SECTION

1

Haematology

Chapter Outline

- Introduction to Haematology
- Study of Compound Microscope
- Methods of Collection of Blood
- Haemoglobinometry
- Determination of Total WBC Count
- Determination of Total RBC Count
- Determination of Differential WBC Count
- Determination of Bleeding Time and Clotting Time
- Determination of ESR
- Determination of Blood Indices
- Determination of Packed Cell Volume (Haematocrit)
- Determination of Blood Groups
- Determination of Osmotic Fragility and Specific Gravity of Blood
- Determination of Reticulocyte Count
- Determination of Platelet Count

1

Introduction to Haematology

Learning Objectives

After completion of this practical, the students shall be able to:

- Define haematology
- Name routine haematological tests
- Enumerate functions and components of blood

INTRODUCTION

- Hematology is the science that deals with the study of blood.
- In a 70 kg normal adult person, blood volume is about 5–5.5 litres. There are several haematological investigations, that are routinely performed in laboratories. All haematology tests do require adequate skill; however, recently most of the investigations have been done by automated machines. Blood samples for all these haematological tests are obtained by taking a prick (capillary blood) or by puncturing the vein (venous blood). Arterial blood samples are required for some specific blood investigations.

COMPONENTS OF BLOOD

Blood has two types of major components—(1) cells, and (2) plasma.

Cellular Component

- Cellular component contain red blood cells, white blood cells, and platelets (thrombocytes).
- Red blood cells (RBCs) constitute the highest number, they are present in millions (4–5.5 million/mm^3 of blood). RBCs help in the transport of gases. White blood cells (WBCs) are in the range of thousands (4,000–11,000/mm^3 of blood). WBCs assist the defence function of the body.
- Platelets are in 1 to 3 lakhs/mm^3 of blood. Platelets help in the arrest of bleeding (hemostasis).

Plasma Component

- The fluid component of blood is plasma. Plasma contains fibrinogen and clotting factors.
- Plasma carries various substances like nutrients, hormones, waste products, etc.

SERUM

When fibrinogen is removed from plasma (during the process of coagulation), what remains is serum. Various investigations do require serum for tests (e.g. serum protein, serum glucose, serum triglycerides, etc.).

BLOOD SAMPLES

- Blood samples for various haematological tests can be obtained by capillary puncture (collection of blood from capillaries) and venepuncture (collection of blood from veins).
- For some investigations, one requires arterial blood, e.g. arterial blood gas (ABG) analysis.

■ ROUTINE HAEMATOLOGICAL TESTS INCLUDE

- Estimation of haemoglobin (Hb)
- Total RBC count
- Total WBC count
- Differential WBC count
- Erythrocyte sedimentation rate (ESR)
- Packed cell volume (PCV)
- Platelet count
- Blood group
- Bleeding time and clotting time
- Osmotic fragility of blood
- Specific gravity of blood.

CHAPTER

2

Study of Compound Microscope

Learning Objectives

After completion this practical, the students shall be able to:

- Identify and give uses for different parts of a compound microscope
- Enumerate precautions during the use of a microscope
- Microscopic adjustment for viewing under low power, high power, and oil immersion
- Handle the microscope carefully
- Enumerate different types of microscopes.

■ INTRODUCTION

The microscope was invented by Leeuwenhoek. A microscope is known to magnify the image of an object. We should know about the basic construction of a microscope.

Various types of microscopes are available; a compound microscope is frequently used in medical laboratories. In physiology, it is mainly used to study different cell counts and morphology of different cells.

■ COMPOUND MONOCULAR MICROSCOPE

It has the following parts **(Fig. 2.1)**

Base

It supports a microscope on the working table. The base of the microscope has a horseshoe-shaped foot, which gives stability to the microscope. To the foot of the microscope, the limb is attached which bears an optical system.

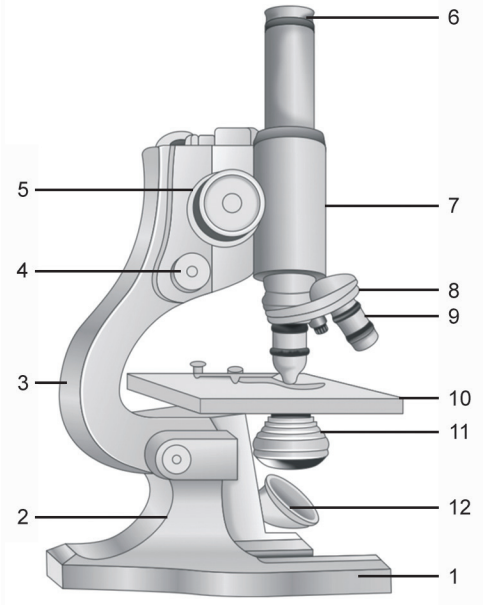

Fig. 2.1: Parts of the compound microscope (1: Base; 2: Pillars; 3: Handle; 4: Fine adjustment screw; 5: Coarse adjustment screw; 6: Eyepiece; 7: Body tube; 8: Fixed and revolving nose piece; 9: Objectives; 10: Stage; 11: Condenser; 12: Mirror)

Body Tube

It is a vertical tube, which can be raised or lowered when required.

Coarse and Fine Adjustment Screws

- Body tubes can be raised or lowered by these screws. With a coarse adjustment screw, the body tube is

raised or lowered quickly. With a fine adjustment screw, the body tube is raised or lowered slowly. These adjustments help to focus the object properly.
- Coarse and fine screws are mounted on top of the handle. One pair of each is present on either side. Even if one screw is rotated, the member on the opposite side automatically rotates simultaneously. Usually, the left hand is used for moving coarse or fine adjustment screws.

Fixed Stage

It is a square platform with an aperture in the center on which the slide is placed. Light passes through the aperture and then through the slide. It is attached to the limb below the objective lens.

