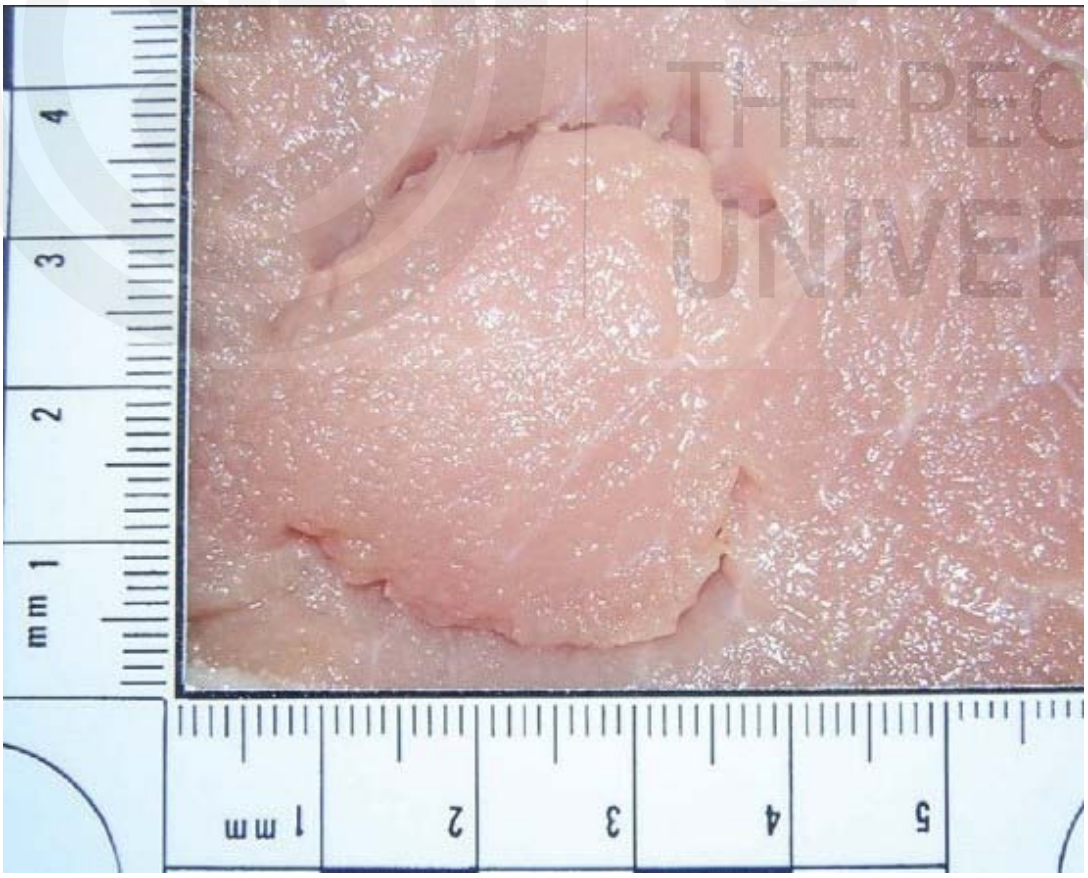


Figure 10: Bite Mark

Source: Fonseca, et al.: Polyether impression material and bite mark analysis

Collection of Bite Mark: Mostly morphological changes occur in the first 24 hours of bite marks. The indentations may disappear within some hours. So this is the primary requirement that all the data of a bitten mark should be secured as soon as possible. All the data including abrasions, bleeding, site, the severity of injury, should be noted and photographed with and without scaling. The American Board of Forensic Odontology (ABFB) established guidelines for the collection and examination of bite marks. All the details like type of injury, shape, size, color, location, etc., need to be proved. Collection methods include photography (with and without scaling), salivary swabbing, impression casting. Techniques like test bite technique, dental records, etc. are employed for the purpose of matching and identification of bite mark. There are some factors which are responsible for the identification of bite mark. These factors include changes with the passage of time, damage in soft tissue, biting site.

The serological evidential value from the bite mark is very high. A bite mark is also a source of saliva stains. Secretor and non-secretor status can also be determined by the saliva sample. Swabbing is done for the collection of latent saliva stain. For the purpose of swabbing a sterile cotton swab should be used.

Examination of Bite Mark: Firstly overall supervision of the dentition pattern of the accused is done by the forensic odontologist. After supervision, several casting methods are employed. As much as possible casting should be done on the same material where suspected bite mark is found. Besides this many other materials like silicon-based material or fast setting rubber, etc. are also employed. During casting, several impressions are taken from different angles on the test surface.

After successful casting, forensic odontologist examines and compares the bite mark. Following are the unique and important features of the dental pattern which are useful in bite mark analysis.

- Odontogram or number of teeth in the upper and lower jaw
- Abnormal position, missing teeth, the abnormal gap between the teeth
- Broken or fractured teeth
- Biting pattern
- Any prominent teeth
- Shape and size of the dental arch.

Hitler's Teeth Confirm He Died in 1945

At the end of World War II, rumors were rampant that Adolf Hitler had escaped with Eva Braun, his wife. But it is a fact that they both died together in 1945, and their bodies were burned and buried by a Russian soldier. It was a challenge to dispel the rumor, due to lack of antemortem and postmortem records. Finally, remnants of a bridge were identified in the pieces of Hitler's jaw because of the unusual form of reconstruction and evidence of periodontal diseases. Hitler's dentist Hugo Blaschke's record work was matched with dental work of Hitler and hence, was confirmed the death of Hitler's.

Check Your Progress 2

4) Define Forensic Odontology.

……

……

……

……

……

5) Discuss the significance of teeth in DVI.

……

……

……

……

……

6) Describe the examination of bite marks.

……

……

……

……

9.3 SUMMARY

Forensic Odontology can play most important role in criminal justice system. Teeth are the most hardest and robust tissues of the human body. They are

often resistant to decomposition even in major accidents, crime, burial, or other severe exposure to the elements. Teeth can provide important information about age, gender, race, occupation, etc.

Teeth always play major role in Disaster Victim Identification (VDI). Bite Marks can give important information about personal Identification and also contain saliva which may be used as DNA Profiling. Teeth are basicly divided in two categries: temporary and permanent and further divided in four type: Incisors, Canines, Premolars, Molars.

The unique nature of the dental anatomy and the custom restorations ensure accuracy when the techniques are appropriately employed. Tooth development is a sequential development process in which dental lamina is the first sign of tooth development.

9.4 REFERENCES

Amedo, O. (1897). The role of dentist in the identification of the victims of the catastrophe of the *Bazar de la Charite*. Paris, 4th May. *Dent Cosm.* 39:905–12

Balachander, N., Babu, N.A., Jimson, S., Priyadharsini, C., & Masthan, K.M. (2015). Evolution of forensic odontology: An overview. *Journal of Pharmacy & Bioallied Sciences*, *7* (Suppl 1), S176–S180. doi:10.4103/0975-7406.155894

Body identification guidelines. (1994). American Board of Forensic Odontology (ABFO). *J Am Dent Assoc*. *125*(9):1244–1246

Chowdhury, R., Singhavi, A., Bagul, N., Bhatia, S., Singh, G., & Goswami, S. (2018). Sex determination by amplification of amelogenin gene from dental pulp tissue by polymerase chain reaction. *Indian Journal of Dental Research*, *Volume 29*, Issue 4. 470-476

Devide, R.S., & Richard A.W. (2013). *Manual of forensic odontology* (5th ed.). CRC Press.

Dilnot, G., & Bles, G. (1928). The trial of professor Webster. *Famous trial series.* LLC, London: Literary Licensing.

Grady, R. (1884). Personnel identity established by the teeth: The dentist as a scientific expert. *Am J Dent Sci.* 17:384–405.

Kolude, B., Adeyemi, B.F., Taiwo, J.O., Sigbeku, O.F., & Eze, O.U. (2010) The role of forensic dentist following mass disaster. *Annals of Ibadan postgraduate medicine*, 8(2): 111–117

Krishnan, K., Kanchan, T., & Garg, A. (2015). Dental evidence in forensic identification – An overview, methodology and present status. *Open Dental Journal*, *9,* 250-256, doi: 10.2174/1874210601509010250

Manjunatha, B.S., & Soni, N.K. (2014). Estimation of age from development and eruption of teeth. *Journal of Forensic Dental Sciences*. doi: 10.4103/975-1475.132526

9.5 ANSWERS/ HINTS TO CHECK YOUR PROGRESS

1) Dental formula is the arrangement of teeth in jaw. Refer to section 9.1.

2) Vestibular lamina is also known as lip furrow band. Refer to section 9.1.6.

3) There are four stages of tooth development i.e. bud stage, cap stage, bell stage and advanced bell stage. Bell stage is also known as Histo differentiation. Refer to section 9.1.7

4) Study of teeth is known as 'Odontology' while the Forensic Odontology is considered as use of teeth for legal purpose i.e.uses for law enforcements. Refer to section 9.1.

5) Teeth always play major role in Disaster Victim Identification (VDI). Bite Marks can give important information about personal Identification and also contain saliva which may be used as DNA Profiling. Teeth are basicly divided in two categries tempreri and permanent and further divided in four type, Incisors, Canines, Premolars, Molars.

6) Bite marks are the impression of teeth found on skin or items left at crime scenes. It usually outlines teeth placement but the dentition pattern of every individual is unique. Refer to section 9.2.7.

UNIT 10 RECENT TRENDS IN FORENSIC ANTHROPOLOGY*

Contents

10.0 Introduction

10.1 DNA Fingerprinting

- 10.1.1 DNA (Deoxyribonucleic Acid)
- 10.1.2 Basics of DNA Fingerprinting
- 10.1.3 Applications of DNA Fingerprinting

10.2 Other Bodily Identifications

- 10.2.1 Somatometric and Somatoscopic Identification
- 10.2.2 Skeletal System
- 10.2.3 Retina and Iris
- 10.2.4 Ear
- 10.2.5 Fingerprint

10.3 Summary

10.4 References

10.5 Answers/Hints to Check Your Progress

LEARNING OBJECTIVES

After studying this unit, you will know the:

- basics of DNA and how DNA fingerprinting is done;
- forensic significance of DNA fingerprinting with amalgamation of its various applications; and
- other bodily identification systems.

10.0 INTRODUCTION

Anthropology is the study of every aspect of human kind. Human species have seen the evolution for thousands of years to achieve their present version which is the best among all their predecessors. The evolutionary process over several millenniums has brought huge number of complexities in the human species. These complexities reflect in their biological, anatomical and genetic features. All of these three aspects are the criterions of consideration in forensic context. Anatomical and biological features including DNA are the best source for the identification and discrimination of human individuals from each other. Forensic anthropology has entirely focused on the personal identification of suspected persons and victims of a crime or mass disaster. This unit thoroughly focuses on the fundamentals of anthropological features including DNA as well as their application for the personal identification concerning forensic science.

* ***Contributor***: *Dr Ankit Srivastava, Associate Professor (Forensic Science), The West Bengal National University of Juridical Sciences, Bidhannagar, Kolkata*

10.1 DNA FINGERPRINTING

10.1.1 DNA (Deoxyribonucleic Acid)

Have you ever been amazed over the fact that how every human from all the parts of the world is unique from the other? Have you ever seen two identical individuals even in the same family? Except the identical twins!!!

We cannot find a single pair of humans around the world having entirely identical phenotypic and genotypic characteristics. The reason for this wonder is DNA. 'Deoxyribonucleic Acid' is the fundamental factor behind the uniqueness of every individual. Predominantly, it is present inside the nucleus of every cell of the body. Mitochondria of a cell also consist of its own DNA. Nuclear DNA carries the genetic material to the next generations.

After knowing this, following questions will surely arise in your mind; let's have a look at these:

Do all the cells of our body have the same DNA?

Yes!! DNA replicates itself to each cell of the body except reproductive cells where only half of the DNA is present.

Are DNA and Chromosome same?

DNA makes a dense coil-like structure with a protein called '**Histone'** to make chromosomes. During the extraction process the protein is removed by chemical process to separate the pure DNA.

DNA Structure

It is made up of two strands, as it is known as a double helix. DNA is a linear polynucleotide which consists of four monomeric nucleotides. Each nucleotide contains three components: a deoxyribose sugar, a nitrogenous base, and a phosphate group. Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) are the four bases. The phosphate group is attached to the deoxyribose sugar that is further attached to the nitrogen of a base. Each nucleotide is linked by phosphodiester bonds with the other.

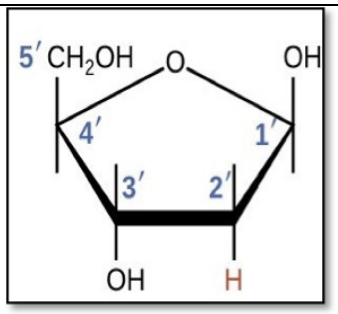


Figure 1: Deoxyribose Sugar
Source: courses.lumenlearning.com/microbiology/chapter/structure-and-function-of-dna/

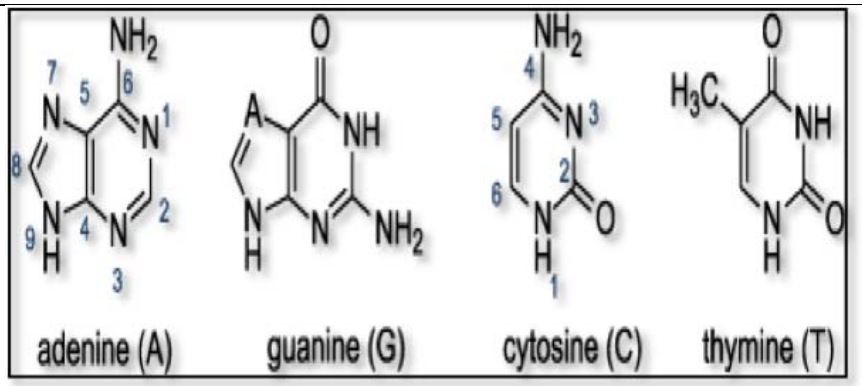


Figure 2: Nitrogenous Bases
Source: www.atdbio.com/content/5/Nucleic-acid structure

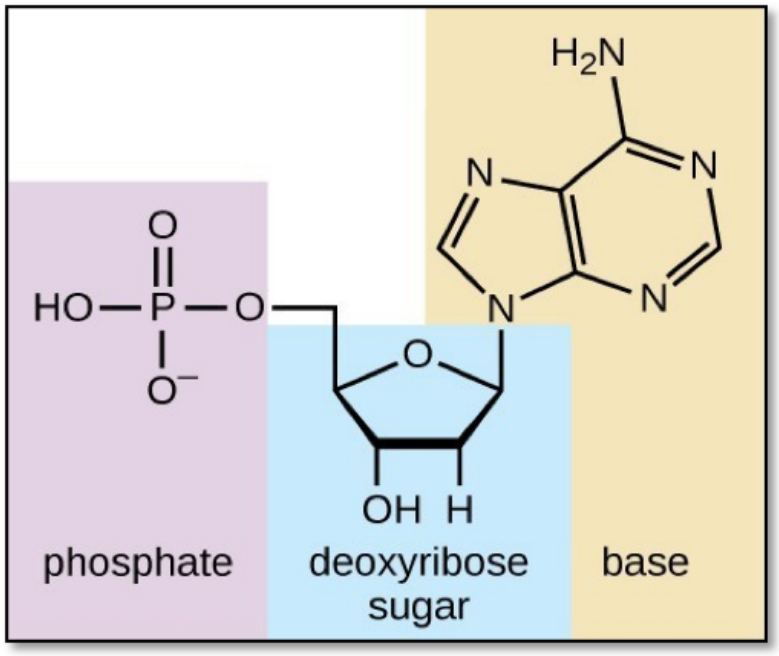


Figure 3: A Nucleotide

Source: blogs.ubc.ca/mrpletsch/2017/02/04/dna-structure/

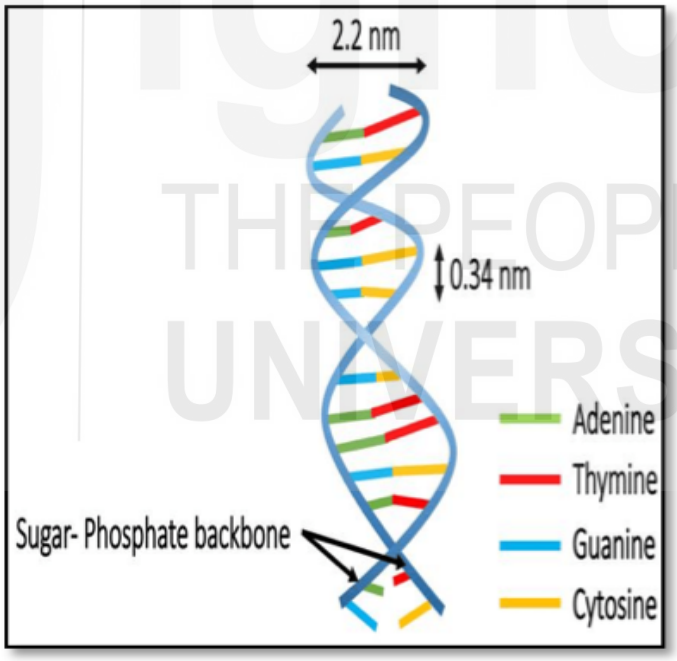


Figure 4: A Double Helix Structure of DNA

Source: www.researchgate.net/figure/2-The-double-helical-structure-of-double-stranded-DNA-The-schematic-is-adapted-from_fig2_313798200

Nitrogenous bases are complementary to each other. Adenine (A) only binds with Thymine (T) by double bond and Guanine (G) only binds with Cytosine (C) by triple bond. For example; if the order of the bases in a section of one strand is GTCCAT, then the order of bases in the complementary section of DNA in the other strand will be CAGGTA.

