Section

1

Haematology Experiments

Experiment 1.1

To study the compound microscope and observe common interfering objects under low power and high power

OBJECTIVES

At the end of the practical class student should be able to—

1. Identify the various parts of microscope and the objective lenses with their magnification power.
2. Focus the objects under low power, high power and oil immersion lens of the microscope.
3. Tell the function of condenser and its position while using low power, high power and oil immersion objectives.
4. Tell various types of surfaces of the mirror (reflecting mirror) present in the microscope and in which situation they are used.
5. tell the types of images formed in the microscope.
6. Identify the interfering objects may be present in the visual field (in slide), e.g. sand particles, starch particles, cotton fibers, hair, fat globules and air bubbles.
7. Tell basic principle of use of plane and concave mirrors in compound microscope.

APPARATUS

A compound microscope, glass slide, coverslip, sand, milk, wool thread, cotton fibers, hair and starch granules (crushed potato).

Compound Microscope

Microscope was invented by Antony Leeuwen Hoeck. Various parts of the microscope (Fig. 1.1.1) are as follows:

1. **Base:** Horseshoe-shaped base which provides stability to the microscope.
2. **Limb:** It joins the base to the optical part of the microscope by hinge joint.
3. **Handle:** It is a curved part which joins body tube to the stage of microscope.
4. **Body tubes:** Outer vehicle tube is attached with the handle. It can be moved up and down by coarse and fine adjustment screws, present on the upper part of the handle. Upper end of the inner tube has eye-piece of ten times (10x) magnification lens. Lower end of outer tube has revolving nose piece which contains three objective lenses with low power (10x), high power (40 or 45x) and oil immersion lens (100x).

   Identification of objectives: Each objective marked with its magnifying power, that is 10x, 40x, 45x and oil.

Magnification by using various objectives:

Total magnification = Magnification by eyepiece × magnification by objective

i. Low power (10x): 10 × 10 = 100 times
ii. High power (45x): 10 × 45 = 450 times
iii. Oil immersion lens (100x): 10 × 100 = 1000 times

5. **Fixed stage:** It is a fixed square platform with a hole in the centre. Slide or counting chamber is placed on it and light rays fall on the slide through central hole.
6. **Mechanical stage:** Two clips present on the fixed stage, are used to shift the slide side to side and

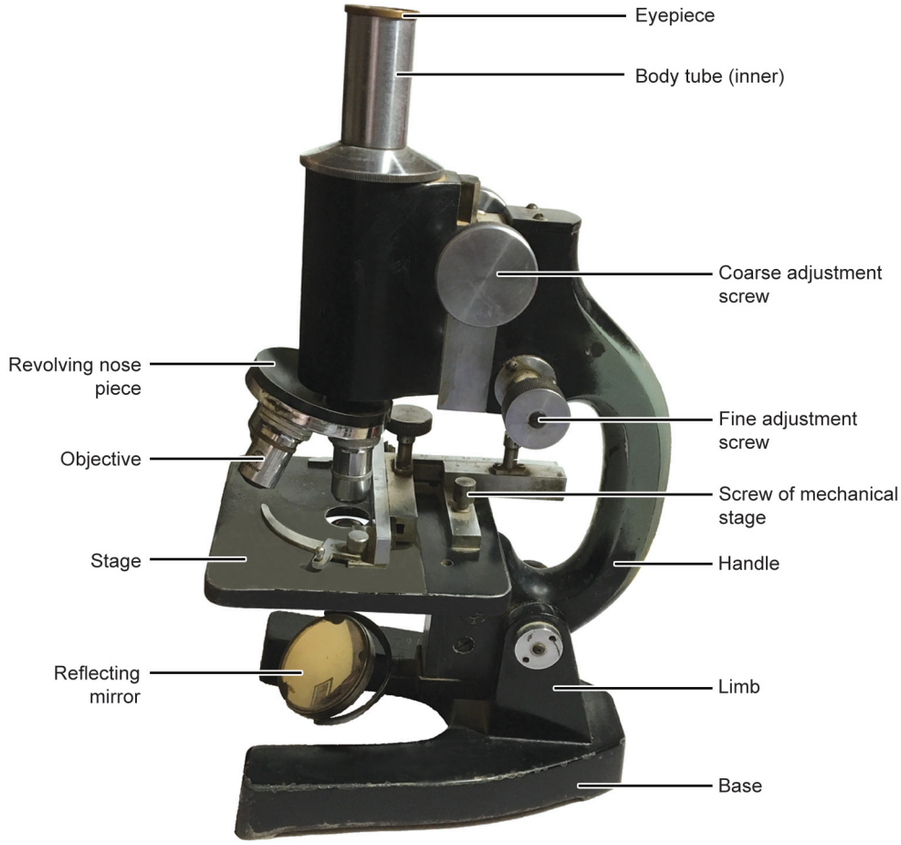


Fig. 1.1.1: Compound microscope (monocular).

backward and forward with the help of two screws present on the stage.

7. **Substage:** Under the fixed stage there is movable stage which has a diagram and a condenser. Aperture size of the diaphragm can be adjusted with the help of a knob present on its side.
   - **Condenser:** It is made up of two convex lenses. It condenses the light rays on the object. It can be moved up or down with the help of a screw present at the lowest part of the handle.
   - **Reflecting mirror:** There is a reflecting mirror having two reflecting surfaces, plane and concave below condenser. It reflects the light from light source to the object.

     Plane mirror is used when natural light is used and concave mirror when artificial light is used. The parallel rays come from plane mirror when light rays are coming from distant source (natural light).

     Concave mirror also gives parallel rays when rays are coming from near source of light (artificial light) but it does not give parallel rays when light rays are coming from distant source (Fig. 1.1.2).

Image Formation in a Compound Microscope

i. Objective forms real, inverted and enlarged image.
ii. Eyepiece forms virtual, erect and enlarged image (Fig. 1.1.3).

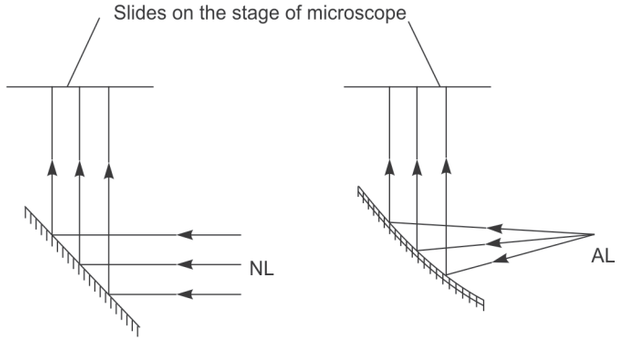


Fig. 1.1.2: Reflection of light rays from plane mirror and concave mirror of a compound microscope when the source of light is natural (NL) or artificial (AL).

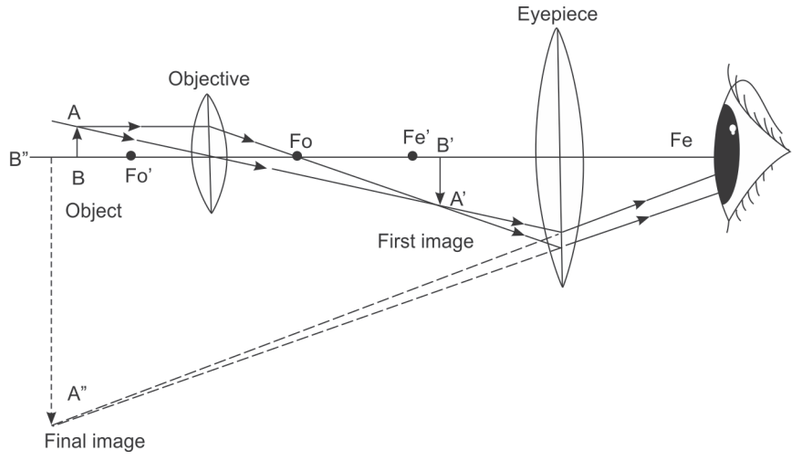


Fig. 1.1.3: Image formation in a compound microscope.

Adjustment of Intensity of Illumination

General Principle

When we use low power (10×) objective, a large area of field is visualized, so we need less illumination. When oil immersion lens (100×) is used, very small area of the field is visualized, requiring highest illumination so that sufficient light reaches up to the eyepiece (Fig. 1.1.4).

Means to increase the intensity of illumination:

1. Aperture size of diaphragm
   Small size—less illumination
   Big size—more illumination
2. Position of condenser
   Lowest position—minimum illumination
   Highest position—maximum illumination

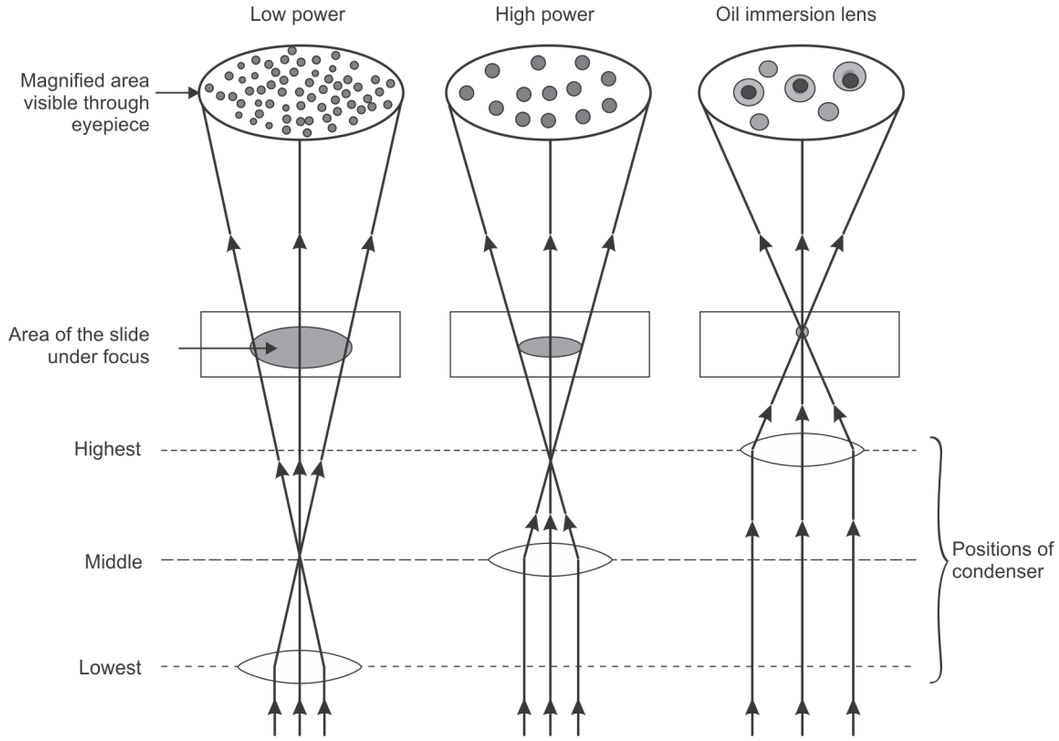


Fig. 1.1.4: Position of condensers in relation to examination of slide under various objectives of a compound microscope.

3. Type of reflecting mirror
   Plane mirror—less illumination
   Concave mirror—more illumination
4. In case of binocular microscope there is no reflecting mirrors and light source is electrical, so illumination can be adjusted with the help of light switch available in the base of microscope and condenser.

Use of Cedarwood oil or Liquid Paraffin in Oil Immersion Objective

Refractory index of cedarwood oil or liquid paraffin is equal to that of glass, so it prevents the scattering of light rays and the image will be more clear when we use it with oil immersion lens (Fig. 1.1.5).

Binocular compound microscope (Fig. 1.1.6)

- Two eyepieces are there in the microscope.
- No reflecting mirror
- Inbuilt light source which can be recharged.
- ***At the time of focusing, stage move up or down not the body tube holding eyepiece.***

Focusing of an Object under Microscope

a. Low Power

i. Place the slide on the stage of microscope and bring the area of the slide over the hole present in the stage with the help of screws attached with mechanical stage.
ii. Then bring low power objective over the slide and adjust its position a few mm above the slide. During this process constantly look from the side of the microscope so that it will not touch the slide.
iii. Adjust illumination by adjusting the aperture of diaphragm, position of condenser and selection of proper mirror.
iv. With the help of coarse adjustment screws move the objective up and simultaneously look into the microscope till the object is visualized.
v. Now with the help of fine adjustment screw further focus the object till it becomes clearly visible.

b. High Power

i. First focus the object under low power.
ii. Turn the nose piece and bring high power objective over the slide.
iii. Adjust the fine adjustment screw till view becomes clear.
iv. Adjust the illumination so that object becomes very clear.

c. Oil immersion Lens

i. Put a drop of cedarwood oil after placing the slide on the stage just over the area which has to be focused.
ii. Dip the oil immersion lens very carefully with the help of coarse adjustment screw by constantly looking from the side of microscope so that it will just dip in the oil without touching the slide.
iii. Do fine focusing with the help of fine adjustment screw by raising the objective till its view becomes very clear.
iv. Illumination is adjusted to the maximum by all the ways discussed (fully open diaphragm and highest position of condenser).

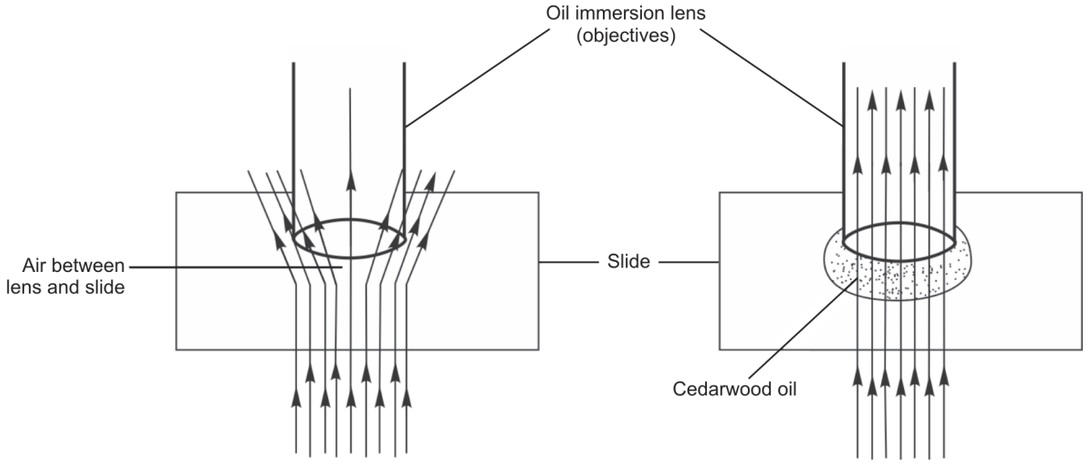


Fig. 1.1.5: Cedarwood oil prevents scattering of light rays.