Mechanical Stage

- It is fixed stage and is calibrated. It has two screws—one for moving slides horizontally and the other for moving slides forward and backwards.
- Stage is fitted with condenser and iris diaphragm.
- The condenser has two lenses mounted into a short cylinder. The condenser can be raised or lowered with the screw. The iris diaphragm is placed below the condenser and controls the amount of light entering the microscope.

Mirror

A mirror is fitted below the condenser to reflect light from the source into the condenser. Mirror has two surfaces: Plain (flat surface) and concave. The mirror can be rotated in all directions. A plane mirror is used in daylight and a concave mirror when the source of light is from one point, like a bulb.

Nose Piece

It is attached to the lower end of the body tube. It has objective lenses of different powers. By revolving the nose piece, any lens can be placed over the slide to be observed.

Objective Lenses

There are three objective lenses with different magnifying powers as given below:

1. **Objective labeled "10x"** magnifies the image 10 times. It is called as low power.
   Magnification with low power would be 100 times (10 × 10).
2. **Objective labeled "40x or 45x"** magnifies the image 40–45 times. It is called as high power. Magnification using high power would be 400 times (40 × 10).
3. **Objective labeled "90x or 100x"** magnifies the image 90–100 times. It is called an oil immersion lens. Magnification with oil immersion lens would be 900–1000 times.

Eyepiece

- The eyepiece is placed at the top of the body tube.
- Usually, two or more eyepieces with different powers are supplied. An eyepiece may have a movable pointer such an eyepiece is called a demonstration eyepiece.
- **Formation of image**: Lenses initiate the magnifying process. A real, inverted, and enlarged image is formed in the upper part of the tube. A field lens is present near the lower plane of the eyepiece, which collects diverging rays of the primary image. These are further magnified by an eye lens from the eyepiece.

■ USE OF MICROSCOPE

The slide to be examined is put on the fixed stage. Objects can be seen under low and high power.

Low Power Adjustment

- Low power objective is placed in position.
- A concave mirror is used.
- Condenser occupies a lower position.
- The diaphragm is adjusted to prevent glare and light is adjusted.
- Body tube is lowered with the help of coarse adjustment so that the low-power objective is about 1 centimeter from the slide.
- Slide is then seen through the eyepiece of the microscope.
- Focusing is done gradually first with coarse adjustment and then finally with fine adjustment. Care is taken not to break the slide.
- The whole slide is scanned and then part of the slide to be seen is selected. The objective is turned to high power.

High Power Adjustment

- High power objective is placed in position. A plain mirror is used.
- Condenser is taken up.
- High power objective is brought near the slide by using coarse adjustment.
- By looking through the eyepiece, light is adjusted.
- Focusing is done gradually first with coarse and finally with fine adjustment to get the best possible view.
- Objective should never be lowered by using coarse adjustment when one is looking through the eyepiece.
- Iris diaphragm is adjusted to cut the thin peripheral rim of rays.

Oil Immersion Adjustment

- Oil immersion lens is placed in position. The plain mirror is used. The condenser is taken high up.

- A drop of cedarwood oil is put on the slide (refractive index of cedarwood oil is same as that of glass).
- Using coarse adjustment oil immersion objective is brought down till it just touches the oil drop.
- Looking through the eyepiece final focusing is done with fine adjustments to get the best possible view.
- Focusing should be done gradually and carefully to prevent the breaking of the slide or damaging of the lens.
- After completing the examination, the objective is raised and the slide is taken out.

PRECAUTIONS

- When not in use, the microscope should be kept covered.
- The microscope must be kept clean and free from dust.
- Oil on the objective should be first removed with a dry, soft cloth and then cleaned with a small quantity of xylene/acetone.
- The lenses should be cleaned with a small quantity of isopropyl alcohol (90%).
- Low- and high-power objectives and eyepieces are cleaned with soft linen or polishing cloth. Glass should never be touched with a finger.
- Lowering of the optical tube should not be done when one is looking through the eyepiece.
- If the microscope has to be moved, it should be held upright using a handle and hand below the foot.

OTHER TYPES OF MICROSCOPES

- **Binocular microscope**: It is used to prevent strain on the eyes, especially when one has to use the microscope for a long time.
- **Dissecting microscope**: Binocular microscope used for microdissection.
- **Electron microscope**: It has a very high resolving power as compared to ordinary light microscopes.
- **Phase-contrast microscope**: It is useful in the examination of living, unstained material, as well as fixed specimens. It is valuable as a research tool.
- **Polarizing microscope**: It is used to detect certain birefringent substances in the tissue, such as suture material, barium, etc. Any ordinary microscope with polarized filters can be used for this purpose.
- **Dark-field microscope**: It is especially useful for studying very minute organisms.
- **Fluorescence microscope**: When ultraviolet light strikes a fluorescent substance the visible light is emitted. Any conventional microscope can be converted into a fluorescence microscope by introducing a light source, rich in ultraviolet radiations by adding a dark-field condenser and a suitable filter system.
- **Differential-contrast microscope**: This microscope helps in the formation of pseudo-three-dimensional images.

COMMON STATIONS – SPOTS IN PRACTICAL EXAMINATION (2/3 MARKS)

- Focus given slide under high power/low power/oil immersion.
- How is the objective of the microscope cleaned?
- Enlist any four adjustments required to be done to adjust the microscope under high power.
- Enlist any four adjustments required to be done to adjust the microscope under low power.
- Enlist any four adjustments required to be done to adjust the microscope under oil immersion.
- Enlist different types of microscopes you know.

OBJECTIVE STRUCTURED PRACTICAL EXAMINATION (OSPE)

Procedure station 1: Make microscopic adjustments for focusing under low power.