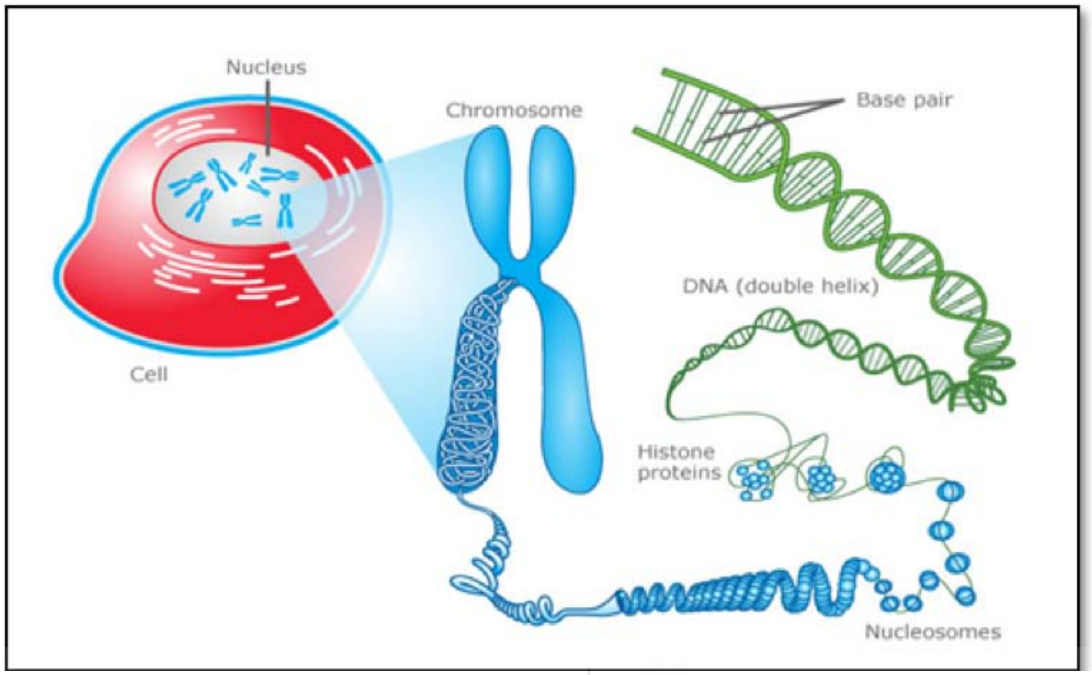


Figure 5: A Pictorial view of the specific location of DNA inside a Cell

Source: www.biotecharticles.com/DNA-Article/DNA-Double-Helical-Structure-History-Gene-its-Molecular-Level-4251.html

DO YOU KNOW?

James Watson and Francis Crick described the structure of DNA in 1953. They received the Nobel Prize for their ground breaking work. The backbone of DNA consists of alternating sugar and phosphate molecules. The double stranded DNA looks like a ladder due to the rungs formed by complementary nitrogenous bases.

Figure 6: Dr. Francis Kirk (Left) and Dr. James Watson (Right)

Source: Francis Crick – Photo gallery. NobelPrize.org. Nobel Prize Outreach AB 2022. Wed. 6 Apr 2022. https://www.nobelprize.org/prizes/medicine/1962/crick/photo-gallery

10.1.2 Basics of DNA Fingerprinting

Let us now discuss about the revolutionary technique of DNA Fingerprinting.

But before proceeding ahead with this learning we need to know the answers of certain basic questions:

Why this technique is named so?

The word ‘Fingerprinting’ is used to signify the uniqueness of the DNA.

It is the analysis and comparison of generated profile of non-coding region of the DNA, collected and extracted from the standard and the questioned biological evidences. The repetitive sequences in these non-coding regions of DNA are polymorphic for every individual except identical twins.

Why DNA is unique?

Two half parts of the chromosomes received from the father and mother respectively go through random recombination process during the formation of the first cell of a new human baby by fusion. The random nature of the process makes the newly generated DNA a unique one from its parent DNAs and also from other individuals.

How much amount of the DNA is unique?

It is very surprising that almost 99% of the DNA of every person around the world is same. The uniqueness occurs only in 1% and it is so huge that makes the difference among each and every individual.

How much percentage of the DNA codes our characteristics?

The DNA in the chromosomes has approximately 3 billion base pairs. DNA in chromosomes is divided into two types i.e., exons and introns. Exons are used to make proteins or other molecules and are called 'Coding DNA' and introns do not contribute in protein production and are called 'Non-coding DNA'. The chromosomes of each human cell contain some 23,688 encoded genes, with each gene averaging 3,000 base pairs. This is less than 1.5 percent of the whole DNA. The rest of the DNA is non-coding, which is approximately 98.5 percent. This non-coding DNA is called 'junk DNA'.

DO YOU KNOW?

Figure 7: Sir Alec Jeffery

Source: https://en.wikipedia.org/wiki/Alec_Jeffreys

The credit of this vital discovery goes to Sir Alec Jeffery, a geneticist from University of Leicester, UK. Sir Jeffrey employed Restriction Fragment Length Polymorphism (RFLP) for analyzing DNA. He observed that DNA has certain repetitive sequences, commonly known as Minisatellite or Variable Number of Tandem Repeats (VNTRs), which are perpetually present in all human beings. However, their length varies among individuals. He utilized these variations in establishing the identity of a person. He soon realized that these variations are unique to each individual except for identical twins and thus, could be used to affirmatively ascertain the identity and individuality of a person.

What are the sources of DNA?

A perpetrator may leave biological evidences at crime scene, which are rich source of DNA and are capable of identifying a specific person. We can find these evidences separately or contaminated/ attached with any other thing. Most common DNA sources are blood, saliva, semen, hair, etc. These samples are commonly found contaminated or attached with toothbrush, glass, any food item, door-knob etc.

After knowing the answers of all the basic questions, let us now learn about this magical technique-

RFLP is the pioneer technique of DNA fingerprinting. It employs a molecular method for genetic analysis i.e., it allows individuals to be identified based on unique patterns generated by single restriction enzyme nicking DNA at specific sites commonly referred to as Restriction Endonuclease Recognition Sites. Polymorphic nature of genetic codes is the key factor behind this technique. Nicked DNA is then separated into different fragments using gel electrophoresis depending on relative sizes of generated fragments. Since DNA carries an overall negative charge, different fragments have a tendency to move towards positive pole. Smaller fragments will obviously move faster. Gel with these bands is then denatured by placing it in sodium hydroxide (NaOH) solution. The single stranded DNA so obtained is transferred into a Nylon or Nitrocellulose sheet by capillary blotting, named after its inventor as Southern blotting. This sheet is then fixed by autoclaving. This single stranded fragmented DNA on Nylon or Nitrocellulose sheet is then allowed to base pair with the labeled RFLP probes. These probes may be labeled with radioactive or chemiluminescent tag. This process is commonly referred to as hybridization. When such hybridized DNA is exposed to X-rays, it produces distinct bands on X-ray film. The autogram so generated is the unique genetic signature of an individual except for identical twins who share the same autogram. For many years RFLP technique served criminal justice system by resolving paternity disputes, ascertaining criminal identity, etc. However, requirement of large quantity of good quality sample, being expensive, tedious and time consuming, were certain limitations associated with this technique. This leads to the need of discovery of more efficient and advance techniques of DNA profiling. PCR and capillary electrophoresis are the further advancements which are presently used throughout the globe for DNA profiling.

Since, the DNA profile of a person is completely unique, which cannot be identical to another person except monozygotic twins, the profile obtained from the crime scene can show complete match with the profile from one of the suspected persons. The figure below shows that the second suspect is involved in the offence that has occurred with the woman because all the DNA fragments of the second suspect match with the DNA fragments found from the crime scene.

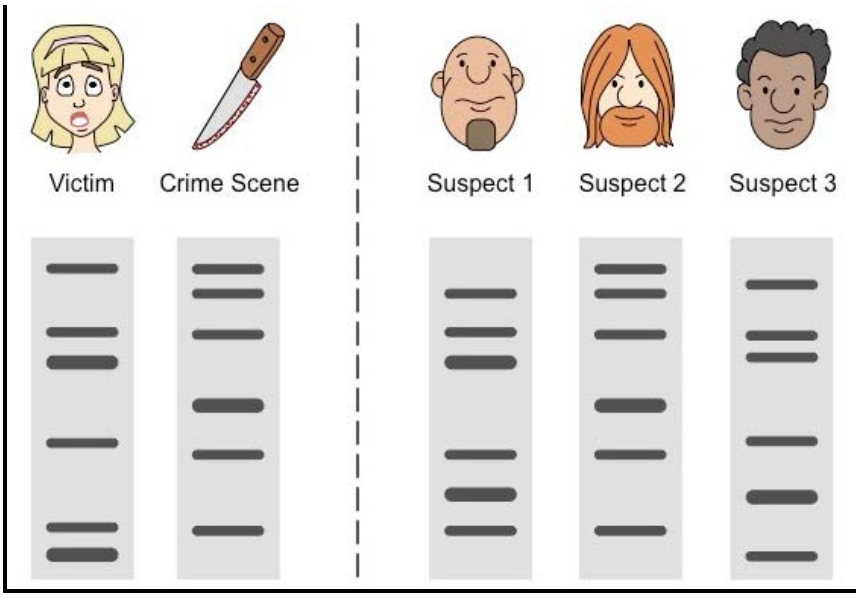


Figure 8: An example of excluding and identifying the suspects by comparison of DNA profiles
Source: http://ib.bioninja.com.au/_Media/str_med.jpeg

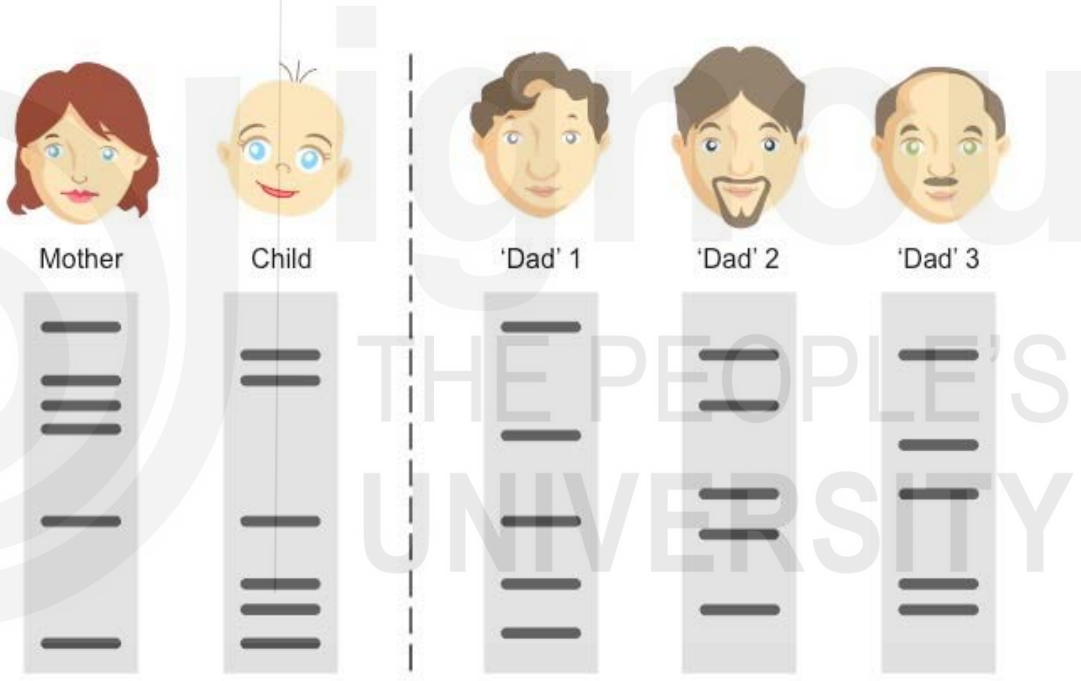


Figure 9: An example of identifying the paternity of a man
Source: http://ib.bioninja.com.au/_Media/str_med.jpeg

Figure 9 shows that the third suspected father is the actual biological father because the remaining half of the DNA fragments of the child matche with the third suspected father's DNA fragments.

After knowing about this technique, you might be wondering what if the amount of DNA recovered is contaminated or too low in quantity. Could we still go for DNA Fingerprinting?

Is it possible to compare a very little amount of DNA from source?

Yes, it is!! We frequently find a very little amount or partially degraded DNA source from crime scene. The answer to this problem lies in Polymerase

Chain Reaction technique. PCR generates multiple identical copies from trace amounts of original DNA evidence in just a few hours. The generated copies are known as 'amplicons'. PCR is an excellent technique for replicating single-stranded DNA from a template with the help of synthetic primers and a polymerase enzyme.

DO YOU KNOW?
Dr. Kary Mullis invented the Polymerase Chain Reaction (PCR) technique, for which he shared the Nobel Prize in 1993.

How a PCR works?

PCR amplification is a chain reaction of series of three steps repeated in a sequential manner, dozens of times-

A) Denaturation

This is the first step of PCR amplification in which helix of DNA unwinds to separate the two strands of DNA by raising the temperature up to 98°C. Now each strand acts as a template for synthesising new strand. Ingredients of this step are a primer (like probes of RFLP), base pairs and a heat stable DNA polymerase usually Taq polymerase.

B) Annealing

During this step the temperature of reaction mixture is lowered to 50–65°C, allowing the primers to cool down and settled to the single-stranded DNA templates. This process is known as annealing. Primers have been named so because they mark the initial location for the synthesis of new strand of DNA.

C) Elongation

In this step, DNA polymerase came into play. Temperature of reaction mixture is again raised to approximately 75–80 °C and Taq polymerase add new nucleotides to the primer complementary to parent strand.

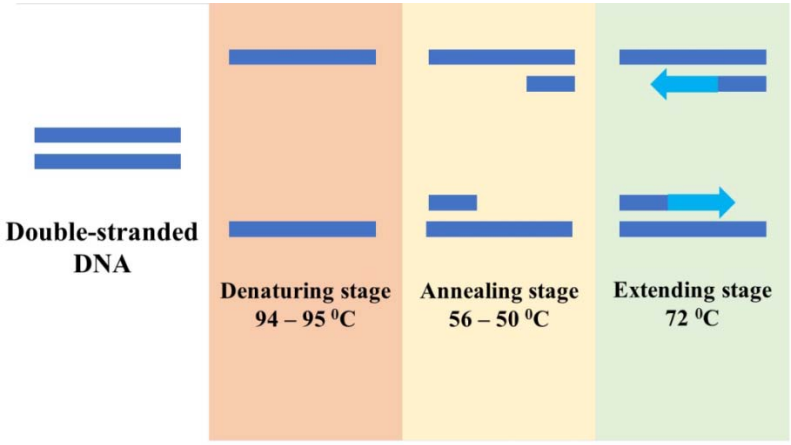


Figure 10: Steps of PCR

10.1.2 Applications of DNA Fingerprinting

There are so many applications of DNA profiling in the field of Forensic science and personal identification. A few are as follows:

1) **Identification of any Individual:** We can identify an unknown person, dead or alive from his/her DNA by comparing it with a pre-recorded DNA database.

2) **Identification of Offender:**

   a) **Violent Offence:** Biological traces left by the offender in a violent offence on the scene of occurrence are used to generate the DNA profile and compare it with the DNA samples from suspected one to exclude the innocents and identify the real offender.

   b) **Sexual Offence:** In case of rape, the seminal stains found on the clothing, body or anything related to the victim are collected to create DNA profile to compare with the profiles of suspected offenders. In case of gang-rape the offenders are identified by generating their Y chromosome profile (DNA from Y chromosome) to separate them as individuals from mixed seminal stains.