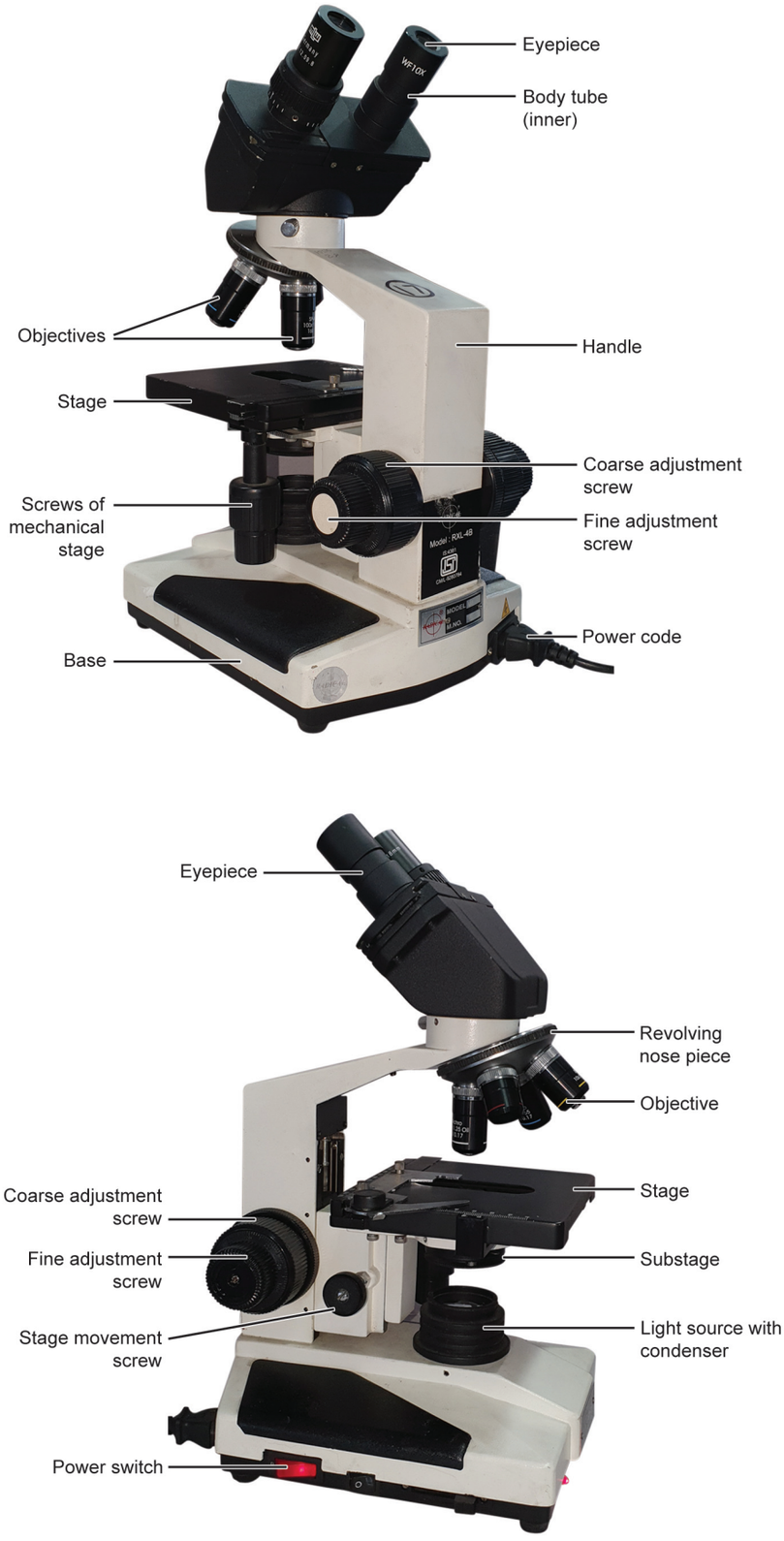


Fig. 1.1.6: Binocular compound microscope.

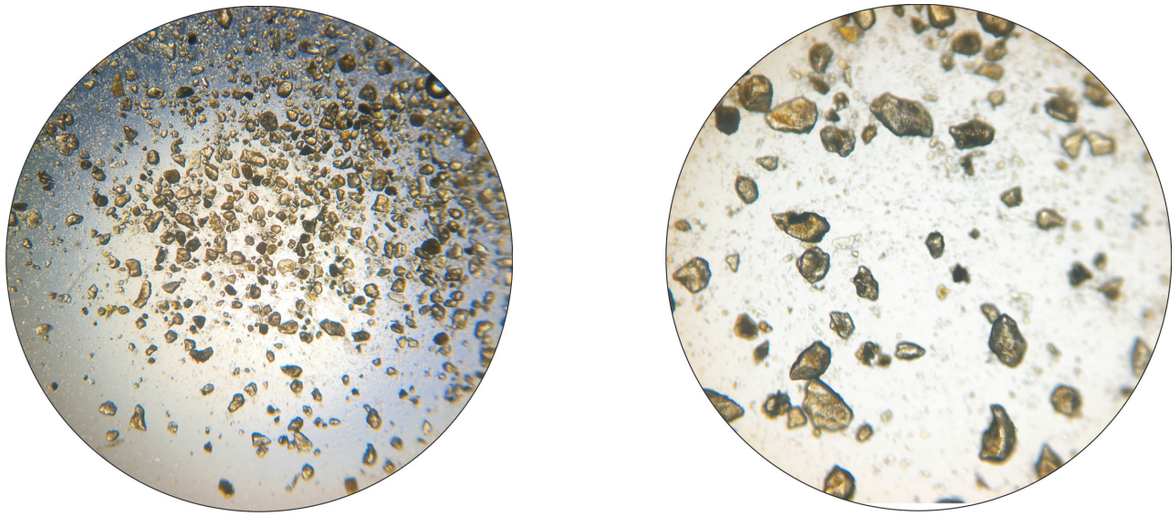
PRECAUTIONS

1. Eyepiece and objective of the microscope must be clean.
2. Do not use dry cotton to clean the lens. Xylene with soft cloth should be used for this purpose.
3. Do not use spirit to clean lenses since it may dissolve the fixing material of the lens.
4. Do not lower the objective grossly without looking from the side of the microscope.
5. Always keep the microscope vertical at the time of shifting it from one place to the other.

Focussing

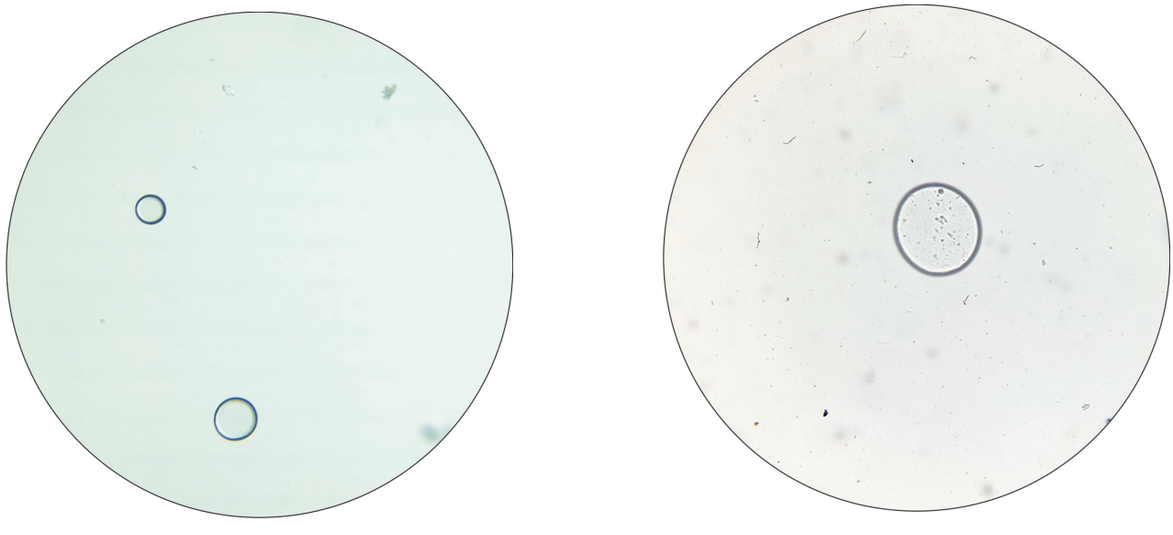
Focus all the objects first in low power and then under high power as follows:

1. **Sand (dust) particles:** Place a little quantity of sand mixed in water on the slide and put a coverslip on it and examine. Translucent and opaque particles of different shapes and sizes are seen. (Fig. 1.1.7)
2. **Air bubble:** Put a drop of distilled water on the slide and place a coverslip over it, in such a way that it will have air bubbles.



(a) (b)

Fig. 1.1.7: Sand (dust) particles under (a) low power (10×) and (b) high power (40×) of microscope.



(a) (b)

Fig. 1.1.8: Air bubble (a) low power (10×) and (b) high power (40×) of microscope.

Air bubbles are clear spaces with dark boundaries because of refraction from the boundaries of air bubble (Figs 1.1.8 and 1.1.9).

3. **Fat globule:** Take a drop of milk on the slide and put a coverslip. Fat globules are seen as circular objects having fine clear boundary (Fig. 1.1.10).
4. **Wool fibers:** Place wool fibers on a drop of water on the slide and put a coverslip over it and examine under microscope.

   These fibers are translucent having no twists.
5. **Cotton fibers:** Put some cotton fibers in a water drop on glass slide, cover it with coverslip and examine under microscope.

   These are translucent fibers having twists (oblique marking) at various sites (Fig. 1.1.11).
6. **Human hair:** Place some hair on a slide, cover it with coverslip and examine.

   These are dark coloured objects some time lighter in the centre and darker in periphery (cortex and medulla) (Fig. 1.1.12).
7. **Starch particles:**
   i. **Unstained:** Take a drop of mixture of crushed potato in water on a slide and put a coverslip on it. Examine under microscope.

      Starch particles are pear shaped objects with concentric rings and pointed hilus on one side (Fig. 1.1.13a).

   ii. **Stained:** Add a drop of iodine solution to starch particles and examine under microscope. Particles appear blue in colour having concentric rings and pointed hilus on one side (Fig. 1.1.13b).

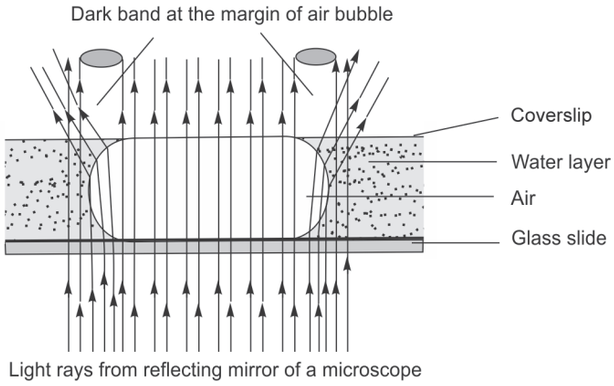


Fig. 1.1.9: Appearance of boundaries of air bubble under oil immersion lens.

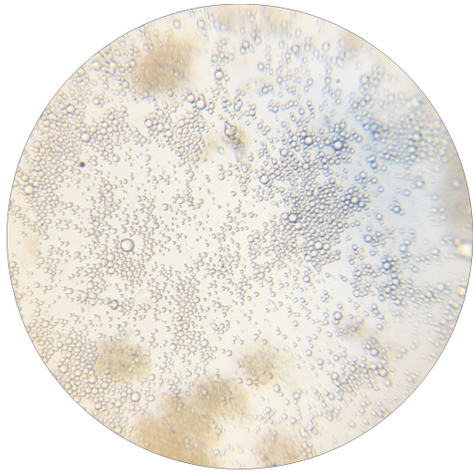
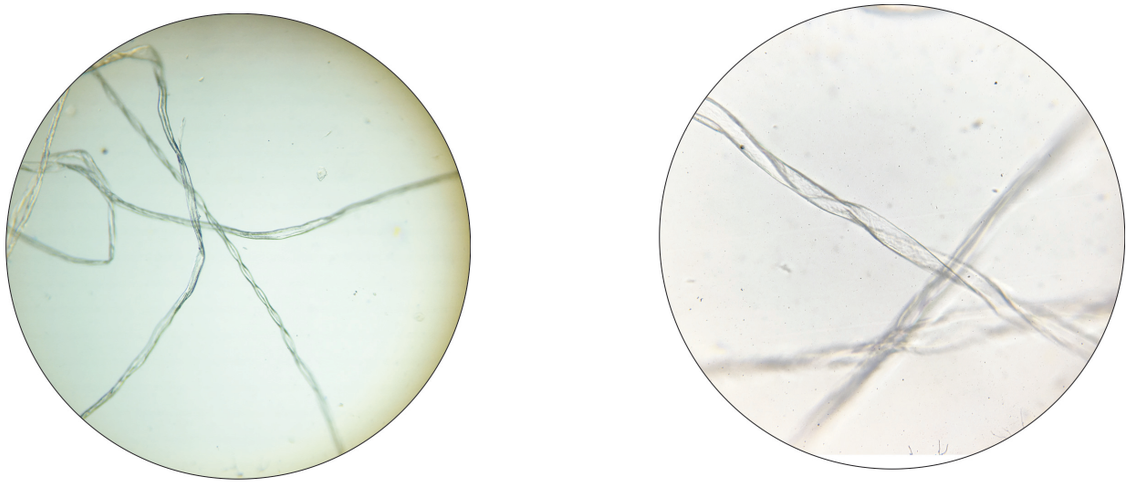


Fig. 1.1.10: Fat globule, high power (40×) of microscope.



(a) (b)

Fig. 1.1.11: Cotton fibers (a) low power (10×) and (b) high power (40×) of microscope.

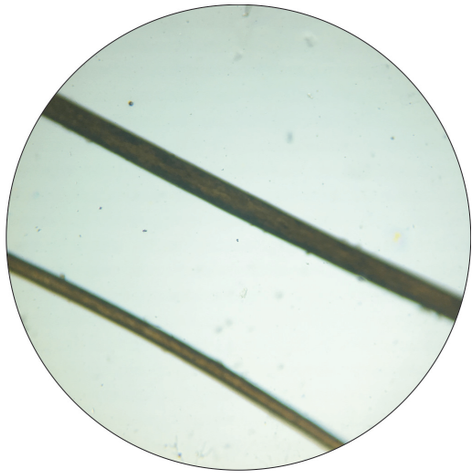


Fig. 1.1.12: Human hair, high power (40×) of microscope.

QUESTIONS AND ANSWERS

Q.1. How will you identity the various objectives?

Ans. *See* text

Q.2. What is the role of cedarwood oil/liquid paraffin in oil immersion lens?

Ans. Cedarwood oil has the same refractory index as that of glass so prevents the scattering of light rays coming from the slide and makes the view more clear.

Q.3. What is the function of condenser and what should be its position while using low power, high power and oil immersion objective?

Ans. *See* text

Q.4. What are various types of surfaces of the mirror present in the microscope and in which situation they are used?

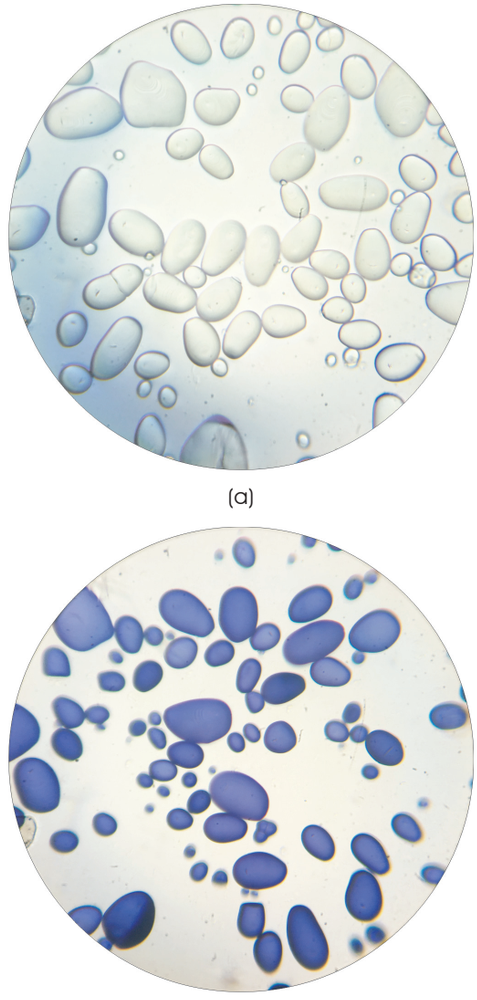
Ans. *See* text

Q.5. What type of image do you observe in the microscope?

Ans. *See* text

Q.6. Why is the boundary of air bubble darker?

Ans. Light rays coming from the boundaries of air bubble divert their path and because of deficiency of light in this region they look dark.



(a)

(b)

Fig. 1.1.13: Starch particles under high power (40×) of microscope (a) unstained and (b) stained.