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
1.	Bring the condenser to the lowest position		
2.	Change to concave mirror and slightly open iris diaphragm		
3.	Place the slide on the stage		
4.	Bring a lower objective in position and make coarse adjustments to focus the image		
5.	Use fine adjustment for final focusing of image		
6.	Report and viva on haematology experiment		
7.	Total		

Procedure station 2: Make microscopic adjustments for focusing under high power

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
1.	Make use of a plain mirror		
2.	Slightly raise the condenser and partially opens the iris diaphragm		
3.	Place slide on the stage		
4.	First focus under low power		
5.	Bring high-power objective in position and make coarse adjustments to focus the image		
6.	Make fine adjustments for final focusing		
7.	Report and viva on haematology experiment		
8.	Total		

Procedure station 3: Make microscopic adjustments for focusing under an oil immersion lens.

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
1.	Change to plane mirror		
2.	Bring the condenser to highest position and open the iris diaphragm completely		
3.	Place slide on the stage and put a drop of oil on the center of the smear		
4.	Bring oil immersion objective into position		
5.	Bring the center of the slide under the view, so that the objective touches the oil		
6.	Make coarse and then fine adjustments to focus the image		
7.	Report and viva on haematology experiment		
8.	Total		

KEY POINTS TO REMEMBER

- A compound monocular microscope is used commonly for all haematology practicals.
- 10X is the power of the lens when one focus under low power and 45X when focus under high power.
- Cedarwood oil is used when you focus under oil immersion as it has a refractive index the same as a mirror. Always, any object first has to be examined under low power and then high power.

CHAPTER

3

Methods of Collection of Blood

Learning Objectives

After completion of this practical, the student shall be able to:

- List general precautions for collecting blood
- Enumerate methods of collection of blood
- Collect blood by capillary method taking antiseptic measures
- List precautions while collecting capillary blood
- List commonly used anticoagulants for various haematological procedures
- Enumerate common sites for blood collection

■ INTRODUCTION

- Blood is collected for various investigations, e.g. haematological, biochemical, serological, and culture.
- For different haematological examinations, collection of blood from the vein is preferred.
- When a small quantity of blood is required, capillary blood is used, e.g. estimation of haemoglobin, red blood cell (RBC) count, white blood cell (WBC) count, blood groups, bleeding, and clotting time.
- The composition of venous blood is almost the same as that of capillary blood.
- For some investigations, arterial blood is preferred. Aseptic precautions are very important while collecting blood.

■ VENOUS BLOOD (Venipuncture)

Sites for Collection

Antecubital fossa (median cubital vein), dorsum of hand in adults, and femoral vein in children **(Fig. 3.1)**.

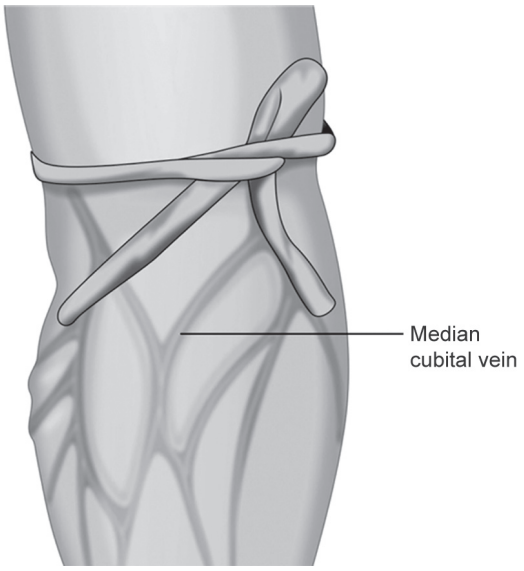

Fig. 3.1: Application of tourniquet for collection of blood by venipuncture.

Precautions

- Before collecting blood, the operator should wash hands and preferably wear disposable plastic or thin rubber gloves.
- Skin over the vein should be cleaned properly. Cleaning of the skin prevents entry of bacteria into the body through the puncture.
- Blood should be withdrawn slowly and delivered gently in the container and mixed properly with an anticoagulant.
- Syringe and needle should be disposed of properly.

Procedure

- Patient is asked to sit on a chair.
- The antecubital vein of the left arm (or right arm) is usually chosen for collecting the blood. Alternatively, veins of the back of the hand or leg can be chosen.
- A tourniquet is placed around the arm. The patient is asked to open and close the fist repeatedly to cause engorgement of the vein.
- After washing hands with soap, the operator should wear disposable plastic, thin rubber gloves. Operators must take care while handling syringes and needles to avoid injury.
- Disposable and dry plastic syringe is used. A disposable needle (sharp and dry) of 18 or 19 G is used. A syringe with a needle is held in the right hand in such a way that the nozzle is in opposition to the patient's skin.
- Skin is cleaned with 70% alcohol and is punctured 0.5 cm below the point where the vein is to be punctured and along the same line of the vein (it prevents counterpunching of the vein).
- Then needle is pushed along the line of the vein to puncture it. When the vein is punctured, blood appears in the syringe.
- The piston of the syringe is withdrawn slowly to collect the required quantity of blood. The tourniquet is removed and the index finger is put on the butt of the needle, and the syringe is withdrawn. A piece of sterile cotton swab dipped in 70% alcohol is firmly pressed at the puncture.
- Blood is transferred to a bulb containing anticoagulant and is thoroughly mixed with anticoagulant by holding the bulb in the palms of hands and rotating it. The bulb is closed either by a cork or a cotton swab. Blood collected is stored in bulbs and bulbs differ according to investigations to be carried out.

CAPILLARY BLOOD (Finger Prick Method)

When a small quantity of blood is required, capillary blood is used, e.g. for estimation of haemoglobin, RBC count, WBC count, differential count, blood groups, bleeding, and clotting time **(Fig. 3.2)**.

Sites for Collection

- The ball of the finger, usually the ring finger of the left hand is used
- Ear lobe
- Heel or big toe in infant

Precautions

- Before collecting blood, the operator should wash hands and preferably wear disposable plastic or thin rubber gloves.