3) **Prove the Paternity:** In a case of suspected paternity, a man can be proved to be the biological father of a child by the comparison of his and the child's DNA profile.

4) **Prove the Maternity:** In a case of suspected maternity, a woman can be proved to be the biological mother of a child by the comparison of her and the child's mitochondrial DNA.

5) **Ancestry:** The ancestral links of a person can be identified from the Mitochondrial DNA and Y-Chromosome profiling in cases of historical importance.

6) **Ancient DNA:** Skeletal remains found from war-field or tombs can be useful due to the higher resistance of DNA extracted from bones. This can be very useful to identify soldiers and famous persons in the history.

Check Your Progress 1

1) What is the structure of DNA?

……

……

……

……

……

……

2) What is PCR and how can it be applied in DNA fingerprinting?

……

……

……

……

3) What are the applications of DNA profiling in forensic science?

……

……

……

……

10.2 OTHER BODILY IDENTIFICATIONS

It is not that we always need DNA for personal identification. Sometimes, other bodily features are enough to identify a person conclusively. To identify a person, usually two types of characteristics are used-

i) Class characteristics, that belong to a group of people and
ii) Individual characteristics, those are unique for each person.

In this context, skeletal remains, retina, iris, fingerprints, etc., are the most accepted means for personal identification.

10.2.1 Somatometric and Somatoscopic Identification

The measurement of various aspects of the human body through landmarks is known as Somatometry. Before discussing the various landmarks for Somatometric measurements, let us have a look on some of the precautions that should be taken care off while taking these measurements-

1) Measurements should be taken in minimum clothes.
2) The person should follow the posture as per the requirement of the measurement
3) The feet should run parallel and the heels touching the wall.
4) Shoulders should not rise upwards.
5) The head should rest in the eye-ear plane.

Different Aspects of Somatometric Measurements

A) Landmarks:

There are several landmarks all over the human body for making these somatometric measurements. These landmarks are some fixed and prominent points of human body and can be very useful to distinguish or identify a person because the position shape and size of these landmarks varies within individuals

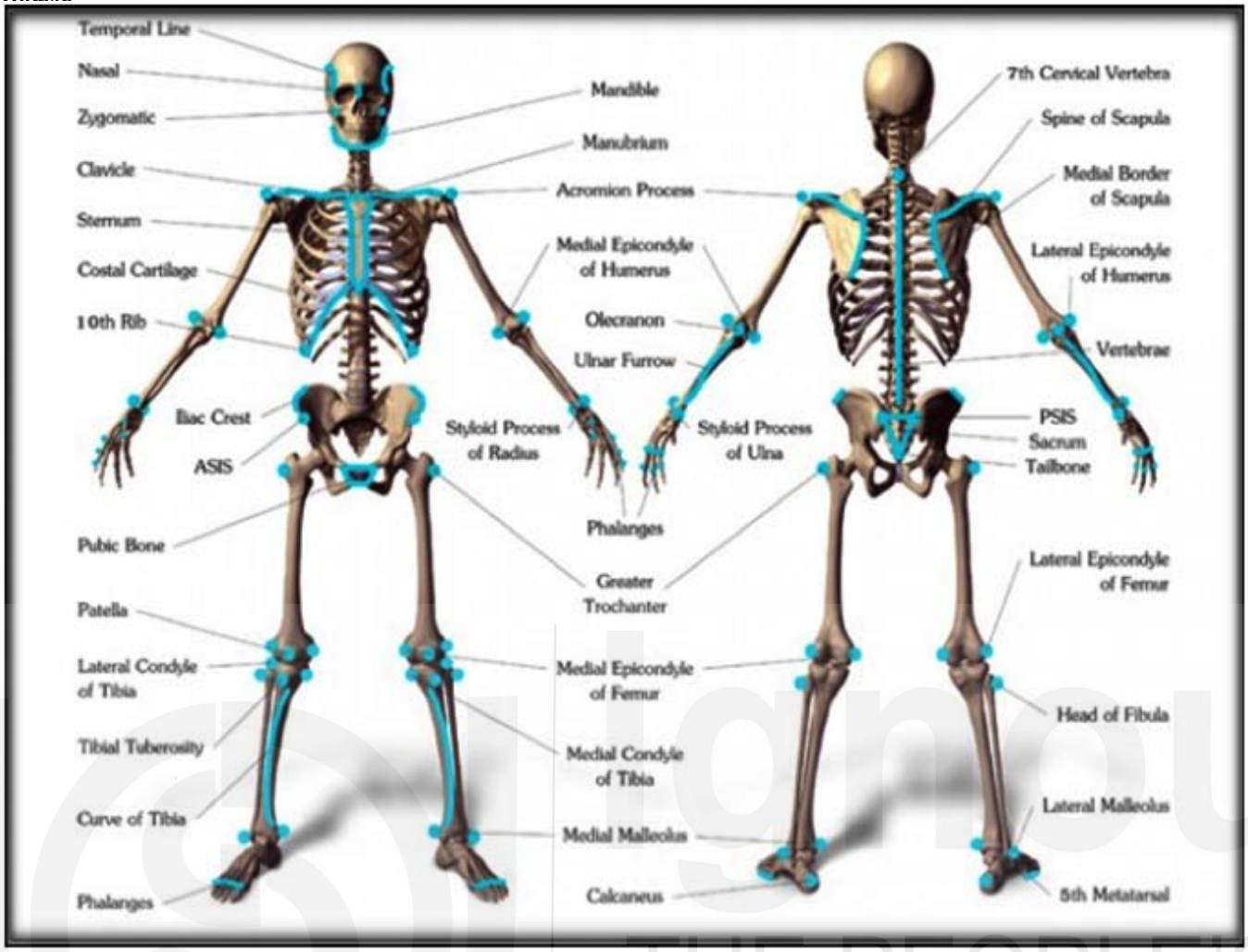


Figure 11: Landmarks of Human Body

Source: www.porko.com

B) Measurements of Body:

Different distances of one point to another throughout the human body are measured. Commonly measured ones are listed below-

- ❖ Projection of height in standing position
- ❖ Measurements of extremities and span
- ❖ Measurements of the dorsal side
- ❖ Projective height in sitting position
- ❖ Linear measurements of frontal trunk and neck
- ❖ Breadths and depth of trunk and waist
- ❖ Length and breadth measurements of the upper and lower extremities
- ❖ Head length and breadth measurements

C) Body Weight and Volume:

Body weight should be recorded in the minimum clothes or naked. Various apparatuses are used to measure body volume. Examination begins with the assessment of the skin color, shape of the chest, abdomen, legs, development

of muscles and condition of the musculo-skeletal system along with other parameters.

The condition of the musculo-skeletal system is evaluated on the overall impression of width of shoulders, massiveness, posture, etc.

Figure 12: Anthropometric Rod
Source: Source: http://www.seritex.com/gpm-anthropometer-100

Figure 13: Sliding Calipers
Source: http://medidentitalia.com/product/boley-sliding-caliper-mm145/

Figure 14: Rod Compass

Source: www.modulor.de/en/ecobra-aluminium-rod-compass.html

10.2.1.1 What is Somatoscopy?

The study of shape, size and position of different parts of the body is known as Somatoscopy. Identification of an individual is done by identifying and measuring shapes and size of the different parts of the body.

A) Body Types:

Body shape is defined on the basis of the shape of head, neck, thorax and limbs of an individual. All individuals are divided into three different body shapes; asthenic, normosthenic and hypersthenic respectively.

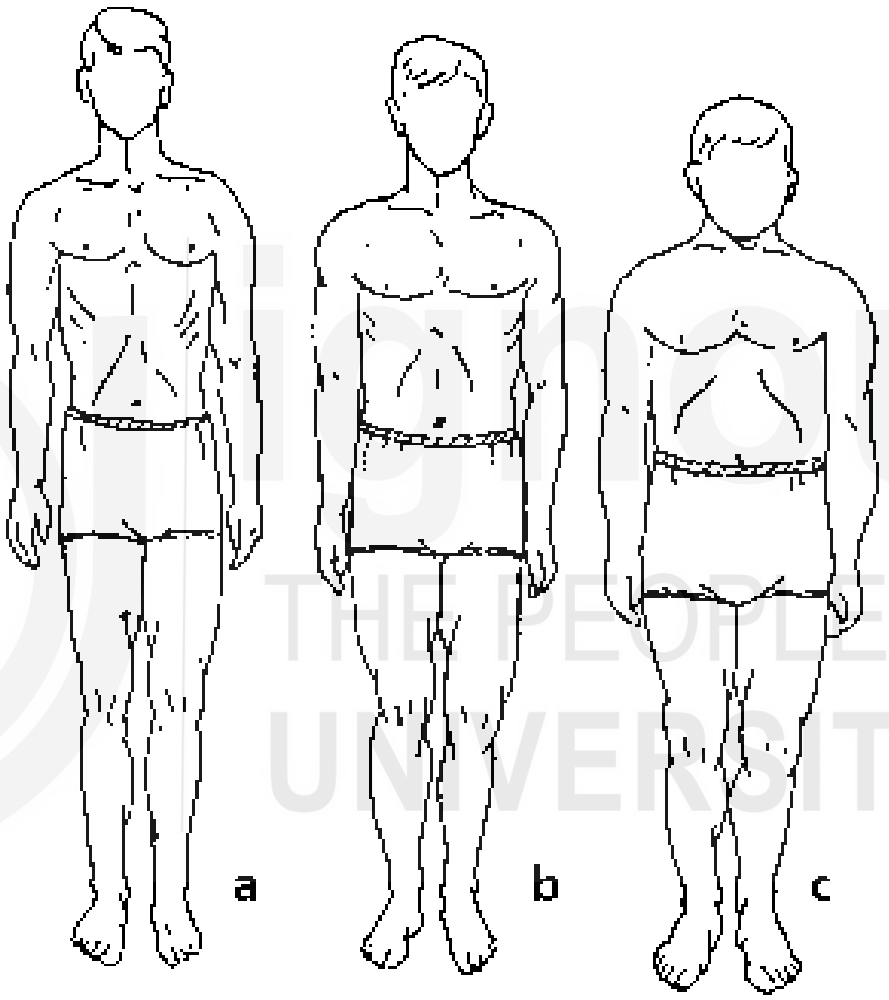


Figure 15: Different Body Types (a) asthenic (b) normosthenic (c) hypersthenic
Source: https://myvaleology.com/english/fiz/statii/physical_growth-en.ht

B) Toe shape:

Toe shapes are defined on the basis of their orientation.

1) Normal (the axis of the lower limb in normal)
2) O-shaped deformity of the lower limbs (bone angled outwards) (Varus)
3) Bone angled outwards (Valgus).

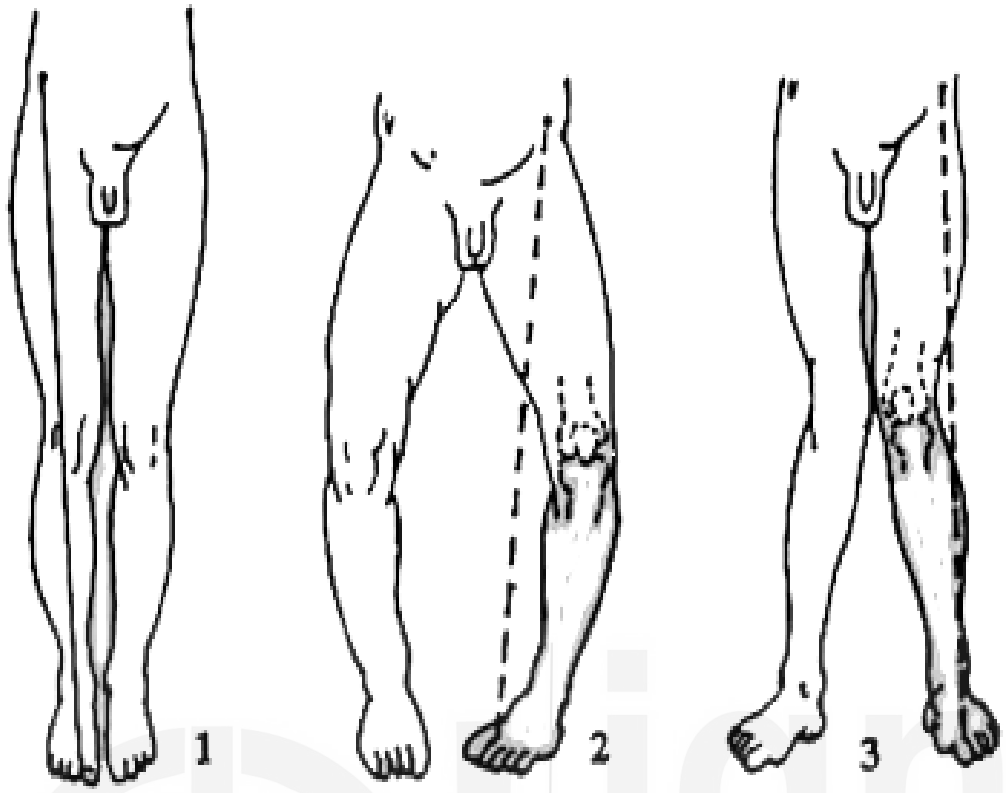


Figure 16: Different Toe Shapes (1) Normal (2) Varus (3) Valgus

Source: https://myvaleology.com/english/fiz/statii/physical_growth-en.ht

C) Neck: Projection of 'Adam's Apple', size and volume of the neck are the main aspects of observation.

D) Extremities: Width and symmetry of upper and lower extremities vary within individuals.

Apart from aspects mentioned above, a large number of external features like Breasts, Buttocks, Hand, Fingers, Finger Nails, Foot, Toe Nails, Skin Colour, Head, Face, Eyes, Nose, Lips, Chin, Prognathism and External Ear are examined for individualisation.

10.2.2 Skeletal System

Skeletal remains not only help in somatoscopic and somatometric examinations, but are also assistive in age, sex and stature determination. Let us know how-

Which parts of the skeleton are used for age determination?

Teeth eruption sequence, appearance and fusion of ossification centers of bones and ossification of sutures of skull are the three fundamental source of age determination.

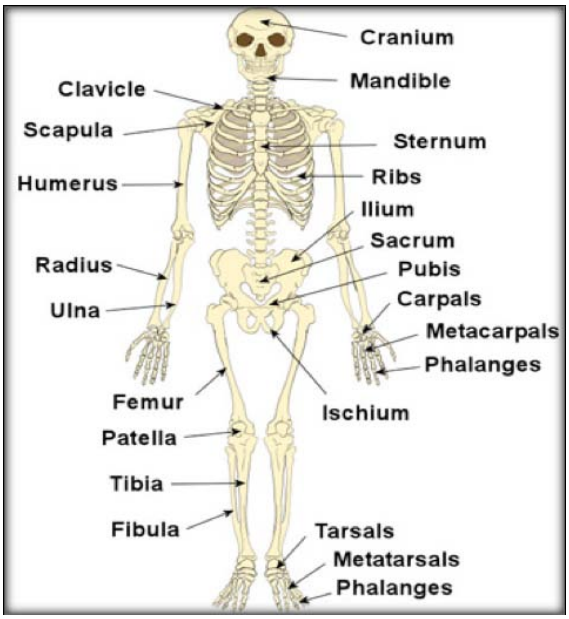


Figure 17: Skeletal System of Human body

Source: www.toppr.com/guides/science/body-movements/skeletal-system/

A) Eruption of Teeth:

Every human has two set of teeth in a whole lifetime. There are 20 temporary and maximum 32 permanent teeth. The eruption and shedding time of temporary teeth and eruption time of permanent teeth are more or less stagnant for all individuals. Stack (1964) developed a method to estimate the age of fetus and newborn from the weight of the erupting teeth.

Gustafson developed a method to estimate the age of a person from six different anatomical changes in the teeth, observed microscopically. The anatomical changes are

- ✓ attrition,
- ✓ periodontosis,
- ✓ secondary dentin formation,
- ✓ cementum apposition,
- ✓ root resorption and
- ✓ root transparency.

B) Ossification of Long Bones:

Age of an individual can be estimated from the age of the appearance and union of long bones of the human body. Each long bone consists of multiple ossification centers with different appearance and union age. From long bones we can estimate the age of a person up to 70 years.