Observations and Diagrams

Observations and Diagrams *(Contd.)*

Student's Notes

Experiment 1.2
To study haemocytometer and to collect blood sample

A. HAEMOCYTOMETER

OBJECTIVES

At the end of the practical class student should be able to—

1. Identify RBC and WBC pipette.
2. Focus Neubauer's chamber under low power, high power and oil immersion lens.
3. Identify the various squares of Neubauer's chamber.
4. Tell the size and volume of big, medium and small squares.
5. Charge the Neubauer's chamber.

Haemocytometer is an apparatus used to do count of various blood cells (RBC, WBC, eosinophil and platelets). It consists of RBC and WBC pipette, and a thick slide (Neubauer's chamber).

1. RBC Pipette

i. This consists of a glass stem having capillary tube in it which opens in a bulb containing red bead and opposite to the bulb again there is a small stem. This small stem is connected to the red coloured mouthpiece with the help of a rubber tube (Fig. 1.2.1).

ii. The stem has three markings, 0.5, 1.0 and 101. From the tip of the pipette to the marking 1.0 there are 10 equal divisions/parts. These are simple divisions not any specific unit like—mm, ml, and cu. mm.

iii. The stem has the capacity of one part and bulb has 100 parts.

iv. Bead of the pipette serves two purposes, one mixing of the blood with diluting fluid and other act as an identification mark of RBC pipette.

2. WBC Pipette

This pipette is similar in shape except size of bulb is smaller, markings are 0.5, 1.0 and 11, and bulb contains white bead and colour of the mouthpiece is white (Fig. 1.2.2).

3. Neubauer's Chamber (Counting chamber)

i. This is a thick glass slide having central platform divided into two positions with the help of H shape groove or trench (Fig. 1.2.3).

ii. On both the sides of lateral groove there are raised ridges of a height of 0.1 mm (1/10 mm) from the central platform. When a coverslip is placed on the ridges, a space of 0.1 mm height is created below the coverslip on the central platform.

iii. Counting chamber (grid) is made up of ruled area of 3 mm × 3 mm size on each central platform (Fig. 1.2.4). Each central area is further divided by triple lines into 9 squares of equal size (1 square mm each).

iv. Four corner squares are further divided into 16 squares of equal size. These four corner squares are used to do total leucocytic count (TLC).

v. Volume of each big square is 0.1 cu mm (1 mm × 0.1 mm).

vi. Central big square is divided into 25 (medium size) squares each having arm 1/5 mm. Area of each medium size square is 1/25 mm^2 (1/5 × 1/5) and the volume of each square is 1/250 cu mm (1/25 × 1/10).

vii. Further these medium size squares are divided into 16 small squares of equal size. Area of each small square is 1/20 mm^2 (1/5 × 1/4). The area of each square is 1/400 mm^2 (1/20 × 1/20) and volume is 1/4000 mm^3 (1/400 × 1/10). Total

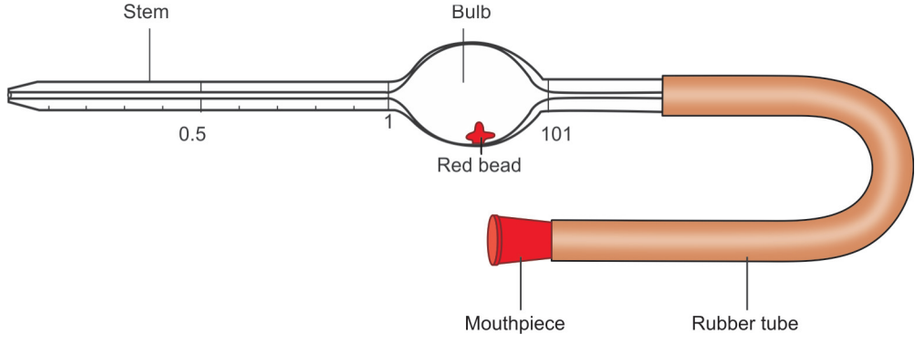


Fig. 1.2.1: RBC pipette.

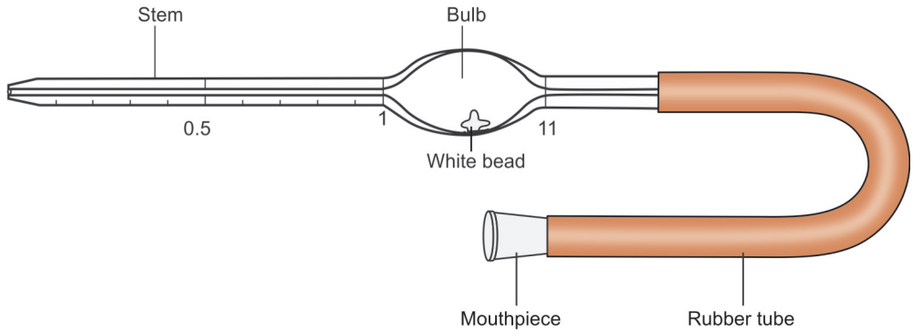


Fig. 1.2.2: WBC pipette.

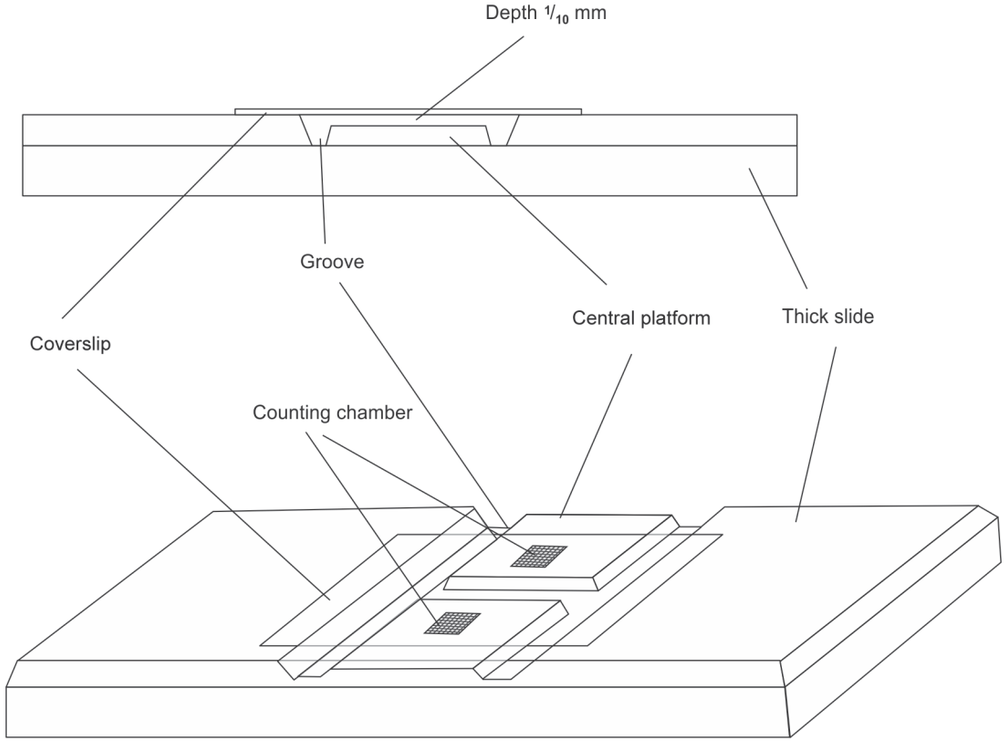


Fig. 1.2.3: Neubauer's chamber (improved), side view (upper) and surface view (lower).

number of smallest squares in big central square are 400 (25 × 16).

viii. RBCs are counted in 5 medium size squares (R1, R2, R3, R4, R5), four corners and one central.

Use of Counting Chamber

RBC chamber: It is used in RBC, platelet and reticulocyte count.

WBC chamber: It is used in WBC and eosinophils count.

PROCEDURE

i. Clean the Neubauer's chamber in soap solution and let it dry.

ii. Place it on the stage of microscope and focus counting chamber in low power and study all the squares carefully.

iii. Turn the nose piece of microscope and focus the chamber under high power. Study all the squares carefully.

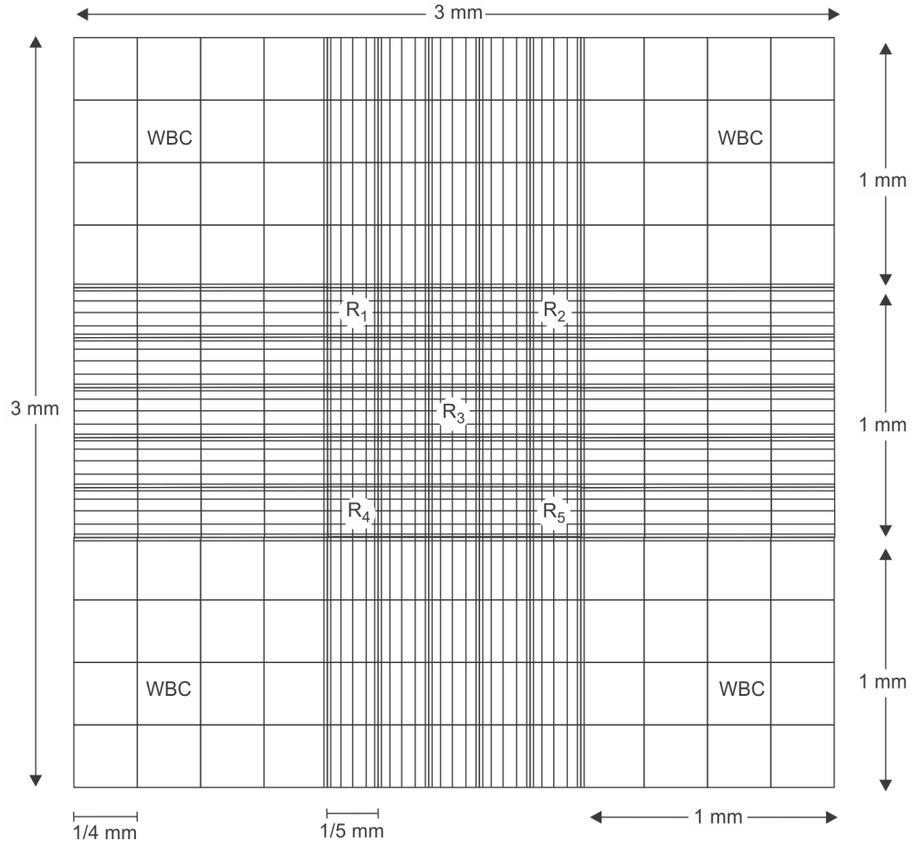


Fig. 1.2.4: Counting chamber (Neubauer's chamber) under low power of a compound microscope.

Charging of Chamber

This involves introducing diluted blood in Neubauer's chamber below coverslip.

Remove the chamber from the stage of the microscope and place a coverslip over the chamber. Now take coloured solution (eosin in water) in the RBC pipette and touch the tip of pipette near the edge of the coverslip on central platform at an angle of about 45 degree. As the fluid enters below the coverslip because of capillary action, immediately remove the pipette.

PRECAUTIONS

1. Chamber should be clean, dry and grease free.
2. There should not be overcharging (flow of fluid in side trenches) or undercharging (incomplete filling of the chamber).
3. Do not hold the coverslip from its flat side (it will leave fingerprints), hold it from the edges.
4. If overcharging is there, wash the chamber and recharge.

B. COLLECTION OF BLOOD SAMPLE

OBJECTIVES

At the end of the practical class student should be able to—

1. Collect blood sample of capillary blood and venous blood.
2. Tell the names of common anticoagulants used and their mechanism of action.
3. Tell the significance of mixed oxalate.
4. Tell the precautions to be taken in collecting and transferring blood.
5. Dispose of the needle, syringe and cotton swab after collection of blood sample.
6. Explain why ring finger of left hand is selected to collect the capillary blood sample.

Blood is collected for various haematological investigations.

Capillary Blood

i. It can be collected from fingertip and ear lobule in adult and heal in case of newborn or infant.
ii. Take 20–24 gauge sterilised disposable hypodermic needle for this purpose.
iii. Clean ring finger of left hand (preferably ring finger) with spirit swab and left it dry. Sterilisation is effective when spirit dries up and spirit causes haemolysis when it comes in contact with blood.
iv. Give 2–3 mm deep prick at centre of the tip of ring finger so that free flow of blood will be there.
v. After collection of blood sample put a spirit swab at pricking site and hold it there for 2–3 minutes (till bleeding stops).
vi. Do not squeeze the finger for collection of blood, it will cause dilution of blood because tissue fluid mixes with blood.

Venous Blood

i. Take 5 ml disposable syringe with 19–20 gauge needle.
ii. Support the arm of the subject on the edge of the table and locate the vein in antecubital fossa (area).
iii. Clean the area with spirit swab and let it dry. Do not touch the area again with finger once it is cleaned.
iv. Apply rubber or cloth tourniquet firmly around the arm to occlude venous return. Ask the subject to close and open the fist repeatedly so as vein gets engorged with blood.
v. Place the thumb of your left hand on the skin about 4–5 cm distal to the vein to be pricked so that vein will not slip.
vi. Introduce the needle into the skin and push the needle on the side of vein, when needle enters the vein resistance felt to cease.
vii. Draw the blood in syringe slowly to the required quantity (slowly means not faster than the filling of vein).
viii. Release the tourniquet and withdraw the needle after putting the fresh spirit swab at the site of the puncture of skin.
ix. Ask the subject to press the swab at the site of puncture for 2–3 minutes (till bleeding stops).
x. Eject the blood from the syringe after removing needle in the vial having anticoagulant. For serum no anticoagulant is required in the vial.

Anticoagulant Used

1. Ethylene diamine tetracetic acid (EDTA):
   - Calcium chelating agent
   - No effect on blood cells
   - 2.4 mg dry powder in a vial for 2 ml of blood. (1.2 mg/ml of blood)
   - Used for ESR and PCV measurement.
2. Sodium citrate:
   - Calcium chelating agent
   - 0.4 ml of 3.8% solution for 1.6 ml of blood
   - Used for ESR and collection of blood from donors
3. Double oxalate mixture:
   - Potassium oxalate and ammonium oxalate in the ratio of 2:3.
   - Single oxalate is not used mainly to measure PCV. Because potassium oxalate causes shrinkage of cells and ammonium oxalate causes increase in volume of the cells.
4. Heparin:
   - Solution or powder may be used according to need.
   - Does not affect cell volume.

PRECAUTIONS

1. Must not interchange the pricking needle with other students.
2. Preferably use a needle for pricking once.
3. If necessary to reuse the needle in the same subject, sterilise it on spirit lamp not with spirit (as spirit does not kill hepatitis virus).
4. Do not use single oxalate anticoagulant for measuring PCV.
5. Destroy the needle before disposing it off in needle destroyer.
6. During collecting blood from blood vessel for investigation must wear gloves.
7. If you get prick accidentally from the needle which has been used to collect the blood sample must inform to the senior doctor immediately for further necessary actions.

QUESTIONS AND ANSWERS

Q.1. Why ring finger of left hand is preferred for pricking?

Ans. Because palmar fascia does not extend up to ring figure. So there are no chances of spread of infection, if occurs during pricking. Other reason is the ring finger of left hand comes in contact least as compared to the other fingers of same hand or of the opposite during working, so the chances of pain and infection are less.

Q.2. Why should we not squeeze the finger for taking the blood?

Ans. *See* text

Q.3. How will you differentiate between RBC and WBC pipette?

Ans. *See* text

Q.4. What are the mechanisms of action of different anticoagulants?

Ans. *See* text

Q.5. What measures will you take to prevent the haemolysis at the time of collection of blood?

Ans. Haemolysis can be prevented by:

i. Using the clean vial to collect the sample.
ii. Taking the sample by wide gauze needle (No. 24 or less).
iii. Withdrawing the blood slowly from the vein.
iv. Delivering the blood gently after removing the needle from the syringe in the vial.