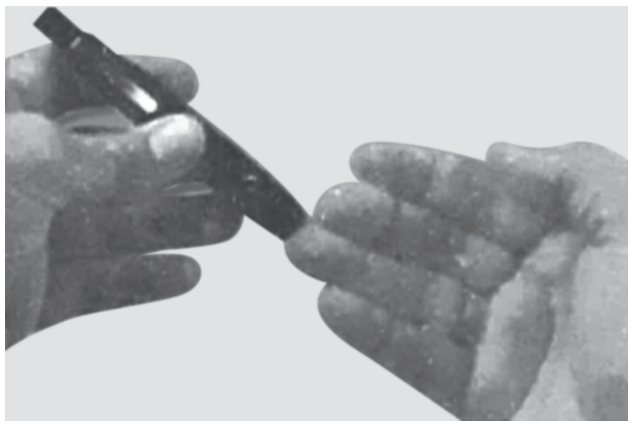

Fig. 3.2: Method of collection of capillary blood

- The pricking needle should be a flat needle with cutting edges having sharp points. It should not be blunt or rusted.
- All apparatus is kept ready before the prick.
- Aseptic precautions should be observed before pricking.
- After a required quantity of blood is collected, a sterile cotton swab should be put and pressed at the site of the prick till the bleeding stops.

Procedure

- The area to be punctured is cleaned with spirit or alcohol. It is allowed to dry.
- Sterile lancets or sterilized disposable needles are used.
- Apparatus (e.g., pipette, slides, etc.) should be kept ready before the prick as blood has to be used immediately after it is withdrawn.
- Bold prick (3–5 mm depth) is given to cause free flow of blood easily so that the finger need not be pressed.
- Fingers should not be squeezed for collecting blood (as it takes tissue fluid out, which dilutes the blood).
- The first drop of blood is wiped with the cotton (as it is diluted with tissue fluid), and then blood is collected.

ANTICOAGULANTS

Anticoagulants prevent blood clotting. They are added to a blood sample (mainly blood collected by venipuncture) and sent to laboratories **(Table 3.1)**.

TABLE 3.1: Anticoagulants

S. No.	*Name of bulb*	*Content*	*Use*
1.	Wintrobe bulb	NH4 oxalate, K oxalate	Haematological investigations
2.	Fluoride bulb	K oxalate, Na fluoride	Blood sugar
3.	Chemistry bulb	K oxalate (0.2 mg)	Blood urea
4.	Citrate bulb	3.8% Na citrate	For ESR
5.	Plain bulb	—	Serological investigation
6.	Paraffin bulb	Double oxalate and 1 ml liquid paraffin	Blood gases

Commonly Used Anticoagulants

Ammonium Potassium Oxalate

A stock of solution of ammonium oxalate 1.2 g, potassium oxalate 0.8 g, and distilled water up to 100 ml is prepared. About 0.5 ml of this solution is placed in a suitable container and dried at 37ºC in a water bath. This much amount of oxalate is sufficient for 5 ml of blood (a bulb prepared in such a way is called Wintrobe's bulb).

Mode of Action

Oxalate precipitates calcium, which is required for clotting.

Uses

It is used only *in vitro*. It is used for the estimation of complete blood count (CBC), erythrocyte sedimentation rate (ESR), and packed cell volume (PCV).

Ethylenediaminetetraacetic Acid

Sodium and potassium salts of ethylenediaminetetraacetic acid (EDTA) are used as anticoagulants for routine haematological work. Excess of EDTA (>2 mg/ml) causes shrinkage of RBCs and degenerative changes in cells.

Mode of Action

Chelates calcium from the blood and acts as an anticoagulant.

Uses

All routine haematological investigations except coagulation studies.

Sodium Citrate

It is the choice of anticoagulant for coagulation studies. It is prepared by dissolving 32 g of trisodium citrate in one liter of distilled water. 3.8% of Na citrate is isotonic with blood but causes its dilution. It is used for the determination of ESR (Westergren's method). The ratio of citrate is 1:4 (One part of citrate is used for 4 parts of blood).

Mode of Action

Chelation of calcium in blood.

Uses

- In a blood bank for storing blood
- Various coagulation studies
- Estimation of ESR by Westergren's method.

Heparin

- It is an effective anticoagulant
- It does not alter the size of RBCs
- It is the best anticoagulant, as it is a natural constituent of blood
- It can be used *in vivo* as well as *in vitro*.

Mode of Action

Prevents action of thrombin and also promotes deactivation of thrombin. By these actions, it prevents the formation of fibrin from fibrinogen.

Uses

- Blood gas analysis and pH assays
- Osmotic fragility test.

Dicoumarol Derivatives

Mode of Action

It competes with vitamin K and therefore a synthesis of clotting factors (Vitamin K-dependent clotting factors) is hampered.

Uses

It can be used only *in vivo*.

Sodium Fluoride

It is usually mixed with oxalate (as fluoride itself is not a very strong anticoagulant). Used for preparing blood specimens for plasma glucose estimation.

Mode of Action

Fluoride inhibits glycolytic enzymes and thus prevents the loss of glucose.

COMMON STATIONS – SPOTS IN PRACTICAL EXAMINATION (2/3 MARKS)

Q.1. Any of the anticoagulants can be kept. Write uses of the same and the mechanism of their action as an anticoagulant.

Q.2. Figure of fingerpick/venipuncture. Identify, and enumerate the precautions, and procedures to do the same.

Q.3. Name anticoagulant that can be used only *in vivo*. What is the physiological basis of them used only *in vivo*? (refer to above).

KEY POINTS TO REMEMBER

- Blood can be collected from capillaries, arteries and veins for different haematological investigations.
- Various anticoagulants are allowed to mix with blood in order to prevent coagulation of blood, once blood is taken out from a patient.
- It is very important to follow all aseptic precautions while collecting blood.
- Following the correct procedure while collecting blood ensures minimum technical errors.