C) Ossification of Sutures:

Age can be estimated from sutures up to approximately 80 years. Following table shows the age of ossification of various sutures:

Table 1: List of common sutures and their age of ossification

Name of sutures	Age of ossification
Metopic	2-4 years
Anterior Fontanelle	1 and half – 2 years
Posterior Fontanelle	At birth – 6 months
Coronal	50 – 60 years
Sagittal	50 – 60 years
Lambdoid	45 - 50 years
Parieto-temporal	60 – 70 years
Basioccipital and Basisphenoid	18 – 20 (Female) 20 – 22 (Male)

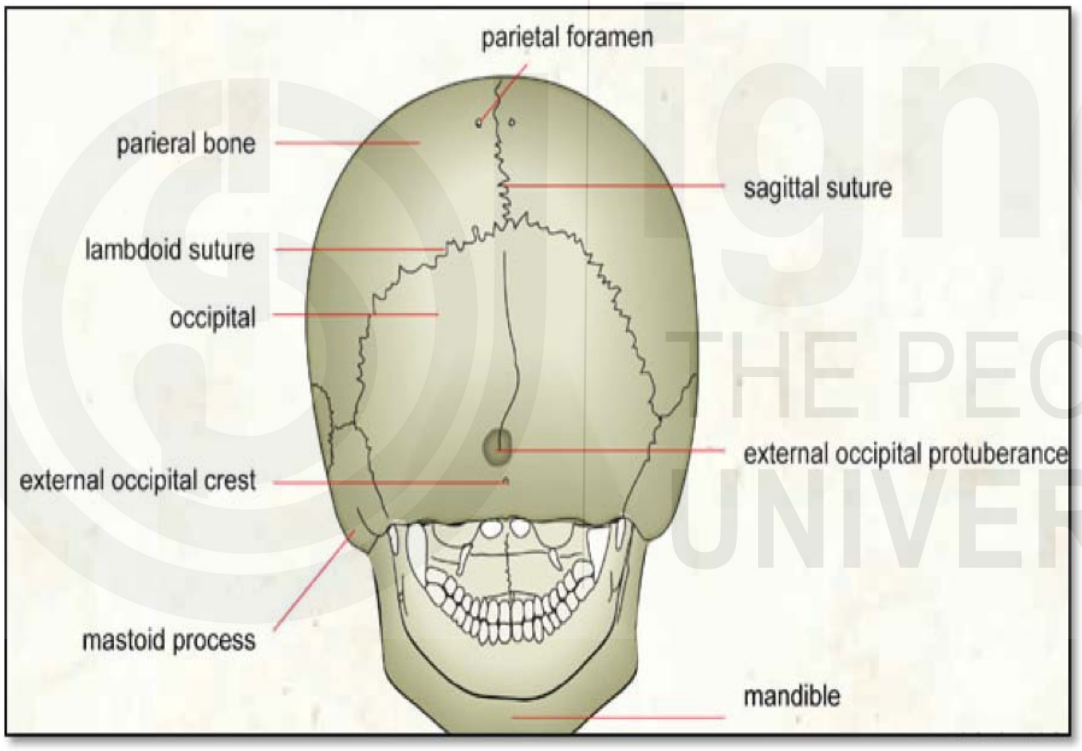


Figure 18: Common Sutures of Human Skull
Source: infovisual.info/en/human-body/skull-posterior-view

How Do We Determine Sex from Skeletal Remains?

Skull and pelvic bone are the two main parts of the skeletal system which are used for sex determination.

1) Skull

Glabella, zygomatic arch, nasion, forehead, mastoid process and ramus are the main distinguishable features among male and females.

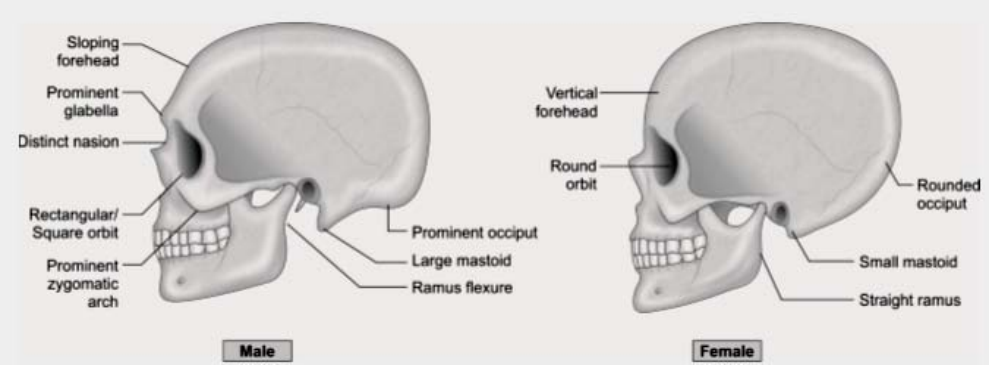


Figure 19: Male and Female Skull of Human

Source: Review of Forensic Medicine and Toxicology, Gautam Biswas, The Health Science Publisher, 3rd Edition

2) Pelvis:

Pelvis is the hip bone of the skeletal system. This bone joints together the upper and lower part of the body. There are a large number of differences between male and female pelvis. Following table shows the difference between some prominent features:

Table 2: Common differences between male and female pelvic bone

S. No.	Feature	Male	Female
1.	General Appearance	Larger, prominent muscular and rough	Smaller, less prominent muscular markings and smoother
2.	Acetabulum	Large, average diameter is 52 mm	Smaller, average diameter is 46 mm
3.	Ischial Tuberosity	Inverted	Everted
4.	Subpubic Angle	'V' shaped, 70^0 to 75^0	'U' shaped, 90^0 to 100^0
5.	Pelvic Outlet	Smaller	Larger
6.	Pelvic Inlet	Heart shaped	Elliptical or circular

10.2.3 Retina and Iris

Use of Retina and Iris as means of personal identification began around 1930. The first commercial version appeared in 1984.

How retina and iris are useful in forensic identification?

The large number of individual characteristics in the retina and iris of human eye makes it suitable for the process of personal identification. The complex structure of blood vessels present in retina is unique for every individual.

The colored part of the eye surrounding the pupil is called the iris. It consists of rings, lines and spots in different colors which are expressed due to the

expression of different alleles of the gene controlling the appearance of iris. It has almost two hundred suitable characteristics for identification. The system for identification based on iris cannot be fooled by lenses, glasses or eyes removed from a dead man. Among these two features, identification through iris is cheaper than retina.

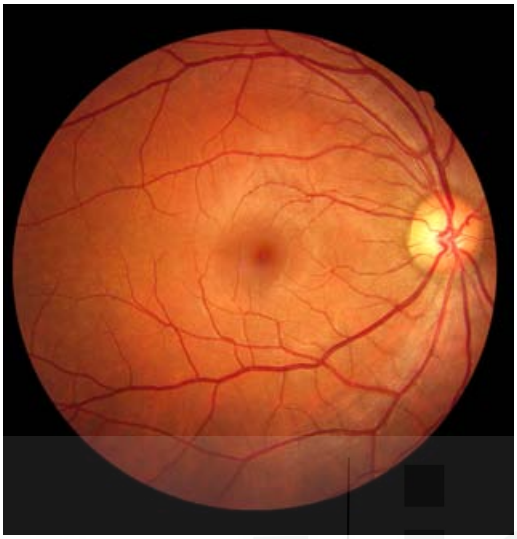


Figure 20: Complex vascular structure in human retina

Source:www.thermofisher.com/blog/biobanking/the-better-to-see-you-with-my-dear-biobanking-human-retinas/

How do we distinguish between the retina and iris of different individuals?

An automated tool is used to distinguish between these two features. This tool is known as 'Biometrics'. Each biometric system consists of four main parts. Input unit is used to measure and register biometric features. The extractor then extracts certain features from the whole. A characteristics identification database is present inside the system. Mathematical and statistical algorithms verify and compare the quantity and quality of the unknown input characteristics and compare them with previously stored ones.

Figure 21: Unique Structural and Optic Appearance of Human Iris

Source: www.medicalnewstoday.com/articles/319767.php

10.2.4 Ear

Ear is one of the five sense organs of human body. It is divided into external and internal ear. External ear helps to receive sounds and the internal ear processes it.

What is the structure of human ear?

The outer rim or helix and the shape of the lobe dominate the structure of ear. The inner helix runs roughly parallel to the outer helix and further forks into two branches at the upper extremity. The inner helix and the lower part of these two branches form the top and left side of the concha that merges into the inter-tragic notch.

What are the unique features of human ear?

Several unique features of ear make it a potential feature of identification. In fact, the two ears of the same person are different from each other and identical twins also have different ear.

The crux of helix, helix rim, auricular tubercle, anterior notch, anterior knob, tragus, inter-tragic notch, anti-tragus, posterior auricular furrow, anti-helix and lower- and upper crux of anti helix are the main variable features of the human ear. The lobulecan have various shapes such as triangular, round, rectangular and lobbed. The ear is classified as kidney-shaped, oval with an unattached earlobe, half-heart shaped and oval with attached earlobe. Shape of the auricle is classified as oval, round, rectangular and triangular.

The distance between the most superior point of helix and the most inferior point of the earlobe on lines parallel to the ear base determines the length of the auricle. The greatest distance between the ear base and the posterior part of the helix, on a line perpendicular to the ear base determines the width of the auricle. Among same age group of male and females, males exhibit higher values for both auricle length and width.

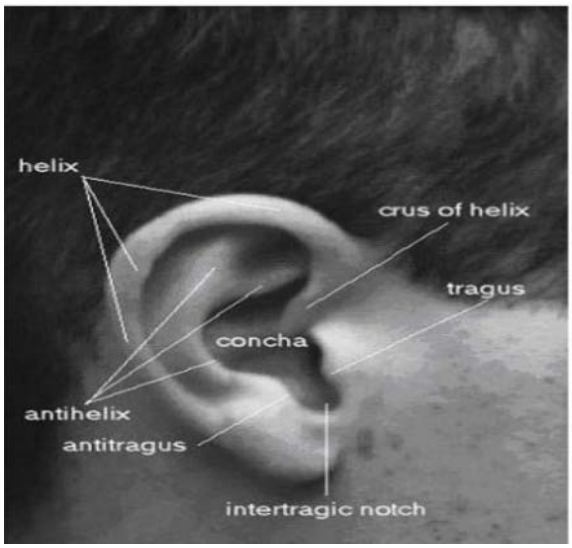


Figure 22: Different Landmarks of a Human Ear

Source: www.slideshare.net/piyushmittalin/ear-recognition-system

How ear can be examined as an evidence?

Ear is found as evidence in two ways. Either it is pressed over a solid surface to leave any structural impression or it may be visible in a video recording through the CCTV. Latent impression of the ear can be recovered by sprinkling fingerprint development powders and lifted through adhesive tapes. In both cases different aspects of the unique features are explored. Nowadays biometric systems have taken over the manual comparison methods for ear identification. A biometric system first extracts the comparable features from the unknown image of the ear and then compares it with previously stored data.

10.2.5 Fingerprints

Fingerprint is the most explored evidence in forensic science for personal identification. It is discovered very often in crime scenes. Fingerprint is a two- or three-dimensional impression made on a surface by the unique pattern like alternative formation of ridge and furrows present on the fingertips.

How many types of fingerprints are found?

Fingerprint can be found in three different states namely, patent, latent and visible.

1) Patent prints are formed when finger is pressed over a soft gradually hardening surface.
2) Latent prints are the most common and formed due to the chemical secretion of different glands present throughout the print region. Latent prints can be found on porous and nonporous surfaces but only visible when treated physically or chemically.
3) Visible prints are formed on any surface when fingerprints physically interact with a colored substance.

Is fingerprints a more conclusive tool than DNA for personal identification?

Yes, it is more conclusive!! The formation of fingerprint is affected by several epigenetic factors unlike DNA. As a result, identical twins possess different fingerprints although they carry entirely same DNA sequences. The collection, analysis and identification steps of fingerprints are much easier than DNA and not require sophisticated equipments.

Can we classify fingerprints?

Yes, we can classify fingerprints into four fundamental patterns as arch, loop, whorl and composite. These four classes are further subdivided into nine subclasses. Arch is divided into plain arch and tented arch. Loop is divided into radial and ulnar loop. Whorl is divided into plain whorl and concentric

whorl. Composite is divided into central pocket loop, twinned loop and accidental.

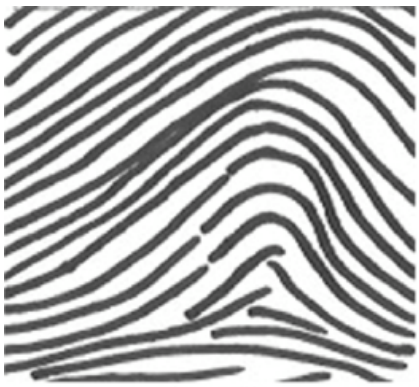


Figure 23: Arch
Source: Walsh, S., Pośpiech, E., and Branicki W. (2016) Hot on the Trail of Genes that Shape Our Fingerprints, Journal of Investigative Dermatology, 136(4):740-742

Figure 24: Whorl
Source: Walsh, S., Pośpiech, E., and Branicki W. (2016) Hot on the Trail of Genes that Shape Our Fingerprints, Journal of Investigative Dermatology, 136(4):740-742

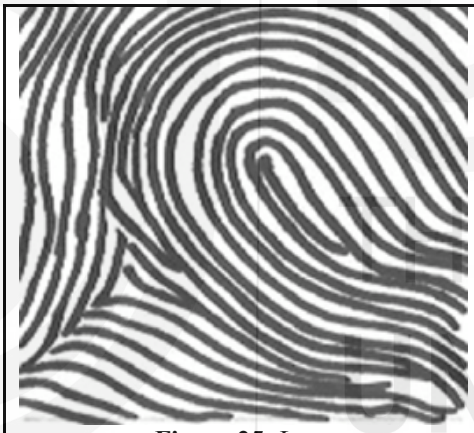


Figure 25: Loop
Source*:* Walsh, S., Pośpiech, E., and Branicki W. (2016) Hot on the Trail of Genes that Shape Our Fingerprints, Journal of Investigative Dermatology, 136(4):740-742

Figure26: Composite
Source*:* academyofhandanalysis.org/tag/composite-whorl/

How fingerprints of two persons are compared exclusively or inclusively?

Fingerprint of a person possess numerous details. These details are classified into first, second and third level details. First level detail consists of general ridge flow and fundamental patterns. Second level detail consists of different characteristic differences in ridge formation known as ridge characteristics. Third level detail consists of microscopic level data from a single ridge such as path deviation, width, shape, pores, edge contour, creases, breaks, scars and other minute permanent details. Comparison and matching of fingerprints are exclusively based on these three levels of details.

How latent prints are developed?

Latent prints can be found on porous or non porous surfaces. Various methods are used according to the nature of the surface. Latent print development methods are divided into three categories; powder method, chemical method and fuming method. Chemical methods are exclusively used for porous surfaces. Powder and fuming methods are commonly used for non-porous surfaces but some of them are applicable on porous surfaces.

A) Powder method:

Fingerprint powders are most common method of latent print development. The powder particles adhere to the lipid and water constituents of latent print residue and are lifted by adhesive tapes.

The first fingerprint powder was the combination of ground chalk and mercury. Lead was used as gray powders through the 1990s. Most modern nonmagnetic powders are composed of an adhesive carrier and a pigment. The adhesive part is a resin extract, such as stearic acid, raven powder, cornstarch, Lycopodium powder, *Acacia* powder, etc. Nowadays, four common types of fingerprint powders are commercially available.

1) Granular powder
2) Magnetic powder
3) Fluorescent powder
4) Metallic flake powder (copper, aluminum)

Figure 27: Fingerprint developed by black powder

Source: www.bvda.com/en/powder-suspensions

B) Chemical method:

Chemical methods are exclusively used to develop fingerprints on porous surfaces. Fingerprint matrix gets absorbed on porous surfaces because it permeates to liquids and gases. The water-soluble components of the fingerprint residue are absorbed and non-water-soluble components remain on the surface for a long time.

Most of the chemical reagents react with amino acids, sodium chloride and lipid components. Chemical fingerprint reagents are applied by dipping or spraying. The latent print change color or fluoresce according to the nature of the reagent. Fingerprint development process on porous surfaces should be in the following order-

1) Visual examination
2) Indanedione
3) Laser or Alternative Light Source (UV or IR)
4) DFO (1, 8-Diazofluoren-9-one)
5) Ninhydrin

Silver nitrate is another chemical used for latent print development on porous surfaces.