Student's Notes

Experiment 1.3
Determination of red blood cell (RBC) count

OBJECTIVES

At the end of the practical class student should be able to—

1. Do RBC count of his own blood.
2. Tell normal value of RBC count.
3. Name the contents of Hayem's fluid and their role in fluid.
4. Tell in which magnification RBCs are counted.
5. Tell rule of counting of cells.
6. Enumerate physiological and some pathological conditions which effect erythrocyte count.
7. Tell the precautions to be taken in this investigation.
8. Tell the possible sources of errors effecting the count.
9. Calculate the dilution factor.
10. Tell the precautions to be taken in this experiment.
11. Tell in spite of all the precautions what are the chances of error.

APPARATUS

Neubauer's chamber (thick slide), RBC pipette, diluting fluid, microscope, coverslip, pricking needle and spirit swab.

RBC Diluting Fluid (Hayem's Fluid)

1. Sodium chloride (NaCl): 0.5 gm, to maintain isotonicity of fluid
2. Sodium sulphate (Na_2SO_4): 2.5 gm, which prevents rouleaux formation.
3. Mercuric chloride ($HgCl_2$): 0.25 gm, acts as preservative (antibacterial and antifungal)
4. Distilled water (H_2O): 100 ml.

PRINCIPLE

Red blood cells are counted in diluted blood and actual count is calculated by multiplying by dilution factors.

PROCEDURE

1. Take about 3–5 ml Hayem's fluid in a watch glass.
2. Prick the ring finger after cleaning it with spirit swab.
3. Wipe off the first drop of blood. Suck the next drop in RBC pipette exactly up to 0.5 mark, taking care that there should be no air bubble. If excess blood has been drawn, remove it by touching the pipette on the cotton swab very carefully.
4. Wipe off the blood sticking around the tip of the pipette with cotton swab.
5. Now suck the Hayem's fluid in the pipette up to mark 101.
6. The pipette is then kept horizontally between palms and rolled gently for a minute to mix the blood with diluting fluid.
7. Focus Neubauer's chamber under low power (10×) objective of microscope.
8. Remove the chamber from microscope and place a coverslip on it.
9. Discard first 2–3 drops of fluid from the pipette which is unmixed fluid present in the stem of the pipette.
10. Charge the Neubauer's chamber:
    i. Small drop of fluid is allowed to form at the tip of the pipette.
    ii. Bring the tip of the pipette near the edge of the coverslip on central platform in such a way that it will make an angle of about 45° with central platform.
    iii. Fluid will be drawn in capillary space below the coverslip. Once the fluid enters below coverslip immediately, remove the pipette from central platform.
11. Wait for 3–5 minutes to settle the cells and put the charged Neubauer's chamber under low of microscope. With the help of fine focussing see that there should be the uniform distribution of RBCs, if it is not recharge the chamber again.
12. Focus the Neubauer's chamber under (40´) high power and count the cells in five medium size square shown in Fig. 1.3.1 (R1, R2, R3, R4 & R5). There are 16 small squares in each medium size square so the total small squares are 80.

Rules for Counting the Cells

i. Any cell which is lying half or more than half or complete is counted in the same square.
ii. Counting is started from left and upper border of square.
iii. Among the tripple line central line should be considered as a main line for counting of cells.
iv. Cells of lower and right border should also counted at the end of each row.

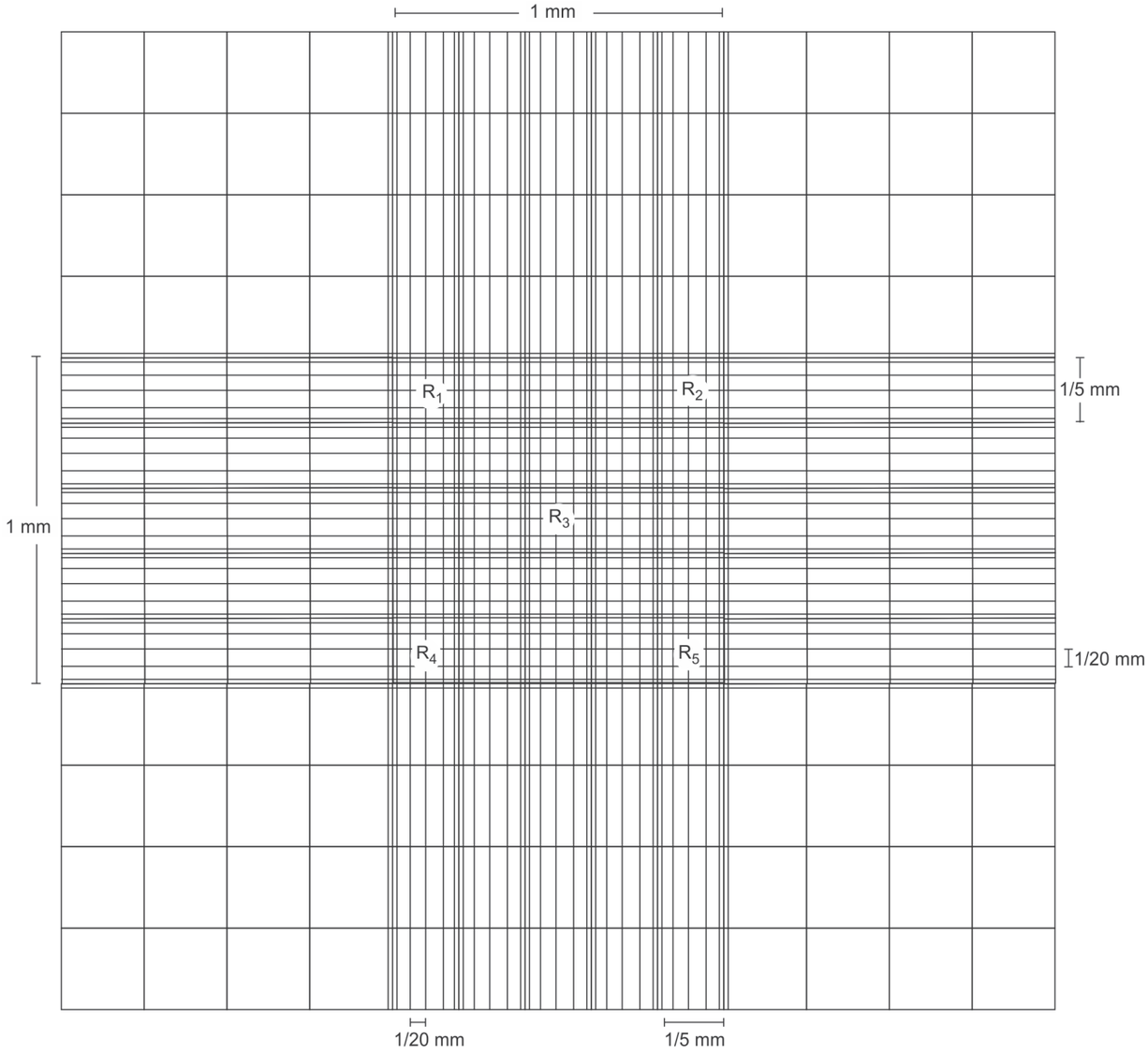


Fig. 1.3.1: Counting chamber (Neubauer's chamber) under low power of a compound microscope.

For example: In medium size square R1 (Fig. 1.3.2) the number of cells are as follows:

Square 1 – 2 cells
Square 2 – 1 cell
Square 3 – 2 cells
Square 7 – 1 cell
Square 9 – 2 cells
Square 11 – 1 cell
Square 12 – 1 cell
Total cells in R1 square = 10.

During counting enters the number of cells present in different small squares in the square drawn on a paper in a similar way as in Neubauer's chamber (Fig. 1.3.2).

CALCULATIONS

Dilution factor: 0.5 part of blood mixes in total 100 parts of mixture (99.5 parts diluting fluid). Fluid present in the stem (1.0 part) does not take part in mixing. This is why 2–3 drops of fluid (present in stem) is discarded before charging the chamber.

$$\text{Dilution factor} = \frac{\text{Total volume of bulb (100 parts)}}{\text{Volume of blood taken (0.5 part)}}$$

$$= \frac{100}{0.5} = 200$$

Area of medium size square (R)
$= 1/5 \times 1/5 = 1/25 \text{ mm}^2$

Depth of chamber $= 1/10$ mm

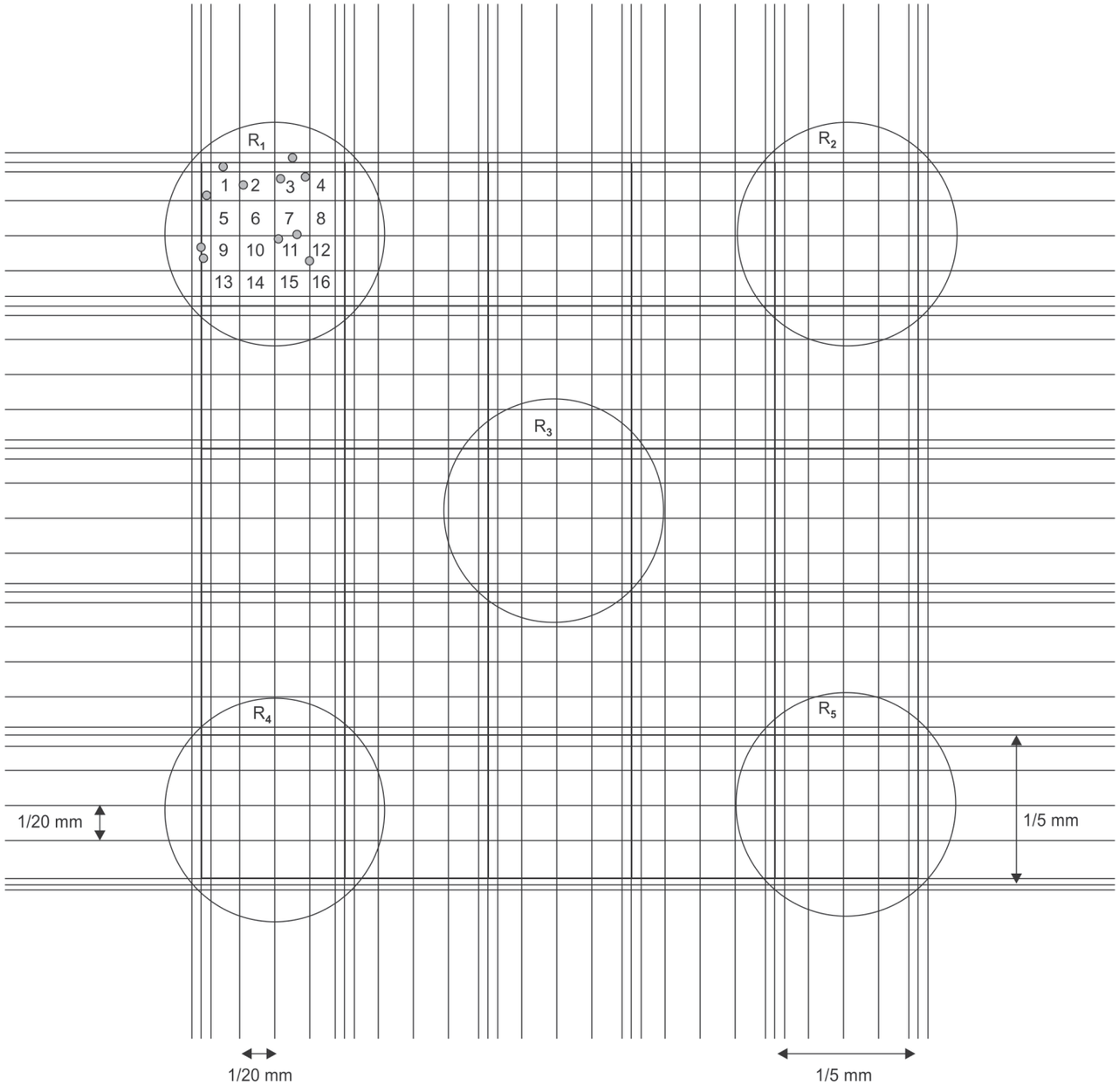


Fig. 1.3.2: Each circle denotes the field which is visualised at one time under high power of a compound microscope.

Volume of each medium size square (R)
= 1/25 × 1/10 = 1/250 cu mm

Total volume of 5 squares (R1+R2+R3+R4+R5)
= 1/250 × 5 = 1/50 cu mm

Suppose total number cell counted in five (R1 + R2 + R3 + R4 + R5) squares = N

Number of cells counted in 1/50 mm^3 in diluted blood = N

Number of cells counted in 1 mm^3 diluted blood
= N × 50

Number of cells in 1 mm^3 undiluted blood
= N × 50 × 200

RBC count = N × 10000/mm^3

Observations and Calculations

Normal RBC Count

In adult male: 5.5 million/cu. mm (5–6 million)
In adult female: 4.8 million cu. mm (4.5–5.5 million)

RESULT AND COMMENTS

Write down the result and comment accordingly

PRECAUTIONS

1. RBC pipette, Neubauer's chamber and coverslip should be clean and dry.
2. Hold the coverslip from its edges not from its flat surfaces.
3. Don't take more time in filling the pipette with blood (blood will clot in the pipette).
4. There should not be undercharging or overcharging.

Physiological Variations

1. Age: In newborn baby it is more.
2. Sex: More in male.
3. High attitude: Increase RBC count at high attitude.

Pathological Variations

1. Hypoxia
2. Polycythemia vera.

QUESTIONS AND ANSWERS

Q.1. What is the normal value of RBC count in adult male and female?

Ans. *See* text

Q.2. How will you differentiate RBC and WBC pipette?

Ans. *See* text

Q.3. What are the functions of bead in the pipette?

Ans. *See* text

Q.4. What are the units of markings on the pipette?

Ans. *See* text

Q.5. How much is the dilution in RBC pipette and why?

Ans. *See* text

Q.6. Why there is a need to dilute the blood for RBC count?

Ans. Because the RBC count is very high and in undiluted blood it is very difficult to count the cells under microscope.

Q.7. What are the other uses of RBC pipette?

Ans. RBC pipette can be used for platelets count and sperm count.

Q.8. Why is it important to discard 2–3 drops of fluid before charging the chamber?

Ans. *See* text

Q.9. How is the RBC count affected, when the chamber is under or overcharged?

Ans. *See* text

Q.10. How will you clean the pipette blocked by a blood clot?

Ans. It should be cleaned with N/10 HCl or hydrogen per oxide.

Q.11. What is the importance of rules of counting of RBC?

Ans. If we follow this rule during counting of RBCs, we will not miss any cell and will not count any cell twice.

Q.12. Will you count the WBCs during RBC count and how will it affect RBC count?

Ans. WBCs should be avoided during counting of RBCs but smaller white are difficult to exclude during counting. Even if these cells are included during counting it will not affect the RBC count because WBC count is very less as compared to RBC count.

Q.13. What is the approximate error in RBC count by this method?

Ans. It is about + 11%.

Q.14. Is there any other accurate method for determination of RBC count?

Ans. Yes, it is done by electronic cell counter which is accurate and more reliable method. By this method error is about 1%.

Q.15. What are the physiological factors responsible for polycythemia?

Ans. *See* text

Q.16. What is the composition of Hayem's fluid and what is the role of various ingredients in fluid?

Ans. *See* text

Student's Notes

Experiment 1.4
Determination of total leukocyte count (TLC)

OBJECTIVES

At the end of the practical class student should be able to —

1. Determine WBC count of his own blood.
2. Tell normal value of WBC count.
3. Enumerate certain conditions in which WBC count increases and decreases.
4. Name the contents of Turk's fluid and their role in fluid.
5. Tell in which magnification cells are counted.
6. Tell rule of counting of cells.
7. Enumerate physiological and some pathological conditions which effect erythrocyte count.
8. Tell the precautions to be taken in this investigation.
9. Tell the possible sources of errors effecting the count.
10. Calculate the dilution factor.

APPARATUS

Neubauer's chamber, WBC pipette, diluting fluid, microscope, coverslip, prickling needle and spirit swab.

WBC diluting fluid (Turk's fluid):

1. Gentian violet: 1% 1 ml, stains nuclei of WBCs.
2. Glacial acetic acid: 3 ml to destroy RBCs and platelets (destroy cell membrane).
3. Distilled water: To make 100 ml.