CHAPTER

4

Haemoglobinometry

Competency:

PY 2.11: Estimate Hb content of blood.

Learning Objectives

After completion of this practical, the students shall be able to:

- Estimate Hb by Sahli's method
- Prick finger with all aseptic precautions
- List the advantages and disadvantages of Sahli's method
- State principle used for Sahli's method
- Name other methods of estimation of haemoglobin (Hb)
- Give normal values of Hb, its functions and its physiological variations
- List common conditions of increase and decrease in Hb concentration
- Define anaemia
- Give common causes of anaemia and classification of anaemia.

Aim

To estimate Hb by Sahli's method.

Apparatus

- Sahli's haemometer, pricking needle or lancet, dropper, spirit or alcohol N/10 hydrogen chloride (HCl), and distilled water.

Sahli's haemometer contains:

- Haemoglobin pipette (with 20 mm^3 mark)
- Haemometer tube (graduated with % and 100 g/dl)
- Glass rod or stirrer
- Coloured standards

Principle

Haemoglobin is converted into a compound called acid hematin by mixing with N/10 HCl. The brown-coloured compound is matched with the standard provided in Sahli's haemometer.

Procedure

- The haemometer tube is first filled with N/10 HCI by using a dropper up to mark 20.
- For estimating haemoglobin, capillary blood is collected by a finger prick with all aseptic precautions.
- When the proper size of drop is formed, the tip of the pipette is applied to it, and blood is filled exactly up to 20 mm^3 **(Fig. 4.1)**. If air bubbles are collected procedure is repeated.
- Blood sticking to the tip of the pipette is wiped off.
- Blood from the Hb pipette is immediately transferred into the haemoglobin tube, in which N/10 HCl is put **(Fig. 4.2)**. Contents are mixed well with a stirrer.
- After waiting for 10 minutes (time required for conversion of Hb to acid hematin brown-coloured compound), acid hematin is formed.
- Drop-by-drop water is added to it every time mixing properly with a stirrer. Against the daylight, it is matches with the coloured standards.

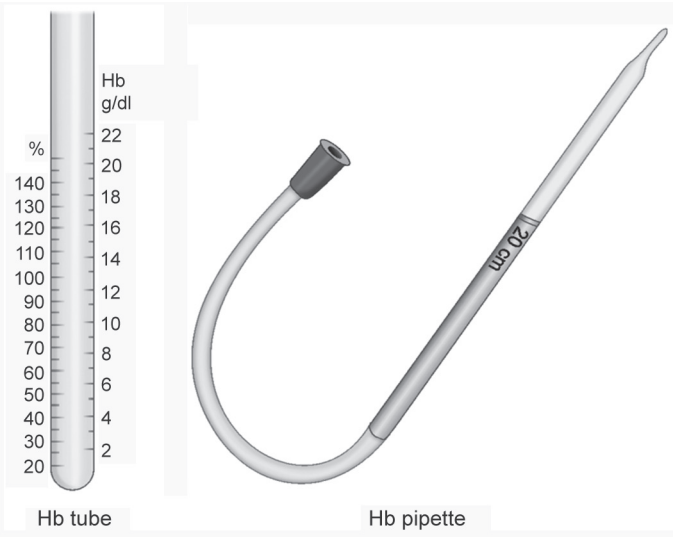

Fig. 4.1: Haemoglobin (Hb) tube and Hb pipette

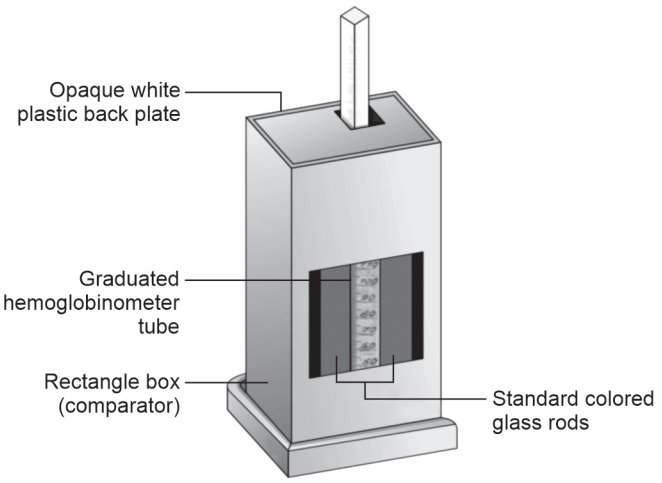

Fig. 4.2: Haemoglobinometer

- Once, it matches with coloured standards, lower meniscus reading is taken as Hb (100 g/dl or %).
- The result is expressed as either % or 100 g/dl.
- According to Sahli's method, 14.5/100 g/dl is considered to be 100%.

Normal Values of Haemoglobin

- **In males**: 14–18/100 g/dl of blood
- **In females**: 12–15/100 g/dl of blood

Advantages of Sahli's Method

- The method is very simple
- Method is less time-consuming

Disadvantages of Sahli's Method

- There is about 8–10% error in the estimation of haemoglobin.
- This method only measures the amount of haemoglobin present in reduced and oxygenated form. Haemoglobin in other different forms (carboxyhaemoglobin, methaemoglobin, etc.) cannot be estimated by this method.
- The brown colour of acid hematin does not remain stable. It begins to fade after some time. Therefore, the method is less accurate. The coloured glass standard of the haemometer also fades with time.

Precautions

- Proper aseptic precautions are a must.
- Once you clean the finger with spirit, allow it to dry on its own (do not blow).
- Squeezing of the finger is avoided.
- Hb tube to be filled exactly to 20 mark.
- Haemoglobin pipette to be filled exactly 20 mm^3.
- Care is taken that blood mixes properly with N/10 HCl.
- For complete conversion of Hb to acid hematin, a 10-minute waiting period is required.
- Matching is done properly against standards provided against the daylight.