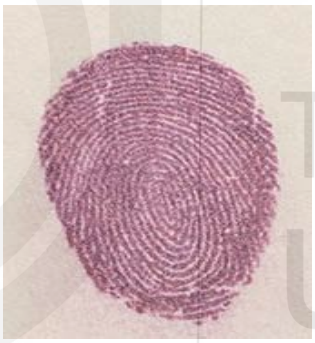


Figure 28: Fingerprint developed
Source:www.latentforensics.com/

Figure 29: Fingerprint developed by Silver nitrate by Indanedione
Source: www.arrowheadforensics.com/

C) Fuming method:

Fuming method is the deposition of the particulate matter of a vaporized substance on the latent fingerprint. This method works on porous as well as on non-porous surfaces. Iodine fuming is historically the first fuming method used for fingerprint development on porous surfaces. Iodine vapour reacts with the fatty acid of the sample and gives brown colored print.

Cyanoacrylate, a very strong adhesive known as superglue is used to develop fingerprints on non-porous surfaces. Vapours of Cyanoacrylate are created by heating and the particles in vapour form deposited on the fingerprint to form polymers of cyanoacrylate. Vacuum Metal Deposition (VMD) is an advanced technique used for print development. Gold and zinc convert into nano-particles in vapour state. These nano-particles deposited in between the

fingerprint matrix corresponding to the ridges.

Figure 30: Fingerprint developed by Iodine Fuming
Source: www.sirchie.com/

Figure 31: Fingerprint developed by Vacuum Metal Deposition
Source: www.goevidence.com/vacuum- metal-deposition-vmd

Check Your Progress 2

4) Is it possible to determine the age of a deceased person from only the skull?

……

……

……

……

……

5) What information can be drawn from the sequence of teeth eruption?

……

……

……

……

……

6) What is latent fingerprint and how can it help in forensic investigations?

……

……

……

……

……

……

10.3 SUMMARY

Personal identification is the most important aspect of criminal investigation and only forensic science can provide adequate information regarding this. A particular human individual can be distinctly identifiable from numerous anthropological features he/she possesses. The identification can be done from the features individually (DNA and fingerprint) or cumulatively (Somatoscopic and somatometric identification). This unit explains the anthropological features individually and also explains their application in personal identification with the processing details. DNA has possessed the highest uniqueness among the evidences generally discovered at crime scenes, although DNA is not readily available at the scenes as it is a microscopic substance. The common sources of DNA are found at the crime scene, including body fluids, hair, nails etc. As it is now established that the DNA of monozygotic twins are also unique in nature, DNA possesses the highest uniqueness among the anthropological features of a human being including fingerprint which was previously proved to be unique in every person. In addition to this, other bodily features can be evaluated by using somatoscopic and somatometric analytical techniques. The distinct characteristic features are studied under somatoscopy, while the measurements and changes in the characteristics with ageing come under somatometry. In addition to identification, somatometric techniques can also estimate the age of a living or deceased person from their skeletal remains.

10.4 REFERENCES

Bertino, A.J., & Bertino, P.N. (2012). *Forensic science fundamentals*. South Western Cengage Learning, USA.

Biswas, G. (2015). *Review of forensic medicine and toxicology* (3rd ed.). The Health Science Publisher, New Delhi.

Daluz, H.M. (2019). *Fundamentals of fingerprint analysis* (2nd ed) CRC Press, Broken Sound Parkway, New York, Suite 300, Boca Raton.

Gunn, A. (2009). *Essential forensic biology* (2nd ed.). Wiley-Blackwell, Singapore.

Harbison, S., & Fleming, R. (2016). *Forensic body fluid identification: State of the art, research and reports in Forensic Medical Science*, 6 (11-23), doi.org/10.2147/RRFMS.S57994

http://onin.com/fp/level123.html

http://study.com/academy/lesson/mannerism-art-definition-characteristics-examples.html

http://www.yourarticlelibrary.com/essay/anthropology/study-of-somatoscopy-22-somatoscopy-techniques-to-study-various-parts-of-human-body/41840

Kannan, K., & Mathiharan, K. (2012). *Modi's A textbook of medical*

jurisprudence and toxicology. Butterworth & CO. (INDIA) LTD., Bakehouse Lane, Fort, Bombay.

Kaushal, N., & Kaushal, P.P. (2011). Human earprints: A review. *Journal of Biometrics and Biostatistics.*

Lee, R. (2012). *Forensic biology*. CRC Press, Broken Sound Parkway, New York, Suite 300, Boca Raton.

Marinovic, D., Njirić, S., Čoklo, M., & Muzic, V. (2011). Personal identification by eyes. *Collegium Antropologicum, 2* (347–350).

Reddy, K.S.N. (2014). *Essentials of forensic medicine and toxicology.* Jaypee Brothers Medical Publishers (P) Ltd., Haryana.

Roewer, L. (2013) 'DNA fingerprinting in forensics: Past, present, future'. *Investigative Genetics, Vol. 4.*

Saad, R. (2005) 'Discovery, development, and current applications of DNA identity testing'. *Baylor University Medical Center Proceedings, Vol. 18*, Issue. 2, p.p. 130 – 133.

Sharma, B.R. (2016). *Forensic science in criminal investigation & trials.* Universal Law Publishing/LexisNexis, Haryana.

www.shsu.edu/~chm_tgc/primers/pdf/CE.pdf

10.5 ANSWERS/HINTS TO CHECK YOUR PROGRESS

1) DNA deoxyribonucleic acid that is made up of two strands, as it is known as a double helix. It is a linear polynucleotide which consists of four monomeric nucleotides. Each nucleotide contains three components: a deoxyribose sugar, a nitrogenous base, and a phosphate group. Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) are the four bases. The phosphate group is attached to the deoxyribose sugar that is further attached to the nitrogen of a base. Each nucleotide is linked by phosphodiester bonds with the other. Nitrogenous bases are complementary to each other. Adenine (A) only binds with Thymine (T) by double bond and Guanine (G) only binds with Cytosine (C) by triple bond. For example; if the order of the bases in a section of one strand is GTCCAT, then the order of bases in the complementary section of DNA in the other strand will be CAGGTA.

2) PCR is the abbreviation of Polymerase Chain Reaction technique. It generates multiple identical copies from trace amounts of original DNA evidence in just a few hours. The generated copies are known as 'amplicons'. It is an excellent technique for replicating single-stranded DNA from a template with the help of synthetic primers and a polymerase enzyme. PCR amplification is a chain reaction of series of three steps repeated in a sequential manner, dozens of times i.e., denaturation, annealing and elongation. The double stranded DNA unwinds in the denaturation stage under very high temperature (98^0C),

while the denatured strands are further cooled to 50-56 ^{0}C to settle down for further chain elongation. The cooled single strands are finally heated to approximately 72 ^{0}C to initiate the elongation process that by addition of primer and individual bases with the help of polymerase enzyme.

The PCR technique solved the problem of analytical limitations due to low quantity of DNA or partially degraded DNA as it can create very huge number of copies of DNA from a very small fragment. In fact, this technique can quantify the initial amount of DNA obtained from the source and the amount of degradation in it. PCR can generate complementary DNA to the RNA strand obtained from a cell which is also used in the identification of body fluids.

3) DNA profiling or DNA fingerprinting is the gold standard of the investigative techniques in forensic science as it gives the highest conclusiveness of the results among all the evidences usually found in crime scenes. Among the several applications of DNA profiling, personal identification is the first and foremost important. As we know that the DNA profile of each human individual is unique, hence, it can distinctly identify a person from mere a cell of his/her body. Addition to this, DNA profiling is widely used in the paternity and maternity disputes. Since, the child inherits half of the profiles from the mother and the father, the profile of the child can be compared with the suspected father and mother to confirm the biological parentship. In sexual assault cases, DNA profiling has proved to be the groundbreaking technique to identify the perpetrator as the semen sample consists spermatozoa which contains the DNA of the person involved in the offense. The DNA profile generated from the semen stains found in the crime scene or from the victim can be compared with the profiles of the suspected persons to confirm the involvement in the crime.

4) The age of a deceased person can be estimated from the skull itself as the skull consists of several irregular joints, known as sutures. Each suture has a specific time period for fusion during the lifetime of the person. Addition to this, the sutures fuses in two parts, as it fuses internally as well as externally due to calcification. Among all the sutures, the posterior fontanelle is the first one to fuse in between the time of birth to 6th month after birth, while the parieto-temporal suture fuses in the range of 60 – 70 years age. IN between these two sutures, there are significant number of other sutures which usually fuse at different age during the whole lifetime.

5) The teeth eruption sequence is basically useful for the age estimation of a person living or dead. There are two types of teeth present during the whole lifetime of a human individual, i.e., temporary and permanent. There are 20 temporary teeth and a maximum of 32 permanent teeth erupts during the whole life. The timeline of the eruption of both type of teeth is more or less similar in all the humans irrespective of their

geographical origin, race etc. These different timeline for different teeth can be useful for the estimation of the age of a person up to approximately 18 years as most of the permanent teeth usually erupts till this age with very few exceptions.

6) A latent print is an impression of the fingers or palms of the hands that has been transferred to any surface which is generally invisible or least visible to the naked eyes as these prints does not consist of any dye or any other colored materials. Latent prints are also two dimensional prints in nature as the three dimensional prints or plastic prints consist of depth which gives them a outlook that is easily visible to the naked eyes. Latent prints are the most common type of fingerprints usually found in crime scenes. Since, we know that fingerprints are unique for each individual as the two fingers of a same individual also not possess the same pattern or characteristics, the prints are used in personal identification. These prints can conclusively verify the presence or involvement of a person to a particular place or a crime.

ignou
THE PEOPLE'S
UNIVERSITY

PRACTICAL MANUAL

ignou
THE PEOPLE'S
UNIVERSITY

PRACTICAL MANUAL*

Contents

1.0 Introduction

1.1 Study of Human Long Bones. Estimation of Age, Sex and Stature from Bones

1.2 Somatometric and Somatoscopic Observations on Living Persons

1.3 Identification of Bloodstain, Urine, Semen and Saliva

1.4 Examination of Fingerprints and Handwriting

1.5 References

LEARNING OBJECTIVES

After going through the manual you will be able to:

- understand the practical aspect of samatometric and somatoscopic observations;
- identify bloodstains, semen, urine, and saliva;
- examine handwriting and fingerprint for personal identification; and
- study long bones by estimating age, sex and stature from skeletal remains.

1.0 INTRODUCTION

Personal identification is referred to as establishing an individual's identity. The need for personal identification mainly occurs in mass disasters such as tsunamis, floods, landslides, etc, and in case of man-made disasters such as bomb attacks, and terrorist blasts, and in cases where the body is highly decomposed or dismembered intentionally to conceal the person's identity. The identification of the individual from the skeletal remains is an important part of forensic anthropology. The main task while personally identifying the individual is to recognise whether the bones are of human or not.

The main aspect of personal identification are determination of sex, stature, age, and ethnicity. The science of measurement of human body bones i.e., Somatometry also has an extreme importance in racial classification, designing clothes and various types of equipment, and also in evolutionary studies. This section will help you with the description of standard techniques, methods, and positions for taking somatometric measurements. These measurements are defined based on various anatomical landmarks and are utilised in describing an individual's morphology.

*__Contributor:__ *Prof. Rashmi Sinha and Ms Kaneeka Joshi, Ph.D Schalor, Faculty of Anthropology, School of Social Sciences, Indira Gandhi National Open University, Maidan Garhi, New Delhi*

Another sub-disciple of Forensic Science for personal identification is Forensic Biology. Forensic Biology and Serology are sub-branches of forensic science that mainly deal with biological evidence and its analysis. While examining crimes such as robbery, murder, and rape, the analysis of biological evidence plays an important role in associating the criminal with the scene of the crime. Such biological evidence may be in the form of stains, body fluids, or other evidence such as blood, semen, saliva, or urine. Fingerprints and handwriting are the types of evidence that cannot be changed throughout life and no two persons have the same kinds. Fingerprints possess both class and individual characteristics discoverable by the forensic expert. They both have immense importance in personal identification.

1.1 STUDY OF HUMAN LONG BONES. ESTIMATION OF AGE, SEX, AND STATURE FROM BONES

Forensic osteology is a discipline under forensic anthropology and is ideally related to the examination of the human skeleton for medico-legal objectives. They are required for the human remain investigation that results from an undetailed and unexplained suicide, homicide, natural death, mass disaster, or genocide. Forensic anthropologists are asked to offer assistance to the forensic medical expert regarding the age confirmation of human beings for the goals of both immigration status and judicial accountability.

The fruitful macroscopic identification of human skeletal bone will majorly depend on the expertise of the forensic odontologist and also on what type of constituents are present in the skeleton. Minor sections from the rib fragments or long bone shafts are difficult to differentiate from animal skeletons, especially from sheep or pigs.

While determining the age, it is a good idea to classify changes in the skeleton into 3 different levels of the lifetime of an individual: development and growth, senescence, and equilibrium. The 1st level is majorly under environmental and genetic influences. It involves young adults and children who undergo the alterations that proceed in a comparatively well-recorded pattern at some predictable rate.

The diversity of the composition of the adult skeleton provides a comprehensive array of growth-associated factors from which estimating age and accuracy are practically high. After reaching maturity, the age-associated changes in the skeleton of adults differ and are more population and individual-specific, majorly at the phase when maturity goes towards senescence and then to degeneration.

Determination of sex is generally the first step of the biological identification procedure. Sex determination reliability depends on the wholeness of the skeletal remains and the extent of sexual dimorphism present in the

population from which the person originates. There are mainly two morphological differences between female and male skeletons. Male bones are more robust and larger than female bones because of the larger attachment and more strong muscle mass, and time of delayed pubertal growth compared to a female. The pelvis of a male is firstly adopted to a bipedal gait whereas the shape and size differences of the female population reflect the biochemical compromise between walking, and changes necessary for the large fetal head passage from the pelvic canal.

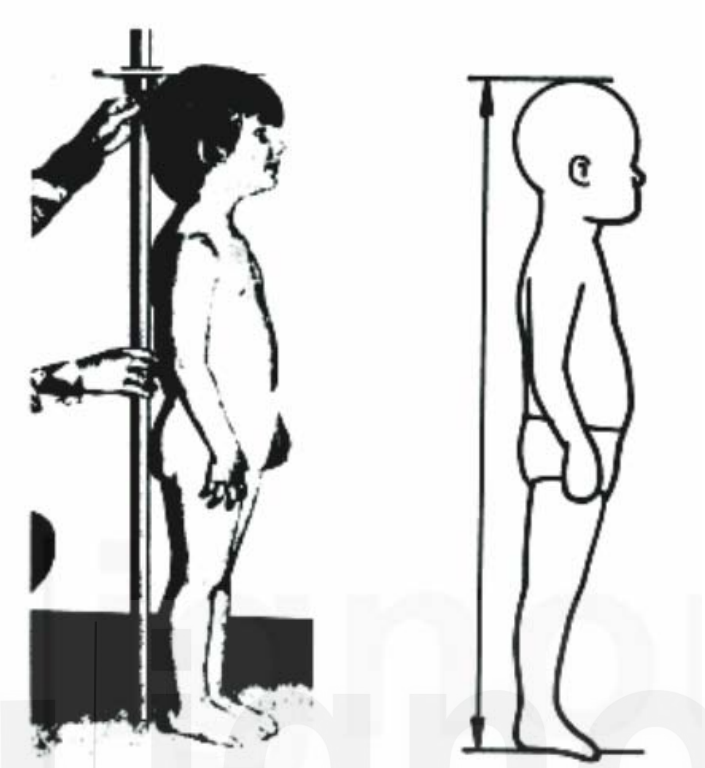
The individual's height can be estimated from the human skeletal remains using two different approaches. The first one is an anatomical method that includes measuring the height of every element of the skeleton contributing to the stature of an individual in life. Estimation of stature is obtained from the long bones' measurement. The long bones are the femur, tibia, and humerus. In case of non-availability of bones, the radius, fibula, and ulna also offer a good range for the expected individual's height. Impartial body fragments can be used to predict height, first by estimating the full bone length using a regression equation before applying any formula to estimate the stature.

The accuracy comes with undamaged bones of known origin and sex, as the individual's height is dependent on race and sex. Most of the forensic concepts use regression equations using which the stature of adult males and females is computed. These formulae have the lowest standard error for the tibia and femur as these bones form an important part of the height of an individual.

Lets understand the procedure

- The participant stands erect, barefoot on a level floor towards the wall with the back and buttocks touching the wall.
- Ensure that the heels are directly touching the wall and the toes are at a 45-degree angle to each other
- The shoulders should not be elevated in the air.
- The arms should be placed in the standard arm hanging spot and the hand's palm should directly touch the thighs.
- The anthropometer rod is placed on the subject's back if the vertical wall is not there
- The subject's head should rest without any stress in the FH plane (right orbitals or tragion should lie in the same plane horizontally) or eye-ear plane.
- The subject's position is fixed. You as an expert stand on the subject's right side with an anthropometer in the subject's median sagittal plane and permits the moving cross-bar to touch the location of the vertex gently.