PRINCIPLE

White blood cells are counted in diluted blood and actual count is calculated by multiplying it by dilution factor.

PROCEDURE

1. Take 3–5 ml Turk's fluid in a watch glass.
2. Prick the ring finger of left hand after cleaning it with spirit swab.
3. Wipe off first drop of blood. Suck next drop of blood in WBC pipette exactly up to 0.5 mark, taking care that there should be no air bubble. If excess blood has been drawn, remove it by touching the pipette on cotton swab very carefully.
4. Wipe off the blood sticking around the tip of the pipette with cotton swab.
5. Now suck the Turk's fluid in the pipette up to mark 11.
6. Hold the pipette in between palms and mix the blood with diluting fluid by rolling it gently.
7. Focus the Neubauer's chamber under low power and charge the chamber after discarding 2–3 drops of fluid from the pipette in the same way as in RBC count.
8. Let the cells settle for 3–5 min, and keep the Neubauer's chamber again under low power of microscope and count WBC in outer four corner squares.
9. During counting WBC enter the number of cells counted in different squares in the squares drawn on a paper (Fig. 1.4.1).

CALCULATIONS

Dilution factor: 0.5 part of blood mixes in total 10 parts of mixture in bulb (9.5 parts diluting fluid). Fluid present in the stem (1.0 part) does not take part in mixing. This is why 2–3 drops of fluid is discarded before charging.

$$\text{Dilution factor} = \frac{\text{Total volume of bulb (10 parts)}}{\text{Volume of blood taken (0.5 part)}}$$

$$= 20$$

Area of a big square = $1 \times 1 = 1\ mm^2/mm^2$

Depth of chamber = 1/10 mm

Volume of a big square = $1 \times 1/10 = 0.1\ mm^3/mm^3$

Volume of four squares = $0.4\ mm^3$

Total number of cells counted in four corner squares = N

Number of cells in $0.4\ mm^3$ of diluted blood = N

Number of cells in $1\ mm^3$ of diluted blood = N/0.4

Number of cells in $1\ mm^3$ of undiluted blood = $N/0.4 \times 20 = N \times 50$

Normal WBC count: 4000–11000/cu mm.

Observations and Calculations

Observations and Calculations (*Contd.*)

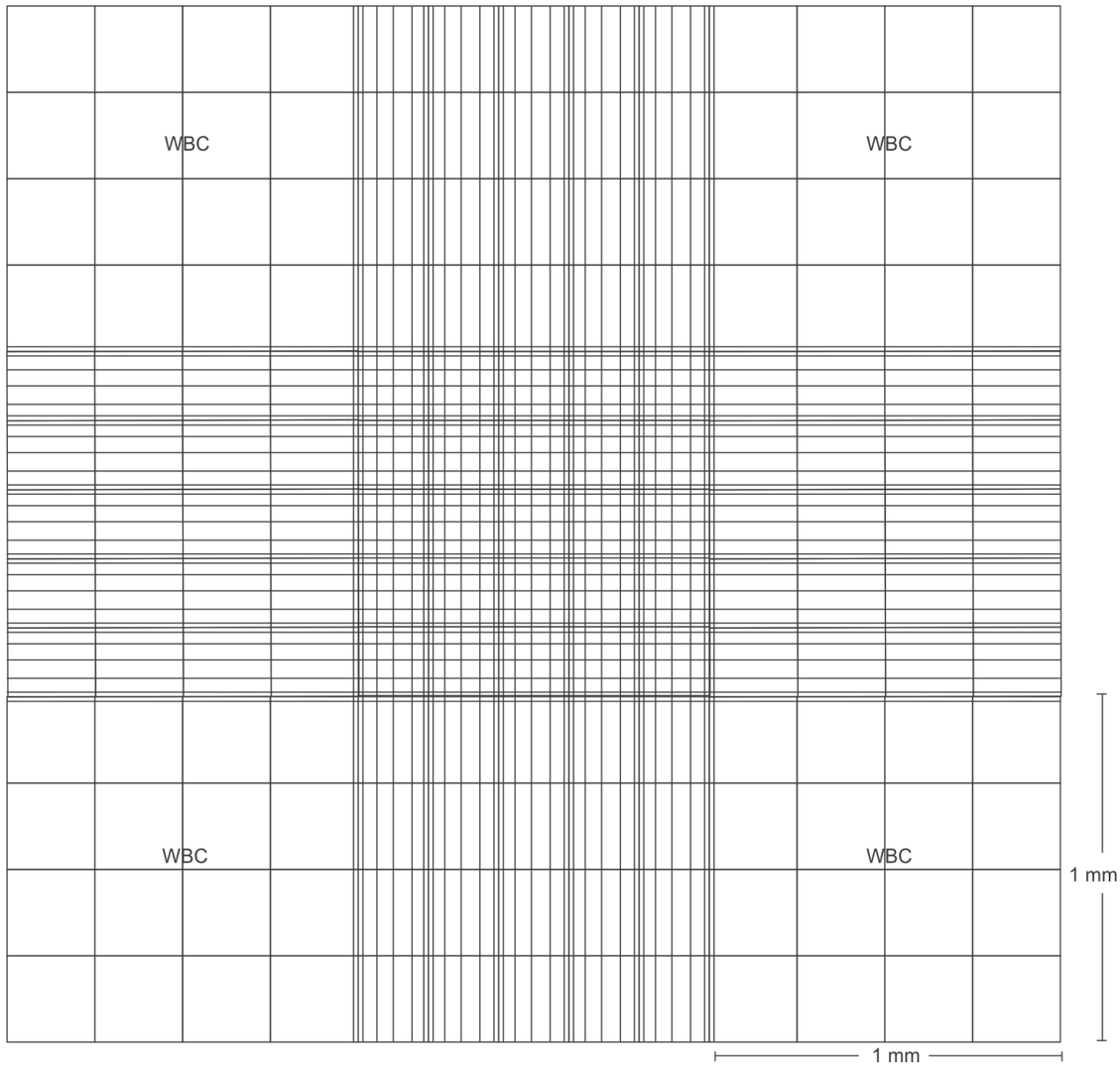


Fig. 1.4.1: Counting chamber (Neubauer's chamber) under low power of a microscope.

Precautions

All the precautions are similar as in RBC count.

Variations in WBC Count

Leucocytosis

Physiological: Severe muscular exercise, pregnancy and food intake.

Pathological: Acute pyogenic infection, e.g. abscess, pneumonitis, appendicitis.

Leucopenia

Pathological: Bone marrow depression, e.g. chloramphenicol, aplastic anaemia.

Result and Comments

Write down the result and give your comments accordingly.

QUESTIONS AND ANSWERS

Q.1. What is the normal value of WBC count?
Ans. *See* text
Q.2. How much is the dilution in WBC pipette?
Ans. *See* text
Q.3. What are the constituents of Turk's fluid and what are the functions of each ingredient?
Ans. *See* text
Q.4. What is the leucocytosis and leucopenia?
Ans. *See* text
Q.5. How will you differentiate between leucocytosis and leukemia?
Ans. In leucocytosis WBC count is less than 50000 per cu mm and in leukaemia it is more. In leukaemia immature white cells are seen in the blood film.
Q.6. What are the causes of leucopenia?
Ans. *See* text
Q.7. What are the physiological and pathological causes of leucocytosis?
Ans. *See* text

Student's Notes

Experiment 1.5
Estimation of Haemoglobin by Sahli's method

OBJECTIVES

At the end of the practical class student should be able to—

1. Tell the principle used in determination of Hb by Sahli's method.
2. Estimate Hb of his own blood.
3. Tell the normal value of Hb.
4. Enumerate other methods of Hb estimation.
5. Define anaemia.
6. Tell the indication of Hb estimation.
7. classify the severity grades of anaemia.
8. Explain absolute and relative scale of anaemia.
9. Explain why the quantity of 0.1 N HCl in Sahli's tube has to be taken up to 20%. Can it be taken more or less?
10. Tell how to prepare 0.1 N HCl.
11. Tell which meniscus of diluted mixture is considered for noting down the level of Hb and why.
12. Tell how to confirm that reading which he has taken for Hb is correct.
13. Tell which method is used to estimate Hb in patients these days in laboratories.
14. Tell which method of estimation of Hb is most accurate.
15. Tell the physiological conditions in which there is rise in Hb level of a person.
16. Tell commonest type of anaemia in India.
17. Name the common causes of decrease or increase in Hb level.

APPARATUS

Haemoglobinometer, pricking needle and spirit swab.

Haemoglobinometer

It is a box which contains:

1. Comparator: It consisted of a box having one slot for Sahli's (haemoglobin) tube and two nonfading standard brown tinted glass pieces for matching the colour of diluted blood. A translucent white glass is fitted at the back to provide uniform illumination for matching the colour (Fig. 1.5.1).
2. Sahli's tube (Haemoglobin tube): It is a graduated tube having markings on two sides. On one side in gm% (gm per 100 ml) from 0 to 24 and other side 0–170%. Gram % means Hb in gm per 100 ml of blood. Simple percent means percentage of haemoglobin in relation to normal value of Hb which is considered as 100%.
3. Haemoglobin pipette: It is a simple pipette having capillary tube in it. This is connected to the teat (mouthpiece) through a rubber tube. There is a marking on the pipette indicating 20 cu mm (0.02 ml) volume.
4. Stirrer: Simple glass rod used to mix the blood with acid or distilled water.
5. Simple rubber dropper: To add acid or water drop by drop.

PRINCIPLE

Known quantity of blood (Hb) is mixed with N/10 HCl leading to formation of acid haematin which gives brown colour. Now mixture containing acid haematin is diluted with distilled water till its colour matches standard colour in the glass fitted in the comparator. Value of Hb is noted down from the Sahli's tube.

PROCEDURE

1. With the help of a dropper, take N/10 HCl in Sahli's tube up to mark 20%.
2. Under all aseptic conditions give a bold prick on the tip of ring finger with the help of pricking needle.
3. Wipe off the first drop of blood and suck the blood from the second drop in Hb pipette up to mark 20 cu mm.
4. Wipe the tip of the pipette with the help of cotton to remove sticked blood around the tip.
5. Blow out the blood from the pipette into acid in Sahli's tube and rinse the pipette with the same.
6. Mix the blood in acid with stirrer and wait for 10 minutes.
7. Dilute the mixture by adding distilled water drop by drop till the colour of mixture matches with standard colour in comparator.
8. Each time you have mix it with help of stirrer after adding the distilled water.
9. Once the colour is matched lift the stirrer up and note down the reading in Sahli's tube by taking the lower miniscus in consideration.

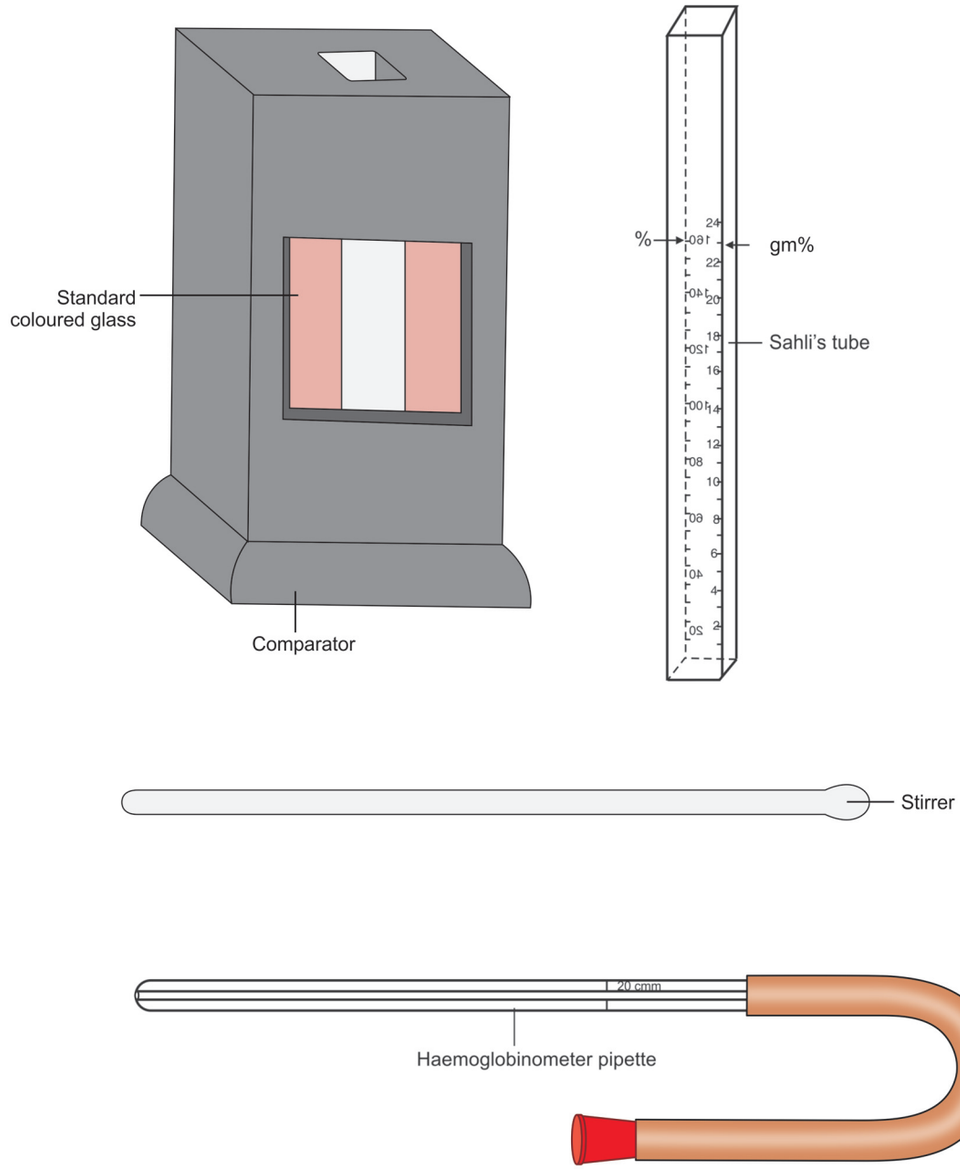


Fig. 1.5.1: Sahli's haemoglobinometer.

10. Usually in coloured solution upper meniscus is considered for taking the reading, but it is a transparent coloured solution so lower meniscus can be recorded.
11. Now add one more drop of distilled water and mix it properly. If colour is still matching note down the reading, if it is lighter, it shows reading taken before dilution was correct. If not, add a drop of distilled water again and match the colour. If it is lighter, it shows that reading taken just before last dilution is correct.
12. Reading is expressed in Hb gm/100 ml of blood.

Normal value of Haemoglobin

Male adult: 15.5 gm/100 ml (14–18 gm%)
Female adult: 14 gm/100 ml (12–15.5 gm%)
Newborn: 16.5 gm/100 ml

Result and Comments

PRECAUTIONS

1. Haemoglobin pipette and Sahli's tube should be clean and dry before use.
2. Suck the blood exactly up to the mark of 20 cu mm.
3. There should not be any air bubble in the pipette with blood.
4. Wait for 8–10 minutes after adding the blood in acid.
5. Add distilled water drop by drop to avoid over dilution.
6. The matching of colour should be done against natural source of light or electrical tube light (white light).

Other Methods Used for Hb Estimation

1. **Cyanmet haemoglobin method:** In this method blood is first treated with potassium cyanide and potassium ferricyanide, which forms methemoglobin. Light absorbed by this is compared with standard solution in photoelectric calorimeter.
2. **Alkali haematin method:** Alkali is used to form alkali haematin and colour is matched with standard.
3. **Iron estimation method:** It is based on the principle that 100 gm Hb contains 347 mg of iron. Value of iron is estimated in blood which gives value of Hb.
4. **Vanslyke's method:** The amount of oxygen combine with Hb is measured. One gm of Hb can carry 1.34 ml of oxygen. So quantity of oxygen is measured in 100 ml of blood.
5. **Tallquist scale method:** In this method a drop of blood is absorbed on an absorbent paper and colour of this paper is matched with standard.
6. **Copper sulphate method:** It is a rapid method for estimation of approximate level of Hb and it is used in large surveys.
7. **Electronic haematology analyzer:** It automatically provides cell count and haemoglobin level of blood. In this method for Hb a haemolyzing agent is added to release Hb which reacts with potassium ferrycyanide and potassium cyanide in solution to produce cyanmet Hb. The cyanmet Hb is then measured by spectrophotometry.