Common Experimental Errors

- N/10 HCl not taken accurately.
- Blood taken more or less in Hb tube.
- Squeezing finger after prick, while collecting blood.
- Not mixing blood properly with N/10 HCl.

IMPORTANT QUESTIONS AND ANSWERS

Q.1. How much is normal Hb?
- **In males**: 14–18/100 g/dl of blood
- **In females**: 12–15/100 g/dl of blood

Q.2. What is the principle of Sahli's method?

Principle: N/10 HCl mixes with Hb to form acid hematin (brown-coloured compound), which is matched with coloured standards.

Q.3. What are the other methods of haemoglobin estimation?

Other methods of haemoglobin estimation are:
- **Cyanmethaemoglobin method (haemoglobin cyanide)**
  - In this method blood is diluted in a solution containing sodium bicarbonate, potassium cyanide, and potassium ferricyanide (Drabkin's reagent).
  - All forms of haemoglobin (except sulfhaemoglobin) are converted to cyanmethaemoglobin (takes about 10 minutes), and therefore an accurate estimation of haemoglobin is obtained. This is one of the commonly practised methods for Hb estimation.
- **Haldane's method**
  - Haemoglobin is converted to carboxyhaemoglobin by passing carbon monoxide gas through blood. Its colour is matched with standard.

- **Tallquist method**
  - A drop of blood is put on a piece of filter paper. The colour of this drop is matched with the standard.
  - This method is fast and so used for mass screening (but less accurate).
- **Alkaline hematin method**
  - This method gives a true estimate of total haemoglobin as all haemoglobin compounds (including meth- and sulfhaemoglobin) are converted to alkaline hematin.
- **Estimation of the iron content of blood**
  - Iron is separated from blood by the action of sulfuric acid and is estimated. 1g of haemoglobin contains 3.35 mg iron.
  - According to iron content, haemoglobin concentration can be calculated. It is an accurate method.
- **Oxyhaemoglobin method**
  - Haemoglobin is fully oxygenated by treating it with ammonium hydroxide or sodium carbonate. This is the quickest method for general use.
- **Direct reading haemoglobinometers**
  - These instruments have a built-in filter and a scale calibrated for direct reading of haemoglobin in g/100 ml or g/litre.
- **Spectrophotometry**
  - This is a very accurate method. In this, blood is diluted (1–200 or 1–250) with cyanide ferricyanide solution and absorbance is measured at 540 nm and haemoglobin is calculated.
- **Determination of specific gravity of blood by copper sulfate method**
  - This is a quick method of approximate estimation of haemoglobin on a mass scale, e.g. during blood donation, camps, etc.
- **Automated haemoglobinometry**
  - Many automated techniques are there to estimate Hb. Automatic dilutors and pipettes are used.
- **Non-automated haemoglobinometry**
  - Disposable, self-measuring dilution micropipettes are available in the market. The pipette gets filled with blood (by capillary action) and then blood is mixed with reagent (provided) and then a reading is obtained on the spectrophotometer.

Q.4. What happens, if N/10 HCl is taken more in quantity?

- When haemoglobin content is normal, if N/10 HCl taken is more, it does not make any difference.
- But, if a person has severe anaemia then it will give an error in the result as by taking more N/10 HCl you are diluting the blood more.

Q.5. What happens, if N/10 HCl is taken in a lesser quantity (<20% mark)?

- In Sahli's method of estimation of haemoglobin, for conversion of entire haemoglobin present in the blood to acid hematin dilute HCl up to 20% mark is required.
- If a lesser amount of HCl is taken, enough amount of HCl is not available to convert Hb into acid hematin.
- The Hb value can come less than the actual Hb value in that person.

Q.6. What are the disadvantages of Sahli's method?

- There can be 8–10% of error in the estimation of Hb.
- All forms of Hb cannot be measured. It estimates only oxyhaemoglobin and reduced haemoglobin.
- Acid hematin colour developed is not stable and it fades with time.
- The coloured standard provided also fades with time.

Q.7. What is the O_2-carrying capacity of blood?

- One gram of Hb combines with 1.34 ml of O_2. Thus, if one knows the Hb values of the person, O_2-carrying capacity can be calculated.

Q.8. Why Hb is more in males? Why Hb count is low in females?

- **In males**, the testosterone hormone stimulates RBC production (erythropoiesis), therefore Hb count is high.
- **In females**, Hb count is low: Due to loss of blood during menstruation and estrogen inhibits erythropoiesis.

Q.9. Why Hb is more in newborns?

- Haemoglobin concentration in a newborn baby is high. It may be as high as 20 g/100 ml of blood. Newborns have more number of active sites of red bone marrow.

Q.10. What are the functions of haemoglobin?

The functions of haemoglobin are:

- It transports oxygen (oxygen gets attached to the iron part of the heme molecule).
- It transports carbon dioxide. CO_2 is attached to the amino group of the protein part of haemoglobin (globin), forming the carbamino compound. It is therefore possible for haemoglobin molecules to carry O_2 and CO_2 simultaneously.
- Haemoglobin is a major protein in the blood. It acts as a buffer and thus prevents drastic changes in the pH of the blood.
- Haemoglobin also acts as a buffer for maintaining the concentration of PO_2 constant in the interstitial fluid (40 mm Hg). This is possible because of the sigmoid shape of the O_2 dissociation curve.

Q.11. What is anaemia?

- Anaemia is defined as the decreased oxygen-carrying capacity of blood due to a decrease in the Hb level of blood below the lower limit of normality for that particular age and sex.

Q.12. Give etiological and morphological classification of anaemia.