Note: The anthropometer should be placed in the vertical location.



Source: www.ovrt.nist.gov

1.2 SOMATOMETRIC AND SOMATOSCOPIC OBSERVATIONS ON LIVING PERSONS

A forensic anthropologist is an expert who is provided the task of determining the gender, age, stature, and race of skeletal human remains, having the final objective of identifying the individual. If sufficient bones and some important bones are available such as the pelvis, skull, and long bones, the work can be done. In case of the availability of few bones, the identification becomes more complicated and only a few characteristics of the decedent can be identified.

There are a number of physical characteristics which cannot be measured easily. Most of the somatoscopic characteristics show marked geographical changes. Somatoscopy refers to the organized visual observations of physical characteristics of different parts of the human body for a correct and accurate detailed description. These observations are qualitative in nature and thus, descriptive in their approach. In order to standardize the approach, different charts have been made by different researchers for determining the color of the eye, skin, hair, etc.

Skin colour: Have you ever realised that the color of our skin is not consistent all over the body, number of physiological parameters such as arterial area or supply of venous blood and other environmental parameters such as contact with sunlight are associated with the appearance of skin color.

There are changes and somewhat a minor difference in the color of the skin of unexposed and exposed body parts. The skin represents two types of pigmentation, one is climatic and the other is inherited. Thus, color of the skin is considered at two important sites: cheek or forehead and the internal side of the upper arm i.e., which is not exposed to the sun.

Hair colour: While looking at someone's head hair so many characteristics come into observation. These are the quantity, colour, and form i.e., texture all forms a component of somatoscopy. For form and colour, hair is majorly studied. As per Fischer and Saller 1928, utilized natural hair to make a chart of hair colour. It should always be noted that color is not dyed and in such scenarios, the colour present in the hairs of the root is to be considered as the hair colour.

The hair colour is affected by oil, perfume, and age. In Indians, the hair colour is classified as light brown/medium brown, dark, and black-brown.

In relation to hair form, it can be broadly classified as wavy, straight, and curly hair. Straight hairs can be smooth, stretched, fat wavy, or smooth. Wavy hair can be narrow, broad navy, narrow wavy, curly wavy. Woolly hairs can also be classified as frizzly, closely knit, spiral, and widely knit. Pluck the hairs from the scalp and it gives the right type of observation.

Nose: There are various morphological characteristics of the nose. Basic detailed terms aid in describing nose parts such as bridge, root, septum, wings, and tip. The nose tip can be downwards or upwards and the profile could be rounded on a certain point, flat or fully rounded. The nose root can be documented as a broad, medium, or narrow i.e., from the side view may look depressed which again may be deep or absent or medium, shallow. The nasal bridge may be documented as a medium, convex-slightly, or wavy-slight. The nasal bridge size may be broad, medium or narrow.

Face: Height-size of the face (short, medium, or long) and face diameter (very broad, broad, medium, narrow), malar prominence, shape, and prognathism aids in describing face. The shape of the face can be elliptical or oval, square or round or flat or quadrangular. Cheek bone prominence (malar) is an important characteristic, it is called as slight, absent, market or moderate. Prognathism is the alveolar protrusion of face.

Lips: The membranous lip thickness is observed with best observations in profile view. It might be medium, thick, thin, everted or puffy. Everted lip is the upper membranous lip is puffy with the convex portfolio, above which the integument lips are concave.

Somatometry is comprised of two words, 'Somato' which refers to living and 'metric' means measurements. It simply means measurements of human beings. Thus, Somatometry is a division of anthropometry is called as methodical method to measure human body involving face and head. Forensic Anthropologists have framed number of measurements for

explaining the man's morphology. All these measurements are not uninformed and depends on anatomical landmarks. They aid in comparing different kinds of men who are living in diverse geographical areas i.e., to study variation in types of body or for racial comparison. Children's physical growth is studied which is based on their measurements of body. The nutritional status of adults and young is also determined with the help of these measurements. It also helps in the assessment of some physiological purposes such as basal metabolic rate, vital capacity etc. The data obtained that is based on the anthropometry surveys of inhabitants has been an important asset for crafting proper instrument for use in defence and industry purposes, garments, spaceships, etc. The surveys of anthropometry also offer standards of the physique of any population and change in trends in morphological characteristics.

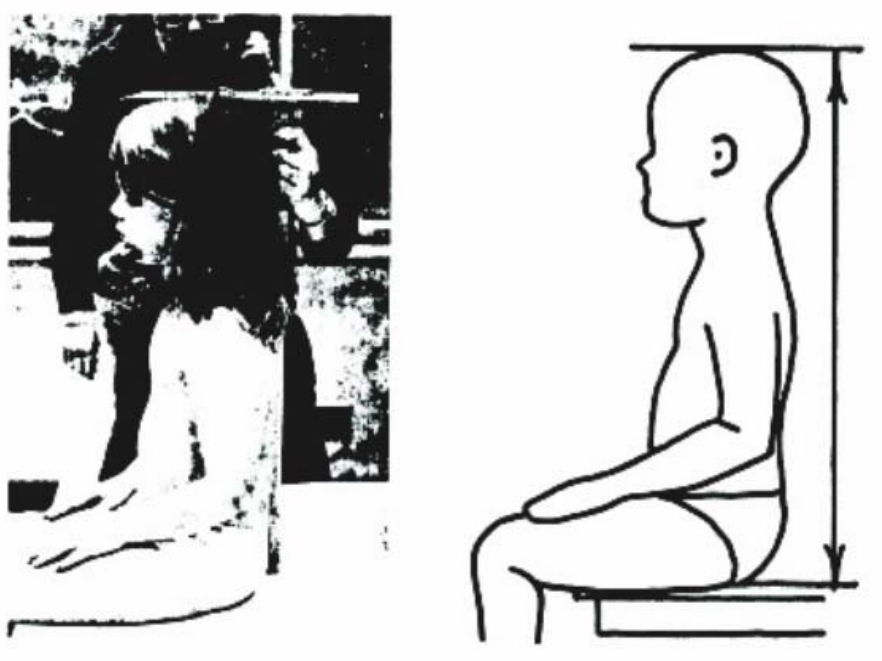
1.2.1 Let's see how some of the common measurements are taken:

Body Weight

- The participant stands with equivalent weights on both the feet.
- The head should be in the forward direction.
- The reading is recorded on the weighing scale when the needle is stationary.

Sitting Height:

- The subject sits on a horizontal position on a 30 to 40 cm height.
- Subject's head is placed in eye-ear plane and the body is overextended to some large extent
- Always observe that the shoulder should be in a parallel position, the thighs must be horizontal and the legs are allowed to be hanged from the table with knee's back touching the vertical area of the table and do not allow the knees to bend.
- The hands must rest on the thighs along with palms were in down position.
- Anthropometer should be positioned at the subject's back aligned to the vertical column and bring down the cross-bar in moving position on to the vertex and record the reading.



Source: www.ovrt.nist.gov

Head Length

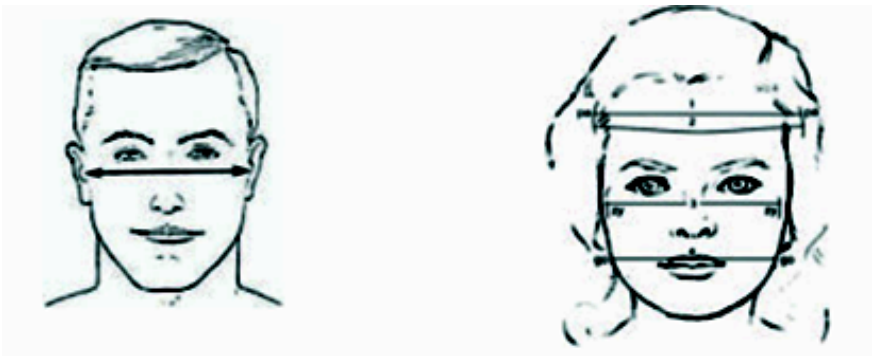
The calliper's left arm is placed on the glabella and move the right arm up and down on the head's back in the mid-sagittal plane, till you get the higher reading in the measurement scale. This is the maximum length of head.

Head Breadth

Place the arms of the spreading calliper tool in such a way that the calliper's joint is in the head's mid-sagittal plane. The calliper tips is slided away from forward to backward and backward to forward in criss-cross way starting with smaller and progressively to large circles till you get maximum reading on the measurement scale.

Bizygomatic Breadth

Take 2 arm tips of the calliper between the first finger and thumb, approx. about 2 cm away from the tragus. The tip is gradually slided over the zygomatic arch in such a way that the thumb should be in touch with the upper margin and the first finger. The maximum reading is documented.



Source: www.amazonaws.com ***Source*** www./ejo.oxfordjournals.org

1.3 IDENTIFICATION OF BLOODSTAIN, URINE, SEMEN AND SALIVA

Materials Required:

- Plastic Tubes – 2ml
- Cotton Swabs
- Glass microscopic slides
- Positive and negative samples
- Testing solutions

1.3.1 Introduction

The science that majorly deals with the reactions and properties of different bodily fluids or serums is called Serology. This is used in forensic science to examine various body secretions, which may found at a crime scene. The science of serology offers an efficient, quick and inexpensive method to determine if a stain had instigated from an organism or not. This sort of evidence also provides framework to the crime scene by recognising which bodily serum is present. The identification of kind of biological fluid present in a crime scene stain provides deep insights to the crime type, which may have been committed. These body fluids involve blood, semen, urine, and saliva.

While examining body fluids, first a presumptive test and then a confirmatory test can be performed to test the questioned sample. A presumptive examination provides an easy and quick examination, whether at the crime scene or in the science laboratory. A confirmatory examination or test offers a ultimate result for the sample that is in question.

These presumptive examinations used in the laboratory involve the phenolphthalein or Kastle-Meyer test for examining blood, the UV Light for detection of urine and the starch-iodine test for examining saliva. The Kastle Meyer test gives an instant pink color when the solution react with the blood because of its pH. The starch iodine test helps in the detection of human saliva through the starch breakdown into simple sugars in case of presence of saliva. And, in case of urine, the presence of a particular compound i.e., urobilin can also be seen giving bright yellow fluorescence under UV light.

1.3.2 Blood

1.3.2.1 Composition

Blood is a intricate vicious fluid having a pH of 7.4. It mainly consists of two i.e., plasma and cells (also called as corpuscles). The liquid section is called plasma whereas the solid part is called erythrocytes (red cells), leukocytes (white cells) and platelets (thrombocytes). In case of blood flowing out of the body, a section separates out as called blood clots comprising of blood cells,

fibrin, and discs. Fibrin helps in blood clotting and comes out of the plasms. The remaining liquid is called serum. The plasma without fibrinogen turns into fibrin.

- **Red Cells**

There are approximately 5 million red cells for a micro litre of blood, each for roughly 10 microns in diameter. These red cells are continuously producing and destroying in the body. The destruction rate is about 10 billion cells for an hour in a healthy adult.

- **Haemoglobin**

The colouring material of the RBCs is haemoglobin. The material is considered as of great importance for performing the bodily functions as it carries oxygen, salts, injected medicines and food to the various tissues of the body. It is useful in case of blood examination.

- **White Cells**

White cells is approx. 4000 to 11000 cells for a micro litre. They also prevents from the attack of ailments or diseases.

- **Platelets**

The platelets number is about 5 times of white cells. The also helps in clotting of blood.

- **Serum**

Serum and plasma are compound mixtures of minerals, proteins and organic components which are dissolved in water.

1.3.2.2 Functions of Blood

Following are the functions of blood:

- It carries oxygen and food to the tissues of body
- It helps to remove waste products of the body tissues by kidneys and other body parts
- It manages the acidity and temperature of the body
- It carries administered medications to the affected body parts

1.3.3 Semen

Semen is found in stains, smears, or in liquid type or it may also be found in anus, rectum or vegina. Semem in fresh form is la gel just like a fluid, that liquifies on contact with atmosphere. Usual semen ejaculation is approx. 3.5ml, that contains roughly 30 million sperms. The semen dry weight is 7% of the liquid weight. The morphological structure of sperm is definite and its identification in a body stain indicates the presence of semen.

The size and shape of human sperm is characteristic but the morphology alone doesn't help in personal identification. If a human sperm does not contain any spermatozoa then it's called aspermic semen. This can be because of some ailment or vasectomy surgery. One of the popular techniques for semen identification is electrophoresis.

1.3.3.1 Composition

It is a mixture of inorganic and organic compounds. Some of the essential semen constituents are proteins involving blood group, enzymes, fructose, choline, uric acid, zinc and citric acid. The composition differs from person to person. The presence of enzyme, acid phosphatase found in the crime scene is in higher concentration than those which are found in other bodily secretions. This enzyme provides a test for the location and identification of semen stains however, positive identification do not depend upon only the acid phosphatase test. Choline present in semen gives crystal tests. Citric acid, fructose, and zinc are less or more absent in other bodily fluids and thus their presence in semen should allow its identification but these compounds have not been used to any considerable amount so far.

1.3.4 Saliva

Stains or smears of saliva may found at the crime scene, in cigarette buds, on handkerchief, tumblers, postage stamps, envelopes, and cups or they might be found at a cloth piece that is used as a gag. Saliva contains an enzyme i.e., called ptyalin which when added to the starch, it gets hydrolysed. Extract of saliva when added to starch prevents its colour reaction with the iodine. Secretor's saliva contains blood group components and can be easily grouped. The saliva present on cigarette buds is frequently in criminal investigations. The evidential value of the saliva stains has increased because of the DNA profiling of the saliva. Food components mixed in saliva may also interfere with blood groups and saliva doesn't give particular precipitin test.

1.3.4.1 Composition

It comprises of three main components:

- 99.5% of water
- Organic Substances such as proteins, immunoglobins, non-immunoglobins, urea, glucose, vitamins, free amino acids
- Inorganic Substances such as sodium, bicarbonate, chloride, phosphate, fluoride, thiocyanate, potassium

1.3.4.2 Functions of Saliva Constituents

- Salivary Amylase: It is one of the main constituents of saliva which acts like an amylolytic which starts the starch digestion in the mouth, and the remaining of which is finished in the lower GIT (Gastro instestinal tract).

The remaining digestion is finished by pancreatic amylase.

- Salivary Lipase: This enzyme is secreted by the gland Von Ebner which aids in the fat digestion.
- Maltase: It aids in converting maltose into glucose
- Immunoglobins: Saliva comprises of IgM, IgG, and IgA. IgA is one of the most pre-dominants that helps in decreasing bacterial growth by binding to a particular bacterial antigen. It affects particular enzymes for bacterial metabolism and prevents colonization of bacteria.
- Lactoferrin: It functions as a bacteriostatic as it decreases the free iron amount in the blood which is needed for metabolism.
- Agglutinin: It possess sticky features which aids in clumping of bacteria which can be flushed away easily by saliva and swallowed.
- Calcium and Phosphate: These are the soluble complexes with carbonate lactate as it bounds to protein.
- Thiocyanate: It possesses antibacterial properties

1.3.5 Urine

Urine is recognised from the relatively large amounts of urine present on it. The stain located with the help of UV rays. Then, it is extracted with water and examined for urea. Stains of urine can now be personally individualised by DNA Profiling.

1.3.5.1 Nature

It is a body's liquid by-product that is secreted by the kidneys by a process called urination and excreted from the urethra. The general chemical composition of urine is majorly water but it also involves nitrogenous substances such as creatinine, urea and other metabolic waste constituents. Other components may be excreted in urine because of infection or injury of the kidney's glomeruli, which can changes the ability of nephron to filter or reabsorb the different constituents of blood plasma.

1.3.5.2 Composition

Urine is a liquid solution having more than 95 percent of water and other remaining constituents.

- Urea (9.3 g/L)
- Sodium (1.17 g/L)
- Chloride (1.87 g/L)
- Creatinine (0.67 g/L)
- Potassium (0.75 g/L)

- Other dissolved inorganic, organic compounds (such as hormones, proteins, metabolites) and ions (Kubic and Petraco, 2003; Mirakovits and Londino, 2016).