Anaemia— decrease in level of Hb in blood from normal value is called anaemia.

WHO Classification of Anaemia

1. Mild: Hb between 10–12 gm%
2. Moderate: Hb between 8–10 gm%
3. Severe: Hb below 8.0 gm%

Variation in Blood Hb Level:

A. Physiological
   i. Hb values higher in —
      Men as compared to women
      – Newborn and infants
      – At high altitude (because of hypoxia)
      – Regular physical exercise
   ii. Hb is less —
      – During pregnancy (because of haemodilution)

B. Pathological
   Increase in polycythemia
   Decrease in anaemia and fall in RBC count.

QUESTIONS AND ANSWERS

Q.1. What is the principle of estimation of Hb by Sahli's method?

Ans. *See* text

Q.2. What is N/10 HCl and how will you prepare it?

Ans. One normal solution of HCl is having 36.5 gm (H:1 + Cl:35.5) HCl in one liter of solution. N/10 HCl is prepared by dissolving 3.65 gm of HCl in one liter of distilled water.

Q.3. What is the normal value of Hb in adult?

Ans. *See* text

Q.4. Which meniscus of acid haematin is considered for taking the reading and why?

Ans. *See* text

Q.5. Why do we wait after adding the blood to HCl for 10 minutes?

Ans. This is the time required to convert the Hb of the blood in acid haematin.

Q.6. Why do we take HCl up to mark 20% in Sahli's tube?

Ans. This is the minimum quantity of HCl which is required to convert total Hb present in the tube. If the quantity of HCl is less, it will be insufficient to convert the whole of the Hb into acid haematin. If the quantity of HCl is more than 20%, it will not affect the result in healthy subjects but it will be the wastage of HCl. In case of patients of severe anaemia it may affect the result. For example, in a patient there is 4.0 gm of Hb and HCl taken in the tube is up to 50% mark, it will be already diluted compared to the standard colour in the haemoglobinometer.

Q.7. Why do we not keep the stirrer outside once we have started to mix the blood and distilled water?

Ans. It may lose the (drop of) mixture present with the stirrer.

Q.8. What are the experimental errors which effect Hb level?

Ans. Experimental error may be because of:

i. Quantity of the blood taken in the pipette may be less or more.
ii. Air bubble in the blood column in the pipette.
iii. Fading of the colour of standard glass rods of comparator.

Q.9. How will you calculate the oxygen carrying capacity of blood?

Ans. It can be calculated after estimating the haemoglobin of the subject. One gram of Hb combines with 3.36 ml of oxygen, with this we can calculate the total oxygen carried by the Hb present in 100 ml of blood.

Q.10. Enumerate some other methods of Hb estimation.

Ans. *See* text

Q.11. What is anaemia?

Ans. Anaemia is decreased Hb level in blood below 12 gm/100 ml of blood.

Q.12. What is the grading of anaemia?

Ans. *See* text

Q.13. What are the physiological and pathological causes of increase and decrease Hb in blood?

Ans. A. Physiological causes:

i. Sex: Higher in male.
ii. Age: Hb decreases with age. In newborn it is about 16.5 gm per 100 ml.
iii. Pregnancy: Hb decreases in pregnancy.
iv. High altitude: At high altitude there is increase in Hb.

B. Pathological causes:

i. Hb decreases in anaemia.
ii. It increases in polycythemia.

Q.14. What is glycosylated (glycated) haemoglobin (HbA1c)?

Ans. It is a form of haemoglobin that is covalently bound with glucose. It is also written as HbA1c. When there is constant high blood glucose level as in uncontrolled diabetes mellitus, glucose combine with Hb and there will be increased level of glycosylated haemoglobin.

Q.15. What is the normal level of glycosylated Hb?

Ans. HbA1c of 6.5 % in blood is recommended as the cut point for diagnosing diabetes mellitus. If it is more than 7%, it means diabetes mellitus is not properly controlled.

Q.16. What is the clinical significance of glycosylated Hb?

Ans. It is an important blood test to determine how well diabetes mellitus is managed/treated. An HbA1c of 6.5 % is recommended as the cut point for diagnosing uncontrolled diabetes mellitus.

Q.17. What is pernicious anaemia?

Ans. *See* text

Q.18. What is the megaloblastic anaemia?

Ans. *See* text

Student's Notes

Experiment 1.6
Preparation of blood smear (film) and identification of various cells

OBJECTIVES

At the end of the practical class student should be able to—

1. Make and stain a blood smear.
2. Identify RBCs, different types of WBCs and platelets.
3. Tell the features of a good smear.
4. Explain the correct method of making the blood smear with spreader.
5. To name the stain used for staining the smear.
6. Name the constituent of stain and tell the functions of each constituent.
7. Tell the identifying features of each cell of blood.
8. Explain what is buffered water.
9. Explain why acetone free methyl alcohol is used in stain.
10. Explain how methyl alcohol fixes the smear on the slide.
11. Explain what is vital and what is supravital stain.

APPARATUS

4–5 glass slides, compound microscope, pricking needle, spirit swab, cedarwood oil/liquid paraffin, Leishman's stain, wash bottle, buffered water and staining tray.

LEISHMAN'S STAIN

1.5 gm powder of Leishman's stain is dissolved in one litre of acetone free methyl alcohol. Leishman's stain contains two dyes, eosin and methylene blue. Eosin is an acidic dye that stains basic structures like RBC and granules of eosinophil. It is pink or red in colour. Methylene blue is a basic dye that stains acidic structures like nucleus or granules of basophils. It is blue in colour. Acetone free methyl alcohol is a fixative for smear.

i. Fixation of smear is because of precipitation of proteins by alcohol which prevent washing off the film.
ii. It preserves the cells in whatever chemical and metabolic state they are at the time of staining.
iii. Acetone, if present, will cause shrinkage or even lysis of the cells.

PRINCIPLE

Blood smear is prepared, stained with Leishman's stain and cells are identified under oil immersion lens.

PROCEDURE

(A) Preparation of Blood Smear

1. Selection of a spreader: Take one slide a spreader which has smooth edge. It should be done by careful look on the narrow edge of the slide or by moving a thumb smoothly on its edge. But, the slide should be washed with soap and water after touching its edge, to remove grease particles from its edges.
2. Take 3–4 clean and dry glass slides and keep them on filter paper or any clean white paper placed on the table.
3. Prick the ring finger of left hand with the help of pricking needle under all aseptic conditions.
   Put a small size of blood drop on each glass slide about half centimeter from its narrow edge on the right side. Place the blood drop on the same way on other slides also.
4. Now put the spreader on left side of blood drop at the angle 45° as shown in Fig. 1.6.1.
5. Give left to right movement to the spreader so that blood comes along the edge of the spreader. Further give side to side movement so that blood comes along the whole edge of spreader.
6. Now spread the blood by giving smooth, uniform and rapid movement to the spreader up to the left edge of the slide. Film should be made by push method, that means spreader should be pushed for spreading the blood on the slide but blood should be pulled along the opposite side of spreader so that it will be pulled along age of spreader and it will be uniformly distributed in the film. Repeat the same procedure with rest of the slides.
7. Dry the smear by waving the slides in the air for some time.
8. Now observe all smears whether they are satisfactory or not (Fig. 1.6.2). A good smear has following characteristics:
   i. It is tongue shaped, having head, body and tail. Head is the area where blood drop is placed. Body

is the area between head and tail. Tail is the last part of the smear.

ii. It should cover two-thirds of the slide.

iii. It should not be thick.

iv. There should not be marks or blank spaces in the smear.

9. After selecting a good smear with naked eyes focus it under low power of the microscope. The smear should be thin enough to have single layer of cells. There should not be rouleaux formation or clumping of cells.

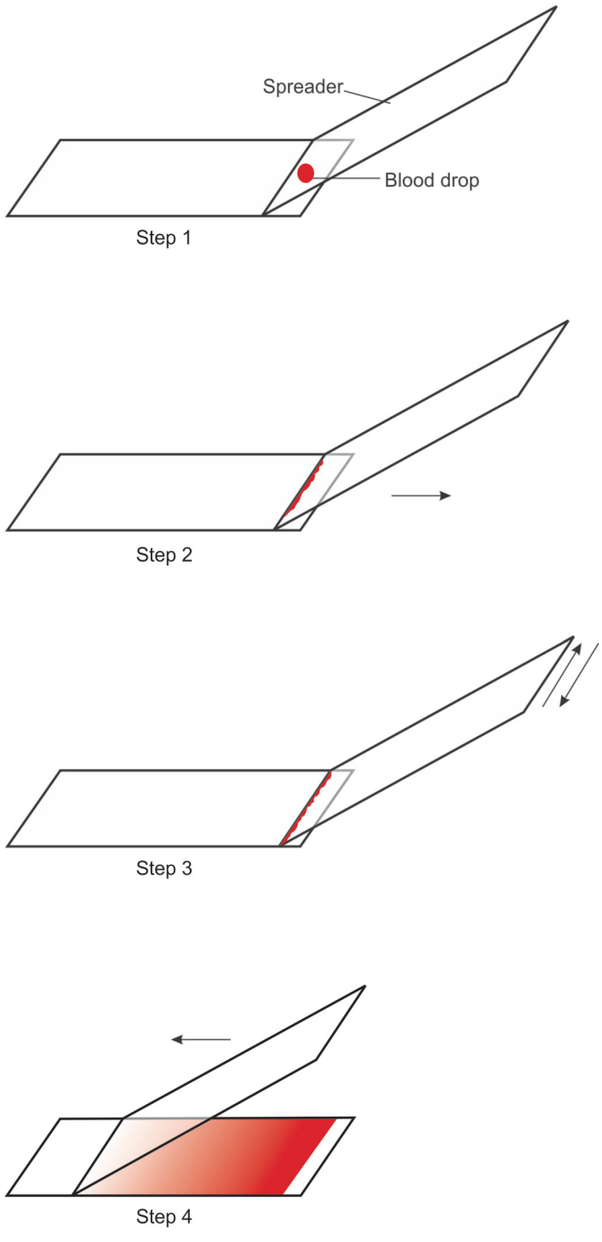


Fig. 1.6.1: To make blood smear.

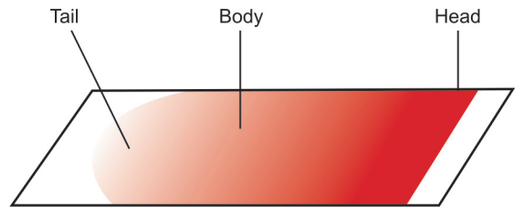


Fig. 1.6.2: A typical blood smear.

(B) Staining of Blood Smear

1. Place 3–4 good slides horizontally on the stand.
2. Add Leishman's stain drop by drop till it covers whole of the smear. Count the number of drops you have put.
3. Leave it for 1–2 minutes for fixation of the smear.
4. Add equal number of drops of buffered water (pH 6.8) on the slide. Mix the stain with water by blowing air with the help of a glass tube or with a dropper.
5. Wait for 8–10 minutes for staining to complete. During this period in the presence of buffered water staining is taking place because of formation of cations and anions of basic and acidic dyes respectively. Methyl alcohol is unable to ionise the stain so unable to stain the cells.
6. Wash the smear in slow running tap water or with the help of wash bottle till the smear becomes pink in colour. Clean the back of the slide to remove the stain from back side.
7. Let the slide dry and focus it under high power. If it is understained, stain it again and if it is overstained, wash it again.

(c) Examination of Smear under Oil Immersion Lens

Focus the slide under high power. Put a drop of cedarwood oil or liquid paraffin on the slide and shift the oil immersion lens by constantly looking from the side of the microscope so that it just dips in the oil.

Now focus with the help of fine adjustment screw and examine the various cells for their identification. (Identifying features of various cells are given in Table 1.6.1.) The various features of different cells are also shown in Figs 1.6.3–1.6.8.

Table 1.6.1: Identifying features of various cells in blood smear.

Cell	*Size (μm)*	*Nucleus*	*Granules*	*Cytoplasm*
RBC	7–8	Absent	Absent	Pink or red colour lighter in the centre as compared to periphery
Neutrophil	10–14	2–5 lobes, purple/blue colour	Fine, few in number, pink/purple	Pink/purple in colour because of fine granules
Eosinophil	10–14	Bilobed, purple/blue colour	Coarse, large in number, red or brown	Almost, not visible because large number of granules
Basophil	10–14	Bilobed, purple/blue colour, not properly visible because of granules	Large number, dark blue colour	Almost not visible because of granules
Small lymphocyte	7–10	Single mass, filling whole of the cell	Absent	Almost absent
Large lymphocyte	10–14	Single mass covering 2/3rd or cell, blue or purple, coarse and lumpy	Absent	Ring of light blue or sky blue colour
Monocyte	10–18	Blue or purple colour, fine chromatin, indented from one side	Usually absent	Light blue/sky blue, more on one side

Diagrams

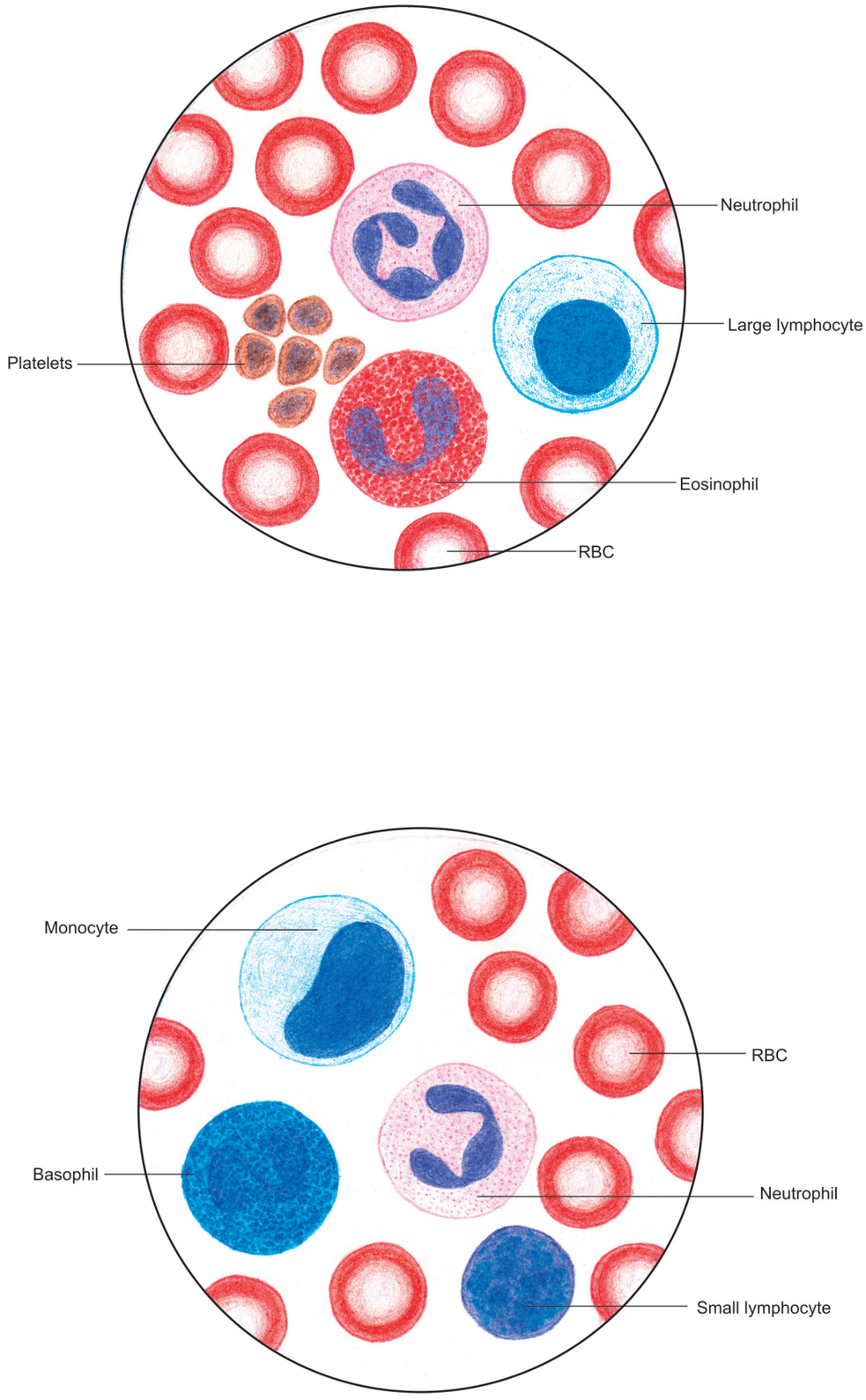


Fig. 1.6.3: Blood cells under oil immersion lens (cells stained with Leishman's stain), manually drawn.