Etiological classification:

- **Blood loss anaemia** caused due to acute or chronic blood loss– acute blood loss as in accident, chronic blood loss as in piles.
- **Anaemia caused due to impaired red cell formation**. Impaired red cell formation is due to the following causes:
  - Inadequate supply of nutrients essential for erythropoiesis (iron deficiency, folic acid deficiency, vitamin B12 deficiency, etc.)
  - Depression of erythropoietic activity of bone marrow (aplastic anaemia and leukaemia).
- **Anaemia due to excessive destruction of red blood cells**. This includes all hemolytic anaemia (extracorpuscular and intracorpuscular defects).

Morphological classification :

- **Hypochromic microcytic anaemia:** RBCs are microcytic and hypochromic, thus MCV, MCH and MCHC values are less than normal. This morphology is typical of iron deficiency anaemia.
- **Macrocytic normochromic/hypochromic anaemia:** RBCs are large in size. Thus, MCV is more, and MCHC may be normal or low. This type of RBC picture is typical with vitamin B12 or folic acid deficiency (megaloblastic anaemia).
- **Normocytic normochromic anaemia:** All blood indices MCV, MCH and MCHC are normal. Such a picture is typically seen when there is chronic blood loss, renal failure (due to deficient erythropoietin secretion) or bone marrow failure.

Q.13. Which blood indices can be calculated with haemoglobin values?

From haemoglobin values, the following indices are calculated:

- Mean corpuscular haemoglobin (MCH) (27–32 pg).
- Mean corpuscular haemoglobin concentration (MCHC) (32–38%).
- Colour index (CI). Its normal value is 0.8–1.2
- All blood indices help in diagnosing the type of anaemia.

Q.14. What are the common causes of iron deficiency anaemia?

Common causes of iron deficiency are:

- Excessive blood loss during menstruation
- Increased demands- infancy, childhood, and pregnancy
- Pathological blood loss due to peptic ulcer, haemorrhoids, ulcerative colitis, etc.
- Deficient diet
- In infants, diminished iron stores at birth
- Daily requirement of iron in males is 0.5–1 mg. In females of reproductive age, it is 1–2 mg
- Diet normally should contain 10–20 mg of iron of which 10% or less is absorbed.

Q.15. What are the common causes of the increase or decrease in Hb count?

Conditions that decrease Hb concentration

- **Physiological**:
  - Hb less in females as compared to males
  - Pregnancy (Haemodilution)
- **Pathological:**
  - Different types of anaemia
  - Excess antidiuretic hormone secretion (hemodilution)
- **Experimental errors**:
  - Finger is squeezed while collecting blood
  - Blood taken less than 20 cumm mark

Conditions that increase Hb concentration

- **Physiological**:
  - High altitude (hypoxia)
  - Newborns
  - Excess sweating
- **Pathological:**
  - Severe diarrhoea and vomiting
  - Congenital heart diseases
  - Emphysema of the lungs
  - Polycythemia vera
- **Experimental errors:**
  - Blood taken more than 20 mm^3 mark
  - Fading of colour plate

Q.16. Describe the cyanmethaemoglobin (Haemoglobin cyanide) method.

- Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. The absorbance of the solution is measured in a spectrophotometer at a wavelength of 540 nm or in a photoelectric calorimeter with a yellow-green filter.
- Diluent (cyanide-ferricyanide) is prepared as follows– 200 mg of potassium ferricyanide, 50 mg of potassium cyanide, 140 mg of potassium dihydrogen sulphate and 1 ml of non-ionic detergent. Then water is added to make a solution to 1 litre.
- This dilution when added to blood, haemoglobin cyanide is formed. About 20 microliter of blood is added to 4 ml of diluent in a tube, is inverted many times and then allowed to stand for five minutes (so that the reaction is complete). Then absorbance of a solution is compared with the standard (in a photoelectric calorimeter).
- Advantages of this method (refer to Q3).

Q.17. How much is the daily requirement of iron, vitamin B12 and folic acid?

- The daily requirement of iron in males is 0.5 to 1 mg and that in females (of reproductive age) is 1 to 2 mg.

A normal diet contains 10 to 20 mg of iron, out of which 10% is absorbed.

- Vit. B12 daily required by adults is 2 to 4 µg and that of folic acid is 200 µg. The body store of vitamin B12 is about 2 to 5 mg and folic acid is 5 to 20 mg. Deficiency of vitamin B12 and folic acid may not appear early due to their storage, even if they are not taken in the diet for long.

Q.18. What are the common causes of deficiency of folic acid?

- It includes decreased intake, nutritional deficiency/impaired absorption (e.g. coeliac disease, tropical sprue, etc.) or increased demands (e.g. pregnancy, haemolytic anaemia, etc.).
- Due to a deficiency of maturation factors RBCs remain large in size (megaloblastic anaemia).

Q.19. Enumerate different varieties of normal Hb.

- **HbA1**: The most common types of adult Hb (its globin contains two alpha and two beta chains
- **HbA2:** This type of adult Hb contains two alpha and two delta chains.
- **Fetal Hb (HbF**): It contains two alpha and two gamma chains, HbF gradually disappears after one year of age.
- **HbA 3**: Altered form of Hb found in old RBCs.
- **Embryonic Hb**: It is made up of two alpha and two epsilon chains in the first three months of intrauterine life.
- **HbA 1c:** Some amount of normal Hb is glycosylated. It can be more than 3 to 5% of normal % of Hb. If it exceeds, it is considered prediabetic or diabetic stage (depending on the level of HbA1c).

Q.20. Give information on haemoglobin.

It is a conjugated protein present in RBCs. It is made up of heme and globin iron part of the haem is recycled while protoporphyrin forms bilirubin (converted to bile salts and bile pigments). Globin component is broken into amino acids and recycled again for Hb synthesis.

OBJECTIVE STRUCTURED PRACTICAL EXAMINATION (OSPE)

Procedure station: Take a known quantity of blood in a Hb pipette and dilute it with acid haematin with the help of the apparatus provided.