1.3.6 Laboratory Procedure

Basic Laboratory Procedure for identification

A) Presumptive Tests

For Blood

Kastle-Meyer Test

- Take 2 swabs of cotton as controls. Then, add 1 drop of the known blood sample to one of swab for a positive control. And, for the negative controls, add 1 drop of distilled water to the other cotton swab.
- After this, apply 2-3 drops of ethanol (95%) and 2-3 drops of phenolphthalein. Wait for about 10 sec. Add 2-3 drops of 3 percent of hydrogen peroxide (3%) to each of the swabs of cotton. Addition of hydrogen peroxide resulting in instant change of pink colour is a positive result. False positive result may arise if no time is there between drops of hydrogen peroxide and phenolphthalein.
- A positive control sample turns pink and the negative sample should not change to any colour. Colour changes to pink is a presumptive test for blood.
- 3 evidence stains are obtained. Using 3 different cotton swabs, moisturized with distilled water and then roll one swab over every stain.
- Then, follow the 2nd step on every cotton swab to assess if the stain tests presumptively positive for blood stain.
- Document your results of the presumptive tests in the table

For Saliva

Starch-Iodine Test

- Put a swab of cotton in your mouth and swab it inside your mouth
- Drop the end of cotton in a microcentrifuge tube. Then break the stick's stem
- Then, add 3 drops of the starch
- Follow the same procedure with a dry swab of cotton i.e., negative control sample
- Incubate the sample for about 1 hour at 3 degree Celsius
- After this, add 2 drops of the solution of Iodine and immediately note the

change in colour.

Note: Change in no colour is a positive result due to the amylase breakdown of starch

For Urine

Ultraviolet Light

- Take a swab of cotton and remove it from the package
- Find your nearest restroom and swab a toilet seat, toilet lip or toilet lid for urine
- Then, take an extra swab of cotton and moistened it with water. This will be a negative control for this test.
- Place both the swabs of cotton to the UV light and look at them under the light. Yellow fluorescence under the UV light signifies a positive test for urine

For Semen

Acid Phosphatase Test

- Put a small piece of 2X2 mm of suspected stains of semen
- Add stain material on whatman filter paper or on other suitable test paper. Use controls and standards involving negative, positive and unstained controls.
- Add one -two drops of Reagent (Buffer -50ml + Sodium alpha-naphthyl Phosphate -126g) and allow to react for 30 sec. No colour development takes place at this stage.
- Note: Buffer is made up of Glacial acetic acid -1ml+ Socium acetate anhydrous-2g + Distilled water -100ml
- Add one drop of reagent (Buffer -50ml and Naphthanil diazo blue B -250g). Document the result after 10 sec.
- Record the positive reaction upon rapid development of a purple colour, which indicates the presence of semen.

B) Confirmatory Tests

1) Sperm Confirmatory Tests

Christmas Tree Stain

- Take a dried semen prepared microscopic slide
- Put 3 drops of Christmas Tree stain A on the semen stain that is dried and allow it to settle for 15 min.
- Carefully rinse the microscopic slide with 95 percent of ethanol to

remain the B stain

- Let the stain air dry
- Add 1 -2 drops of immersion oil to the slide (sample)
- Analyse the slide by compound microscope with the objective of 100X. Recognise the sperm.

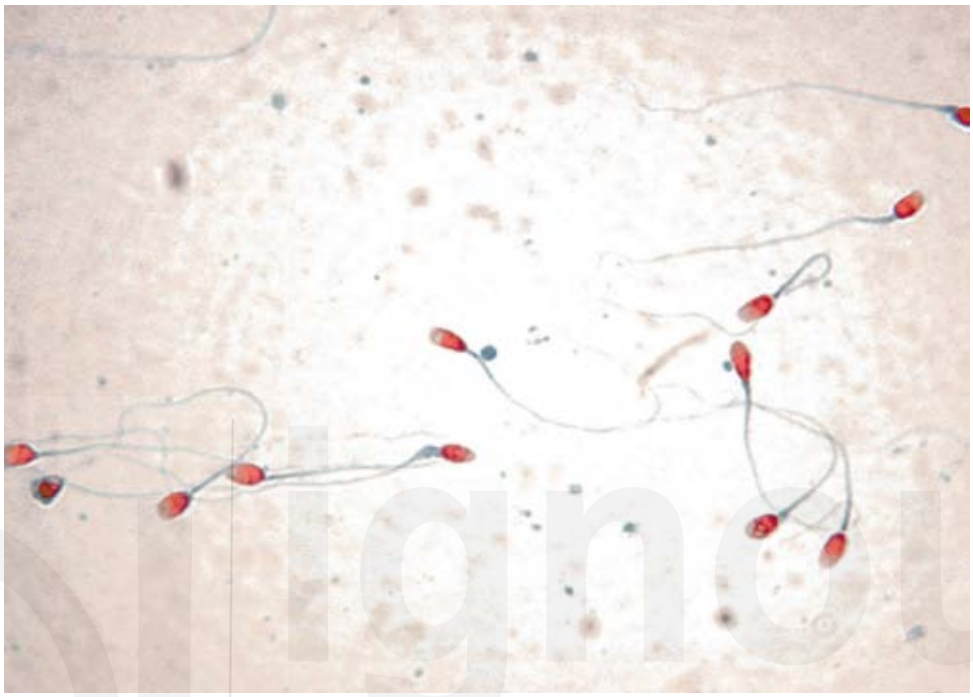


Figure 1: Christmas Tree Stain of Sperm Cells
Source: https://indianlegalsystem.org/indentification-of-semen-on-the-spot-part-2/

2) Takayama Test for Blood

- Put the material in investigation on a microscopic slide and then cover it with a cover slip.
- Add 1 drop of Takayama Reagent and permit it flow under the cover slip
- Gently warm the microscopic slide on a hot plate for 10 to 20 seconds at 65 degree Celsius
- Let it cool and observe it under microscope at 100X
- Pink needle shaped crystals of pyridine are appeared.

1.4 EXAMINATION OF FINGERPRINT AND HANDWRITING

The friction ridges that cover the finger tips, the surfaces consisting the palm of your hands and the outer regions of the soles of our toes and feet are formed at the time of gestation. All these friction ridges are developed fully during birth. The finger patterns created by these friction ridges are known to remain the same throughout the life. Additionally, it is also said that no two individuals have the identical or same fingerprints. So, the friction ridge

patterns on the fingers, soles, toes and palms have been utilised for identifying humans for more than a century. It means that the variables in the ridge patterns and characteristics make it certain statistically that no two people alive will have same fingerprints.

Fingerprints is the most interesting field in the areas of criminal investigation and positive identification. The friction ridge patterns of the fingertips are transferred readily when they exposed to a surface. Secretions coming out from the skin pores i.e., sweat, composed of about 99% of water and 1% of organic and inorganic compounds which are transferred readily from one tip of finger to any items they touch.

The different types of fingerprints that might be found at the scene of crime are hidden/invisible or latent prints, residual or visible and patent or plastic prints. Latent prints have the invisible deposit of body oils or sweat on a surface. Visible prints are opaque deposits of dirt, soot, blood or other materials transferred to other surface from the fingertips. Patent prints are the fingerprint impressions in soft items such as clay or putty.

Invisible or latent fingerprints are made visible using different powders, chemicals, or other ways. Visible prints are first observed by eye or with low magnification having 5X to 10X. They are formed when fingers are covered with any type of opaque article like blood, ink, grease that is transferred onto the area when contact happens between the surface and fingertip. Plastic fingerprints are formed when fingers touch the soft or pliable items that forms an impression like wet paint, wax, putty. Etc. Plastic fingerprints are the reversed impressions of the fingerprint ridge patterns and then they can be cast with a material of silicon casting.

Fingerprints possess both class and individual characteristics discoverable by the forensic expert. The most observed class characteristic of fingerprints is the usual flow of the friction ridge pattern, which comprises of a loop, arch and whorl.

A loop pattern comprises of a friction ridge pattern that enters from 1 finger side and curves to exit from the same finger side. Loop patterns contain 1 delta. A whorl pattern contains at least 1 ridge that forms a full elliptical shape in the centre of the ridge pattern. Whorls possess 2 deltas. Arches have a pattern that enters from 1 side of the finger and exits from the other finger side and no deltas are present in the fingerprint pattern.

Fingerprint's individual characteristics includes the location of smaller characteristics i.e., called minutiae. Cores, enclosures, bifurcations, delta, ridge endings and islands are all the identifiable characteristics that are present in different sections in each person's fingerprint.

There are 3 basic fingerprint patterns:

Loops: These patterns are classified into radial and ulnar and have 1 delta

Arches: These patterns are classified into plain or tented arch

Whorls: These patterns classified into plain, double whorl, central pocket whorl or accidental whorl and possess 2 deltas.

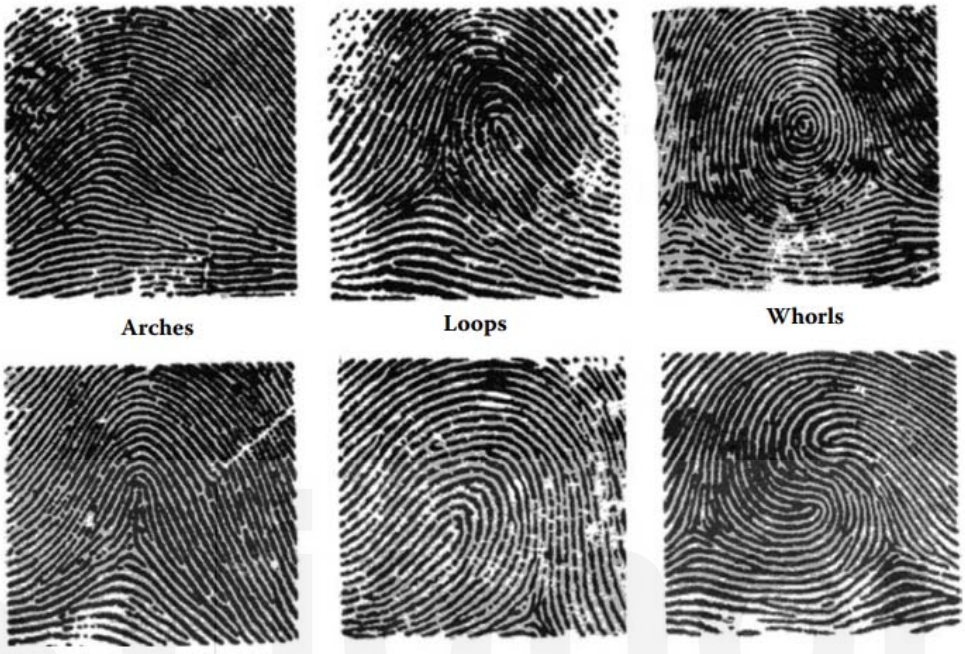


Figure 2: Basic fingerprint patterns

Source: https://epgp.inflibnet.ac.in/

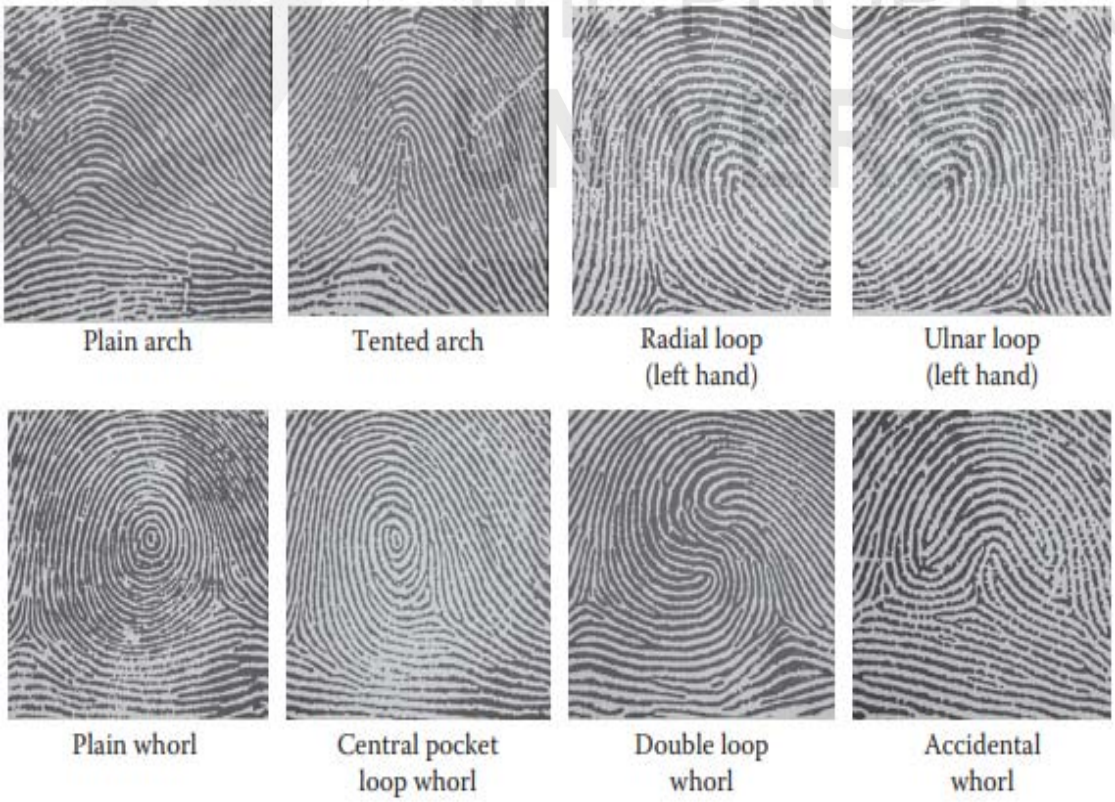


Figure 3: Class characteristics of fingerprints

Source: *Kubic and Petraco, 2003*

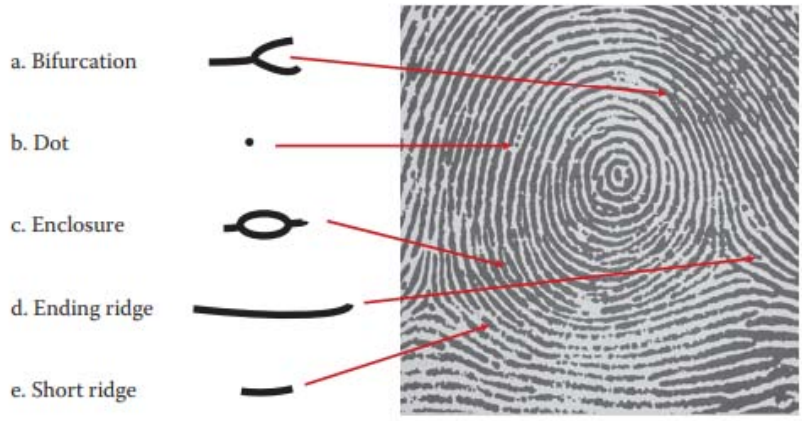


Figure 4: Individual characteristics of fingerprints

Source: *Kubic and Petraco, 2003*

There are number of means to gather and preserve the fingerprints based on the circumstances of how the fingerprints is formed. As discussed, these fingerprints can be patent, latent or plastic. Because of the different means prints can be visible, several techniques need to be used to collect and preserved properly.

Materials Required

- Fingerprint roller and ink with a glass piece of about 10m X 9m or other smooth area to spread the ink
- Fingerprint recording cards
- Magnifying glass
- Ceramic or glass article to dust for latent prints
- Light and dark fingerprint powders or multipurpose fingerprint powders with dusting brushes
- Lifting tape
- Ink remover paper towels or towelettes with denatured ethyl alcohol or waterless hand cleaner.
- A fingerprint safety hood for dust for containing fine particles of dust
- Fingerprint magnifier
- Photograph prints with 1:1.

Procedure

Fingerprints Dusting

- ✓ Deposit few fingerprints on a surface
- ✓ Gently dust the fingerprint using a brush and black powder
- ✓ After collecting the black powder onto the print, remove all the extra power
- ✓ Place a clear piece of tape over the dusted fingerprint
- ✓ Then, remove the tape . Place it over a white index card

✓ Recognise the fingerprint pattern.