Neutrophils

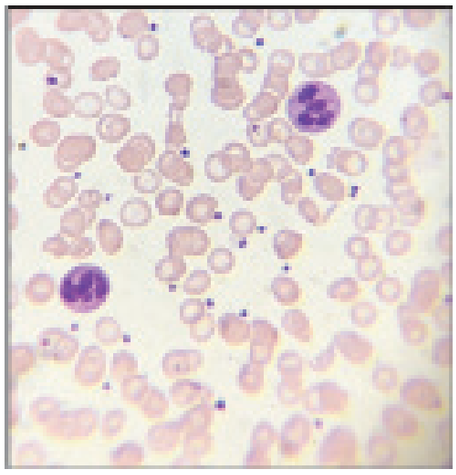
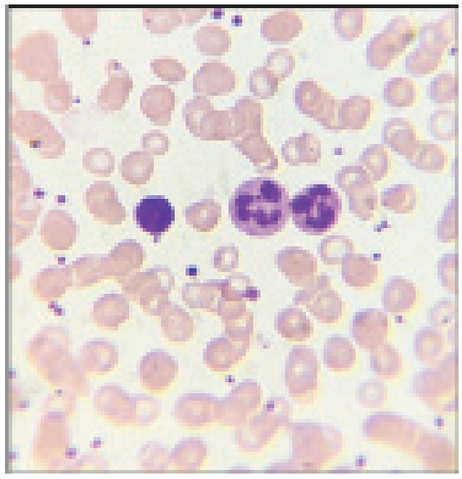


Plate 1



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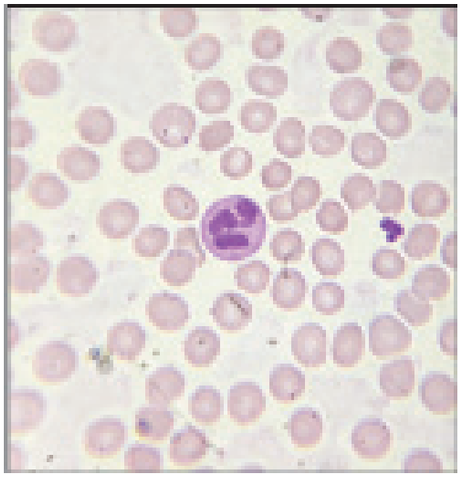

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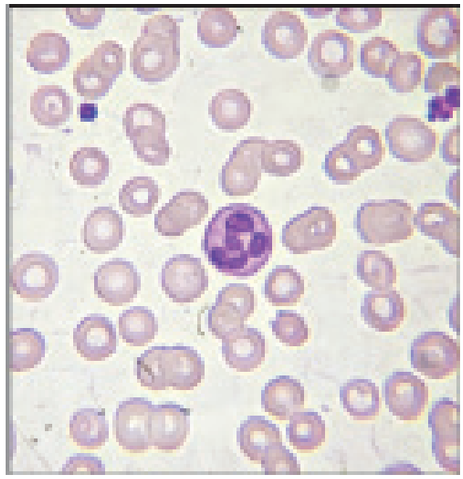


Plate 4

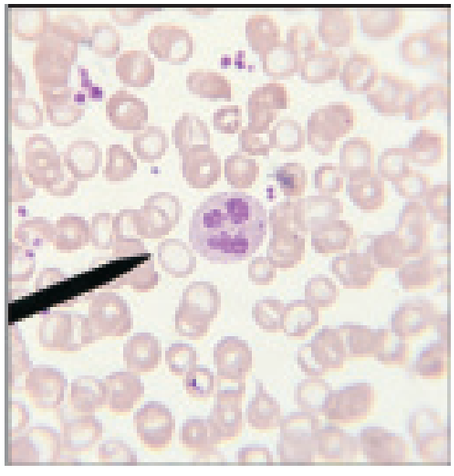


Plate 5

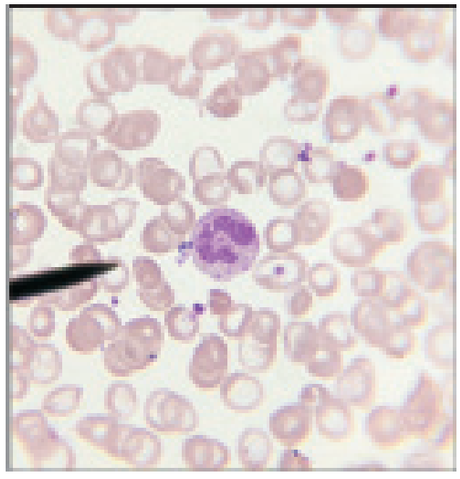


Plate 6

Fig. 1.6.4: Neutrophils with different lobes under oil immersion lens, stained with Leishman stain (Plate 2 shows small lymphocytes in addition to neutrophil and small dots are platelets).

Lymphocytes

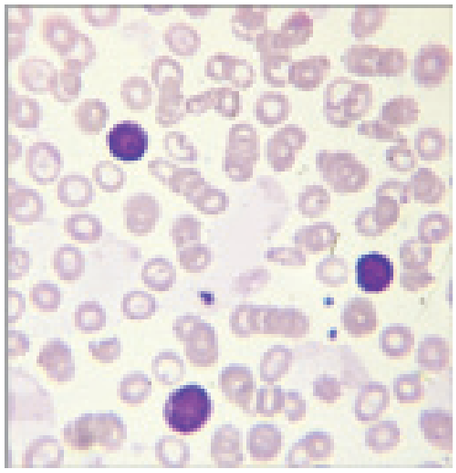
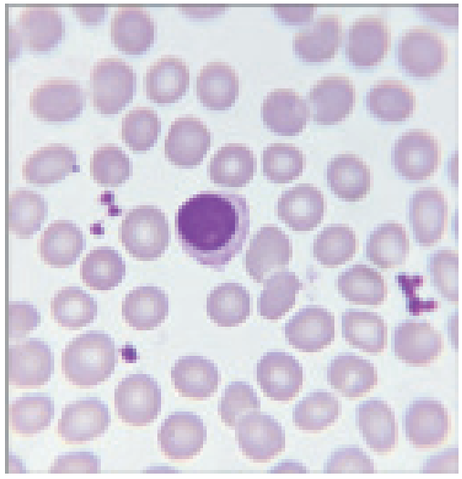


Plate 1



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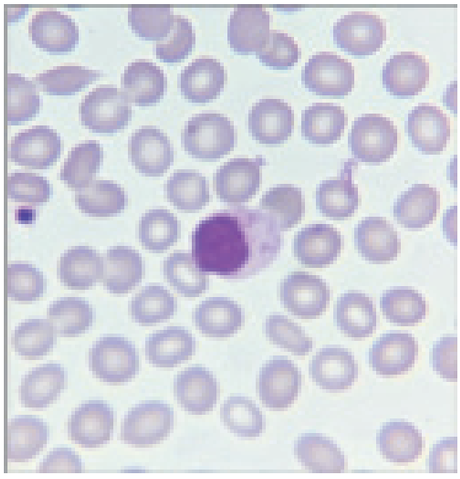

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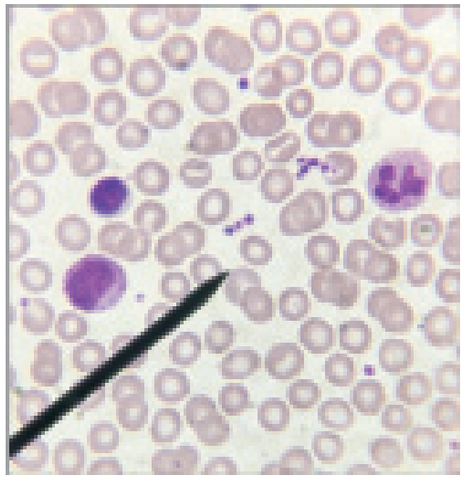

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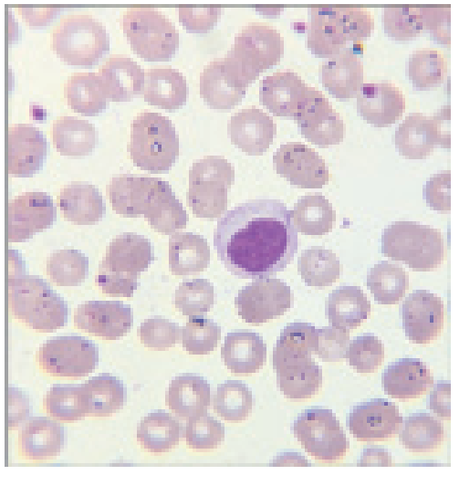

Plate 5

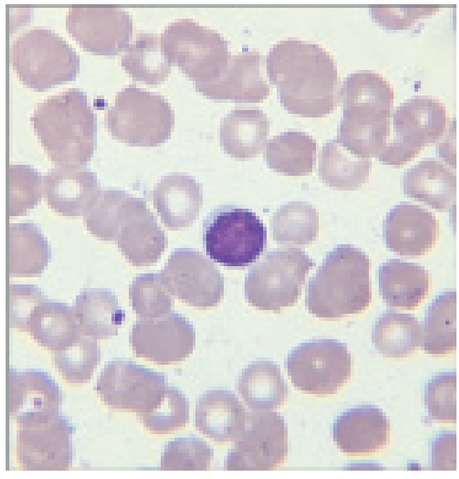

Plate 6

Fig. 1.6.5: Lymphocytes under oil immersion lens, stained with Leishman stain (Plate 4 shows neutrophil in addition to lymphocytes).

Eosinophils

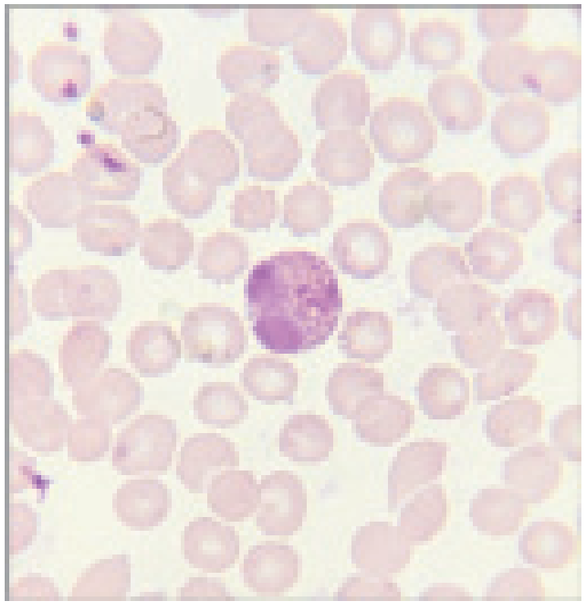

Plate 1

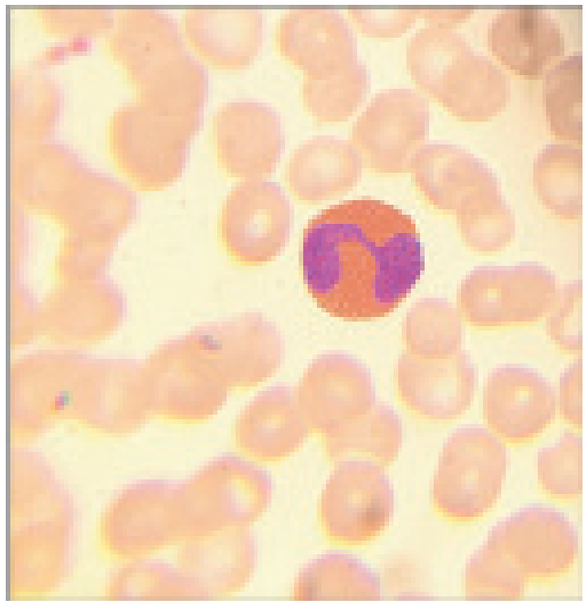

Plate 2

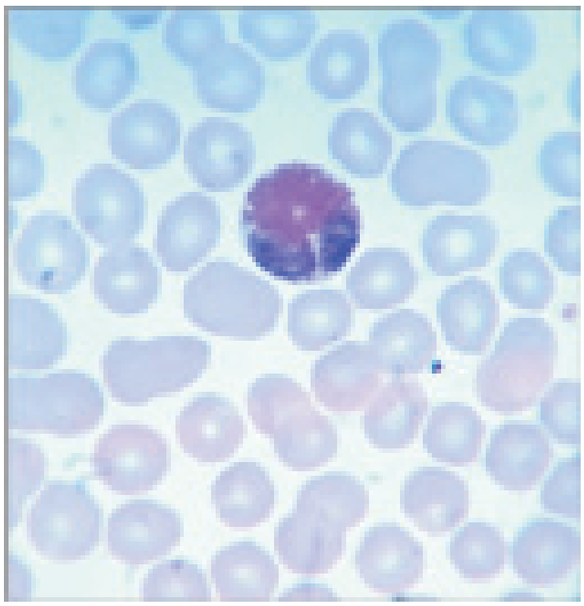

Plate 3

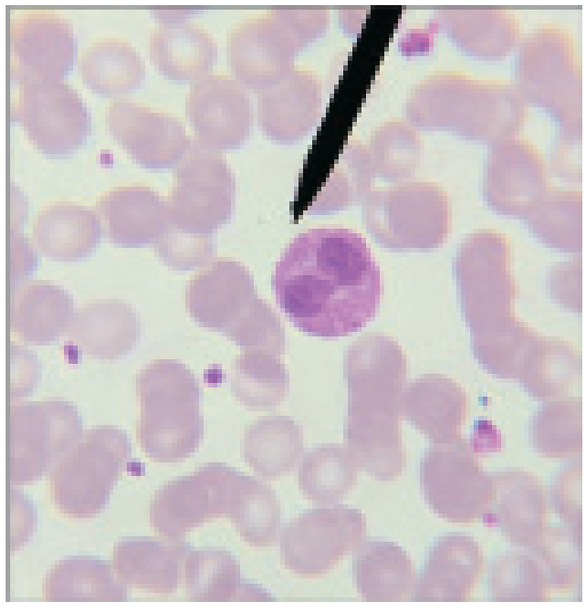

Plate 4

Fig. 1.6.6: Eosinophils under oil immersion lens, stained with Leishman stain.