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
1.	Check Hb pipette, its patency and Sahli's haematometer given		
2.	Check all apparatus for cleanliness and patency		
3.	Take N/10 HCl to the given mark		

Contd...

Contd...

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
4.	With all aseptic precautions (check above for details) take capillary blood up to designated mark on the pipette		
5.	Blow out the blood in Hb tube in which N/10 HCl is taken		
6.	Report and viva haematology experiment		
7.	Total		

Procedure station: Find out the Hb of your own blood with the help of Sahli's haemoglobinometer.

S. No.	*Assessment criteria*	*Marks assigned*	*Marks given*
1.	Check Hb pipette, its patency and Sahli's haematometer given		
2.	Check all apparatus for cleanliness and patency		
3.	Take N/10 HCl to the given mark		
4.	With all aseptic precautions (check above for details) take capillary blood up to the designated mark in the pipette		
5.	Blow out the blood in Hb tube in which N/10 HCl is taken		
6.	Mix blood properly with acid hematin, waits for 10 minutes		
7.	Drop by drop add water in the Hb tube and each time mix the content well		
8.	Match the colour of acid hematin in Hb tube with standards against the daylight		
9.	Report and viva on haematology experiment		
10.	Total		

COMMON STATIONS – SPOTS IN PRACTICAL EXAMINATION (2/3 MARKS)

Q.1. Diagram/instrument of Sahli's haematometer: Identify and answer any one or two questions (check above).

Q.2. Diagram/instrument of Hb tube, Hb pipette: Identify and answer any one or two questions (check above).

Q.3. Picture of pallor: What is the clinical sign? What is it due to? Write morphological classification of anaemia.

Q.4. If in a 20-year-old male Hb is 14.5 g%–find out the O_2 carrying capacity of his blood (clue- 1 g of Hb combines with 1.34 ml of O_2).

■ CASE-BASED SCENARIO/PROBLEM-BASED

Case 1: A 25-year-old lady comes with c/o fatigue, palpitations, and H/o heavy bleeding during menstruation. O/E pallor ++ MCV-60, MCH-17 picograms, PCV 28.

- What is the reason for pallor in this lady?
- What will be the physiological basis of treatment?
- What can be the morphology of RBCs?
- Why PCV is less? (clue-iron deficiency anaemia, microcytic, microchromic, thus less MCV and MCH) less PCV as well.

Case 2: A 58-year-old man comes with c/o weakness and more loss of blood due to piles. He has been on and off bleeding due to piles for the last 8 to 10 years.

- What chronic blood loss will cause?
- What will be the morphological picture of the RBCs in him? (clue- normocytic normochromic with chronic small amount of blood loss).

Case 3: A paediatrician comes for a visit to check the newborn (born one day before). He checks the newborn thoroughly. Newborn's Hb was found to be 20 g%.

- What is the normal range of Hb in a newborn?
- Why is Hb more in newborns as compared to adults? Give its physiological basis.

Case 4: A 25-year-old male comes with c/o pallor and fatigue. He also complains of problems with respect to focus (concentration) and short-term memory. He also c/o intermittent tingling and numbness in his hands and feet. His Hb is 10.9 g, MCV(123 cubic microns), MCH 37.6 picograms and MCHC 33.5 picograms. Serum antibodies for the intrinsic factor test is negative.

Blood smear: Anisocytosis++, Poikilocytosis++, large RBCs

- What condition probably the patient is suffering from?
- What is megaloblastic anaemia
- Why serum intrinsic factor test was done?
- What will be the physiological basis of treatment (clue vit B12 injections)

Case 5: 7-year-old boy c/o fatigue, body ache and dark-coloured stools for last two days. On examination– pulse 90 beats/min other vitals normal, Hb 7g%, retic (reticulocyte) count 4%, no bilirubin present in urine, total bilirubin 6 mg/dl and high indirect bilirubin.

- What could be your probable diagnosis? (Haemolytic jaundice).
- Write steps in bilirubin metabolism. (Haem–biliverdin-unconjugated bilirubin-gets converted in the liver into conjugated bilirubin- bilirubin glucuronides excreted in bile- in the intestine it is converted to stercobilin, remaining 20% converted as urobilin and excreted in urine)
- What is indirect bilirubin?
- What is the physiological basis of raised reticulocyte count? (clue- stimulation of erythropoiesis process).

Haemolytic jaundice: Excess production of bilirubin glucuronide, increasing quantity of stercobilin formation (dark-coloured stools with excess faecal stercobilinogen) and no bilirubin in urine.

Case 6: A 45-year-old female comes with c/o fever and pain in her abdomen for 4 days. She also has c/o dark-coloured urine and pale stool.

On examination– icterus ++ Hb 11.7 g%, serum bilirubin 5 mg/dl, direct bilirubin levels higher than indirect bilirubin levels.

- What is the probable diagnosis? (obstructive jaundice)
- Physiological basis of signs and symptoms.

Obstructive jaundice: Occurs due to obstruction to bile secretion in the intestine. Thus no faecal stercobilin formed leading to pale-coloured stool and conjugated bilirubin is excreted in urine (dark-coloured urine).

■ KEY POINTS TO REMEMBER

- *Normal Hb values in males:* 14–18 g/dl and in females: 12–15 g/dl of blood.
- *Sahli's principle:* When blood is mixed with HCl, acid hematin (coloured compound) is formed and its colour is matched with standards.
- Important functions of Hb include carrying O_2 from lungs to tissues, CO_2 from tissues to lungs, and Hb acts as a buffer.
- Hb values are more in males, and infants, and are less in females.
- The most commonly practised method for Hb estimation is cyanmethaemoglobin and the most accurate method is an estimation of the iron content of the blood.
- We can diagnose different types of anaemia with the help of blood indices. Iron deficiency anaemia is most common in our country. Common symptoms of anaemia are pallor, fatigue, and muscle weakness.