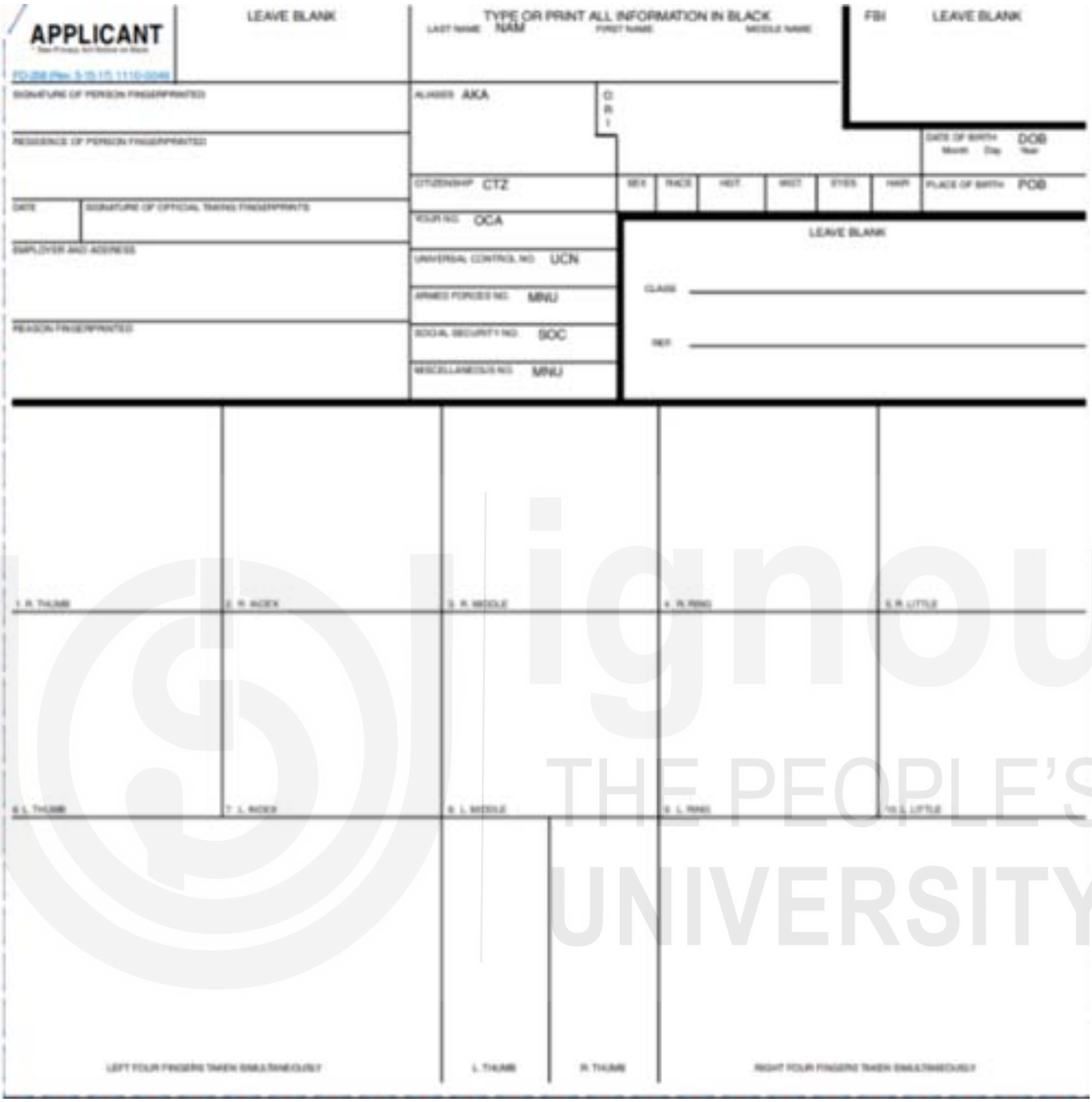


Figure 5: Fingerprint card

Source: https://mn.gov/boards/cbc/fingerprint-card/sample-cards/

Superglue Fuming

- Deposit few fingerprints on a microscopic slide. Then put the hot plate onto the chamber.
- Add a glue on a small dish of aluminium and then place it onto the hot plate. Add a small cup of water in plastic to the chamber. Put the glass slide in the chamber, then plug in the hot plate and put the lid on the chamber.
- Wait for 15 min.

- After the fuming of evidence, apply some Rhodamine 6G dye to the fingerprint with a cotton swab to help visualize the fingerprint and check it under UV light.
- Identify the fingerprint pattern
- Inked Fingerprints
- Complete the fingerprint card using an ink pad
- Exchange the set of your fingerprint and your lifted print on the card with your laboratory partner
- Then, determine the finger that made the lifted print on the fingerprint set of your lab partner (Mirakovits and Londino, 2017; ePG Pathshala

Document analysis involves the examination of handwriting on a document that is questioned or suspected of being altered or tampered with or forged. This mainly comprises of any article that possess numerical or linguistics marking in which there is a no clarity as to who the owner is or if the writing sample is original.

Analysis of handwriting is performed on questioned documents by the principle that each individual has unique features or characteristics at the time of writing and thus an individual's writing cannot be repeated by any other individual.

Handwriting analysis can be performed from 2 different kinds of writing examples: non-requested and requested samples. Requested writing comprises of writing samples that are taken with the objective of comparison to a questioned sample of document. Since these samples of writing are take on request, the circumstances by which these document samples are attained can be controlled. Moreover, to requested samples, non-requested samples of writings attained from usual course of personal or business transactions, can also be then used to compare to the questioned samples. The benefit of non-requested samples involves that the offered writings cannot be tried to be altered or forged when provided, since these samples are written without being aware of use in getting compared to a questioned sample. Thus, it is more likely to reflect an individual's handwriting. Though, these variables like writing tools used, writing surface, and text offered cannot be measured unlike any requested samples.

At the time of analysing the provided examples of writing to a questioned document, class and individual features present in the writing of an individual are examined to assess if the known writing sample belong to a same individual as the questioned sample of writing.

Features like comparative proportion between and within letters, spacing between words and letters, individual formulation of letter, overall writing slant, letter combination formulations, pen pressure, starting and ending strokes connecting strokes, unusual flourishes and pen lifts are analysed between the known sample and the unknown sample to examine the similarity between the two samples. The objective of handwriting examination is to assess if a person could have written a particular questioned document.

The important instrument that is used in questioned document is alternate light source, VSC (Video Spectral Comparator), light wavelengths are presented onto the document. Due to the different pens having different inks, different pens will show and absorb different light wavelengths. This discipline helps a forensic expert to see when the different types of ink were utilized on a document and determined how the document was altered.

Materials Required:

- Known documents
- Unknown questioned documents
- Laboratory Procedure

Basic Procedure in Laboratory

Comparison of handwriting

- Take the given questioned document and assess if an individual write the whole document. Suspect and victim's handwriting examples are allowed to compare to the document that aids in determining this.
- Check the writing style, the letters shape, writing slope, spacing between words and letters, connection, initial, terminal strokes, etc.
- Recognise and observe why the document sample could have or could have not belong from the same individual along with who write the document sample

Advanced Procedure in Laboratory

Alternate Light Source or VSC

- Put the document under the VSC light
- Analyse the document for changes by noting any difference in the colour shades on the ink under individual light wavelengths.
- Repeat 1 and 2 steps by checking at the document with the 3 different ALI
- Recognise if the sample appears to have changes and document what these alterations are on the workbook.

1.5 REFERENCES

Kubic, T., & Petraco, N. (2003). *Forensic science laboratory experiment manual and workbook*. CRC Press.

Mirakovits, K., & Londino, G. (2016). *The basics of investigating forensic science: A laboratory manual*. CRC Press.

Mirakovits, K., Londino, G., & Siegel, J.A. (2017). *The basics of the forensic science: A Laboratory Manual.* Boca Raton: CRC Press.

PGPathshala. e. (n.d.). Retrieved May 1, 2022, from https://epgp. inflibnet. ac.in/

SUGGESTED READING

UNIT 1 INTRODUCING FORENSIC ANTHROPOLOGY

Chadha, P.V. (2004). *Handbook of forensic medicine & toxicology*. New Delhi: Jaypee Brothers.

Modi, J.P. (2001). *Textbook of medical jurisprudence & toxicology*. N.M. Tripathi Pub.

Parikh, C.K. (1999). *Text book of medical jurisprudence, forensic medicine & toxicology*. New Delhi: CBS Pub.

Rao, N.G. (2010). *Text book of forensic medicine & toxicology* (2nd ed.). Jaypee brothers Medical Publisher (P) Ltd.

Sharma, B.R. (2003). *Forensic science in criminal investigation & trials.* New Delhi: Universal Publishing Co.

UNIT 2 BASIC HUMAN SKELETAL BIOLOGY

Boyd, C.C., & Boyd, D.C.(2018). *Forensic anthropology. Theoretical framework and scientific basis*. Wiley.

Christensen, A.M., Passalacaqua, N.V., & Bartelink, E.J. (2019). *Forensic anthropology: Current methods and practice*. Academic Press.

Dirkmaat, D.C. (2012). *A companion to forensic anthropology*. Wiley Blackwell.

Houck, M.M . (2017). *Forensic Anthropology*. Academic Press. Elsevier.

Schmitt A, Cunha E, Pinheiro J. (2006). *Forensic anthropology and medicine*. Human Press.

UNIT 3 DISCOVERY AND TECHNIQUES FOR RECOVERING SKELETONISED HUMAN REMAINS

Klepinger, L.L. (2006). *Fundamental of forensic science.* John Wiley & Sons, Inc, Publication.

Marieb, E.N., & Hoehn, K. (2013). *Human anatomy & physiology (9th ed.)*. San Francisco: Pearson Education.

Nath, S. (1996). *Forensic Anthropology*. Ashtam Prakashan.

Steele, D.G., Bramblett, C.A. (2003). *The anatomy and biology of the human skeleton*. Texas A & M University Press.

Stewart, T.D. (1979). *Essentials of forensic anthropology*. Springfield, IL: Charles C. Thomas.

UNIT 4 BIOMETRICS

Jain, A.K., Bolle, R., & Pankanti, S. (Eds.). (1999). *Biometrics: Personal identification in networked society*. Norwell, MA: Kluwer.

Jain, A.K., Flynn, P., & Ross, A.A. (Eds.). (2008). *TIP Sheet 5 Handbook of Biometrics.* Springer Science+Business Media, LLC

Maltoni, D., Maio, Jain, A.K., & Prabhakar, S. (2003). *Handbook of fingerprint recognition*. New York: Springer-Verlag.

Suggested Reading

Prabhakar, S., Pankanti, S., & Jain, A.K. (2003) Biometric recognition: Security and privacy concerns. *IEEE Security Privacy Mag., vol. 1*, no. 2.

Sinha, G.R., & Patil, S.B. (March 08, 2013). *Biometrics: Concepts and applications*. Wiley India

Tistarelli, M., & Champod, C. (Ed.). (2017). *Handbook of biometrics for forensic science.* Springer Publishing Company, Incorporated.

UNIT 5 ESTABLISHMENT OF COMPLETE AND PARTIAL IDENTIFICATION

Cristensen, A.M., Passalacqua, N.V., & Bartelink, E.J. (2014). *Forensic anthropology: Current methods and practice.* London: Academic press (Elsevier).

Dirkmaat, D.C. (2012). *A companion to forensic anthropology.* West Sussex, UK: Wiley-Blackwell.

Ketzenberg, M.A., & Saunders, S.R. (2008). *Biological anthropology of human skeleton*. Hoboken, New Jersey: Wiley-Liss, John-Wiley & sons.

Krogman, W.M., & Iscan, M.Y. (1986). *The human skeleton in forensic medicine* (2nd ed.). Springfield: Charles C Thomas.

Tersigni-Tarrant, M.T.A., & Shirley, N.R. (2013). *Forensic anthropology: An introduction*. Boca Raxton, London: CRC Press, Taylor and Francis.

UNIT 6 PERSONAL IDENTIFICATION OF LIVING PERSONS–I

Bhattacharya, D. (1968). A Somatometric Study of the Anglo-Indians of India. *Journal of the Anthropological Society of Nippon*, *76*(2), 75-84.

Christensen, A.M., Passalacqua, N.V., & Bartelink, E.J. (2019). *Forensic anthropology: Current methods and practice*. Academic Press.

Francis Galton. (1889). Personal Identification and Description. *The Journal of the Anthropological Institute of Great Britain and Ireland*, vol. 18, pp. 177–91

Harris, H.A., & Lee, H.C. (2019). *Introduction to forensic science and criminalistics*. CRC Press.

Jain, A.K., Flynn, P., & Ross, A.A. (Eds.). (2007). *Handbook of biometrics*. Springer Science & Business Media.

Langley, N.R., & Tersigni-Tarrant, M.A. (Eds.). (2017). *Forensic anthropology: A comprehensive introduction*. CRC press.

Maltoni, D., Maio, D., Jain, A.K., & Prabhakar, S. (2009). *Handbook of fingerprint recognition*. Springer Science & Business Media.

Modi, R.B.J.P. (2013). *A textbook of medical jurisprudence and toxicology*. Elsevier.

Pheasant, S., & Haslegrave, C.M. (2018). *Bodyspace: Anthropometry, ergonomics and the design of work*. CRC press.

Singh, I.P., & Bhasin, M.K. (2004). *A manual of biological anthropology*. Kamla-Raj Enterprises.

Cherril, F.R. (1954). *The Finger Prints.* System at Scotland Yard, Her Majesty's office, London.

Wentworth & Wilder (1948): Personal Identification. R. G. Badger. Boston.

Cummins & Midlo (1943). *Finger prints, palms and soles*. The Blakiston office London.

Harrison, W.R. (1966). *Suspect documents & their scientific examination*. Sweet & Maxwell Ltd., London.

Hilton, O (1982). *The scientific examination of questioned document*. Elsevier North Holland Inc., New York.

Mehta, M.K. (1980). *Identification of thumb impression & cross examination of finger prints*. N. M. Tripathi (P) Ltd. Bombay.

UNIT 8 SEROLOGY

Bevel, T., & Gardner, R.M. (2008). *Bloodstain pattern analysis: With an introduction to crime scene reconstruction (3rd ed.)*. Boca Raton, Florida: CRC Press.

Gunn, A. (2009). *Essential of Forensic Biology (2nd ed.*).Hoboken New Jersey: Wiley-Blackwell.

James, S.H., & Eckert, W.G. (1999). *Interpretation of bloodstain evidence at crime scenes (2nd ed.).* Boca Raton, Florida: CRC Press LLC.

James, S.H., & Nordby, J.J. (Eds.). (2014). Forensic science: An introduction to scientific and investigative techniques. *(4th ed.).* Boca Raton, Florida: CRC Press.

Saferstein, R. (2018). *Criminalistics: An introduction to forensic Science. (12th ed.).* Boston: Pearson Education.

Web: www.finalanalysisforensics.com.

UNIT 9 PERSONAL IDENTIFICATION FROM TEETH

Adams, C., Carabott, R., & Evas, S. (2014). *Forensic odontology: An essential guide.* Wiley.

Ahuja, V. (2014). *Handbook of forensic odontology*. Selective and Scientific Publishers.

Devid, T., & Lewis, J. (2018). *Forensic odontology: Principles and practice(*1st ed.). ELSEVIER.

Kumar, G.S. (2015). *Orban's oral histology and embryology* (14th ed.). ELSEVIER

Masthan, K.M.K. (2009).*Textbook of forensic odontology.* Jaypee Publisher.

Senn, D.R., & Weems, R.A. (2013). *Manual of forensic odontology*. CRC Press.

Taylor, J.A., & Kieser, J.A. (2016). *Forensic odontology: Principles and practice.* Wiley.

UNIT 10 RECENT TRENDS IN FORENSIC ANTHROPOLOGY

Beals, R.L., & Hoizir, H. (1985). *An introduction to anthropology*. New Delhi: Macmillan.

Bridges. (1942). Practical finger printing. New York: Funk and Washalls Co.

Cherril, F.R. (1954). *The finger prints*. System at Scotland Yard. Her Majesty's office, London.

Cummins, H., & Midlo, C. (1961). Finger prints, palms and soles. U.S.A: Dover Publications.

Krogman, W.M., & Iscan, M. (1986). *Human skeleton in forensic medicine.* U.S.A: Charles C. Thomas.

Lee, H.C., & Gaensslen, R.E. (1990). *DNA and other polymorphism in forensic science*. Year book Medical Publishers, Inc.

Lincoln, P.J., & Thomson, J. (Eds.). (1998). Forensic DNA profiling protocols. Humana Press, Inc.

Mehta, M.K. (1980). *Identification of thumb impression & cross examination of finger prints.* Bombay: N. M. Tripathi (P) Ltd.

Moenssens. (1975). *Finger prints techniques.* Philadelphia, New York: Chitton Book Co.

Nath, S. (1987). An introduction to forensic anthropology. New Delhi: Gian Publishing House.

Saferstein, R. (1990). *Criminalistics.*, New Yoek: Prentice Hall.

Wentworth & Wilder (1948). *Personal identification.* Boston: R. G. Badger.

PRACTICAL MANUAL

Bell, S. (2019). *Forensic science: An introduction to scientific and investigative techniques*. CRC Press, Taylor & Francis Group.

Kubic, T., & Petraco, N. (2003). *Forensic science laboratory experiment manual and workbook*. CRC Press.

Siegel, J.A., & Mirakovits, K. (2021). *Forensic science: The basics*. CRC Press.

State, B.E.J. (dept. O. A. C. (2014). *Forensic anthropology - Current methods and Practice*. Elsevier Science Publishing Co.