Monocytes

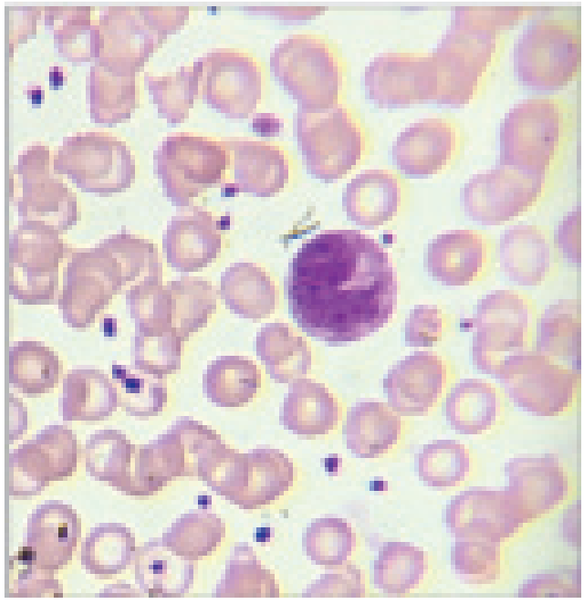

Plate 1

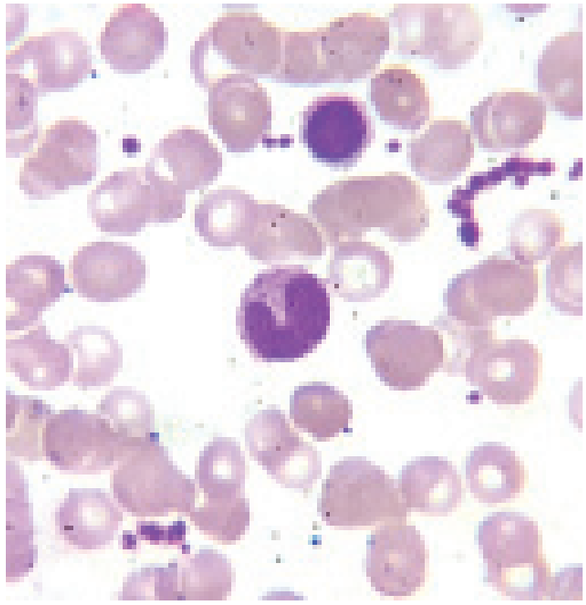

Plate 2

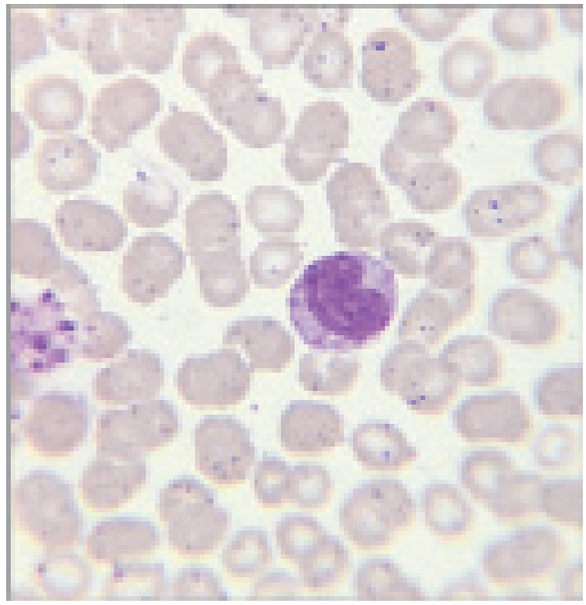

Plate 3

Fig. 1.6.7: Monocytes under oil immersion lens, stained with Leishman stain.

Basophils

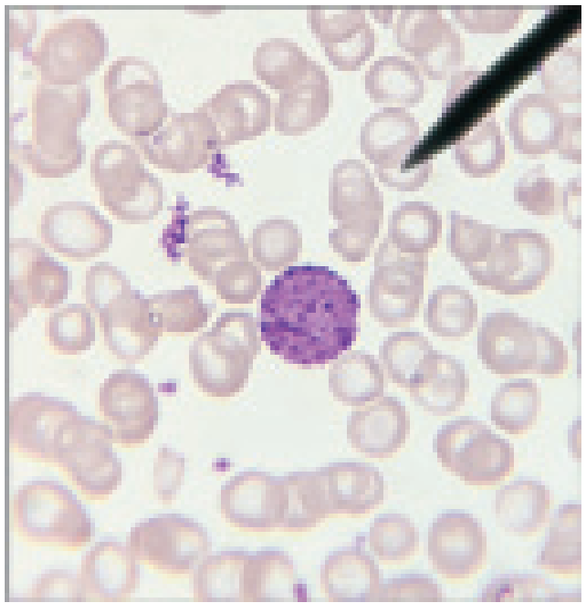

Plate 1

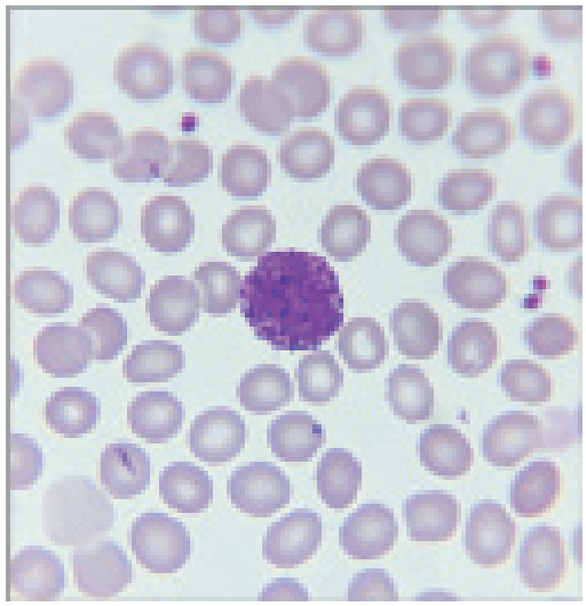

Plate 2

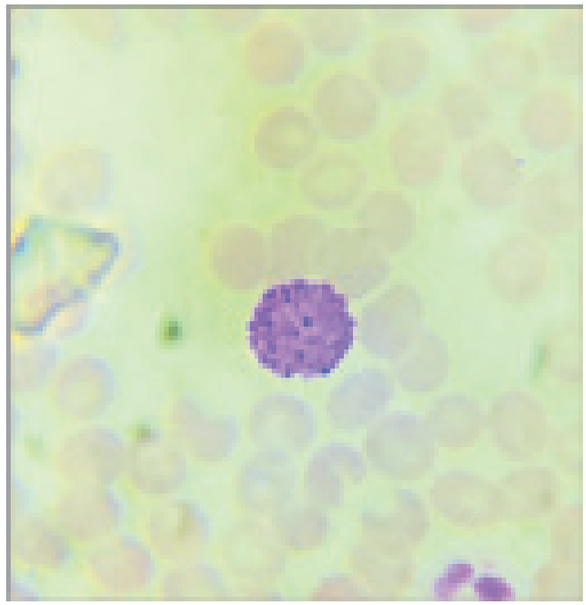

Plate 3

Fig. 1.6.8: Basophils under oil immersion lens, stained with Leishman stain.

Diagrams

PRECAUTIONS

1. Slide should be clean and grease free.
2. Edge of the spreader should be smooth.
3. Do not take much time in making the smear once the blood has been taken on the slide.
4. Stain should not dry on the blood smear.
5. Assess the quality of blood smear both grossly and microscopically before staining it.

QUESTIONS AND ANSWERS

Q.1. Enumerate the characteristics of a good blood smear.

Ans. *See* text

Q.2. What are the constituents of Leishman's stain? Enumerate their functions.

Ans. *See* text

Q.3. Why the cells do not get stained in the first two minutes after adding the stain on the smear?

Ans. Methyl alcohol is unable to ionise the stain so cells are not stain within 2 minutes of adding the stain to the smear. Buffer water ionise the stain and cations and anises allow the staining of cells.

Q.4. Why should the Leishman's stain be acetone free?

Ans. Acetone causes shrinkage of cells and it may cause lysis of cells.

Q.5. What is buffered water and why we use it?

Ans. It is the water whose pH is 6.8. It causes ionization of the stain particles present in the Leishman's stain. Optimal ionization of stain particles occurs at pH 6.8.

Q.6. How can we estimate size of a white blood cell under microscope?

Ans. It can be found by comparing these cells with RBCs. The size of RBC is about 7–8 μ.

Q.7. Why the left ring finger is chosen for giving prick?

Ans. *See* text

Student's Notes

Experiment 1.7
To do the differential leukocyte count (DLC) of your own blood

OBJECTIVES

At the end of the practical class student should be able to tell—

1. What is differential leukocyte count?
2. The method of counting of different cells.
3. Significance of DLC.
4. What is normal value of DLC?
5. Identifying features of various white blood cells.
6. Functions of various white blood cells.
7. Physiological and pathological increase or decrease in percentage of various white blood cells.

APPARATUS

4–5 glass slides, Leishman's stain, compound microscope, cedarwood oil, buffered water and staining tray.

PRINCIPLE

Total 100 leukocytes are studied, identified and recorded from a blood smears.

PROCEDURE

1. Prepare, stain and examine a blood smear under oil immersion lens.
2. Draw one hundred squares on a paper.
3. Identify various types of leukocyte and enter them by first letter (N - Neutrophil, E - Eosinophil, B - Basophil, L - Lymphocyte, M - Monocyte) in all 100 squares.
4. Count the cells in a specific sequence to avoid repeated counting.
5. Cells are counted in one direction and then field is shifted and counting is done in opposite direction. Field is again shifted in the same direction and counting is done in same direction as in the beginning. Direction of counting is shown in Fig. 1.7.1.
6. Percentage of various types of white cells is calculated by counting the different cells in various squares.

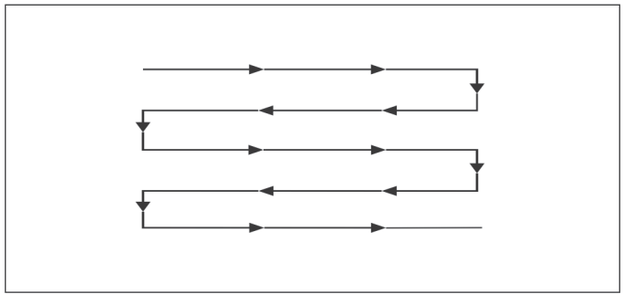

Fig. 1.7.1: Direction of shifting the field during counting of WBCs.

Normal DLC

Neutrophil	40–70%
Eosinophil	1–4%
Basophil	0–1%
Lymphocyte	20–40%
Monocyte	2–8%

PRECAUTIONS

1. Same as in preparation of blood smear and identification of various cells.
2. We should not count the same cells twice.

VARIATIONS

A. Neutrophils

a. *Neutrophilia*: Increase neutrophil count.
   1. Severe muscular exercise, pregnancy and food intake.
   2. Acute pyogenic infectious, e.g. abscess, boils, tonsillitis, etc.

b. *Neutropenia*: Decrease neutrophil count.
   1. Depression of bone marrow: Aplastic anaemia, bone marrow depressant drug, e.g. chloramphenicol.
   2. Typhoid, paratyphoid and malaria.

B. Lymphocytes

a. Lymphocytosis: Increase lymphocyte count.
   1. Newborn and infant lymphocytes are more.
   2. Chronic infection, e.g. tuberculosis

b. Decrease lymphocytes (lymphopenia) ACTH and steroid therapy.

C. Eosinophil

a. Eosinophilia (increase eosinophil count): Allergy condition like pulmonary eosinophilia, worm infestation, hay fever and urticaria.

b. Eosinopenia (decrease eosinophil count): Stress (acute bacterial infection) and glucocorticoid administration.

Observations

D. Basophils

Increase basophil count in chronic myeloid leukemia, smallpox and polycythemia. Decrease basophil count in acute Pyogenic injections.

E. Monocyte

Increase monocyte count in infection mononucleosis, kala-azar and malaria.

QUESTIONS AND ANSWERS

Q.1. What are the physiological and pathological causes of increased neutrophil count?

Ans. *See* text

Q.2. What are the pathological conditions causing neutropenia?

Ans. *See* text

Q.3. What are the causes of increased eosinophil count?

Ans. *See* text

Q.4. What are the physiological and pathological causes of lymphocytosis?

Ans. *See* text

Q.5. What are the pathological conditions leading increase in monocyte count?

Ans. *See* text

Q.6. There is a child of 18 months looking healthy, on examination of his blood film it was found that lymphocytes are about 50%. What is your probable diagnosis?

Ans. Child is normal because this is as a result of physiological variation.

Student's Notes

Experiment 1.8
To determine bleeding time (BT) and clotting time (CT) of your own blood

OBJECTIVES

At the end of the practical class student should be able to—

1. Define bleeding and clotting time.
2. Enumerate methods of estimation of bleeding and clotting time.
3. Perform these tests.
4. Tell the clinical significance of these tests.
5. Name the conditions in which bleeding and clotting times are prolonged.
6. Explain, in haemophilia bleeding time remains normal, still there is bleeding after injury.
7. Explain the effect of environment temperature on BT and CT.
8. Explain the inheritance of haemophilia.
9. Explain purpura and its types.

PRINCIPLE

A. *Bleeding time*: Time elapse between skin prick and arrest of bleeding.

B. *Clotting (coagulation) time*: Time elapse between skin prick and formation of fibrin thread.

A. Duke Method of Bleeding Time

Apparatus

Pricking needle, spirit swab and filter paper.

Procedure

- Get 2–3 mm deep prick after cleaning tip of ring finger of left hand.
- Note down the time (zero time).
- Remove the blood every 15 seconds by touching the finger gently on a filter paper till bleeding stops.
- Note the time when no trace of blood on the filter paper.
- Count the stops of blood on filter paper and express bleeding time in minutes and seconds. Normal bleeding time: 1–4 min.

Other Methods

1. ***Saline beaker method***
   - Prick is given in all sterile conditions and time is noted.
   - Finger is put into the beaker containing normal saline at 37° C temperature.
   - Time is noted when bleeding stops.

   Normal BT by this method: 2–6 min.
2. ***Ivy method***
   - Sphygmomanometer cuff is tide on the left arm and pressure is maintained to 40 mm Hg.
   - About 3 mm deep prick is given on the left fore arm after cleaning the area with spirit swab.
   - Blood is absorbed by filter paper till bleeding stops.

   Normal BT by this method: Up to 9 min.

B. Capillary Blood Clotting Time

Apparatus

Pricking needle, glass capillary tube, spirit swab.

Procedure

- Get a deep prick (3 mm) on the fingertip after cleaning it with spirit swab.
- Fill a capillary glass tube by dipping its one end in the blood drop on the finger.
- Note down the time as soon as blood starts to enter in the tube.
- Break off a small piece (1/2 to 1 cm) of capillary tube from one end of it.
- Capillary tube is held horizontally between palms.
- Break the tube at every 30 seconds time till fibrin thread is seen between the broken ends.
- Note down the time again.
  Normal clotting time: 2–5 min.

Precautions

1. There should be no air bubbles in blood column in capillary tube.
2. Hold the capillary tube horizontally between the palms when you are doing the test in winter.
3. Note down the time when blood starts to enter the capillary tube.

Other Methods

1. ***Drop method***
   - This method is less and accurate.
   - Blood drop is taken on a clean dry glass slide.

- An allpin is drawn at an interval of 30 seconds till fibrin threads are seen.
- Time is noted.

Normal time: 2–4 min.

2. ***Lee and White test tube method***
   - Blood is collected by venepuncture and transferred in three clean and dry test tubes.
   - Keep these tube in a beaker having normal saline at 37°C temperature.
   - Shifting of blood column is checked at the interval of one minute by tilting the test tube at an angle of 45°.
   - Once the blood column is not shifting, second tube is inverted.
   - Finally it is confirmed from the third tube.
   - Time from collection of blood and staying of clotted blood in the inverted tube is noted.

   Normal clotting time: 5–10 min.

Variations in bleeding and clotting time

1. Bleeding time is prolonged in purpura, not affected in haemophilia.
2. Clotting time is prolonged in haemophilia.
3. Fall in environmental temperature decreases bleeding time, but increases clotting time.
   Cold causes vasoconstriction which is responsible for decrease in bleeding time. It also causes slowing of enzyme reactions of coagulation and increases clotting time.

Observation and Results

QUESTIONS AND ANSWERS

Q.1. What do you understand by bleeding and clotting time?

Ans. *See* text

Q.2. What is their clinical significance?

Ans. *See* text

Q.3. What is the effect of environment temperature on BT and CT?

Ans. With rise in temperature (in summer) there is prolongation of bleeding time (vasodilatation) and shortening of the clotting time (enzyme reactions become faster). With decrease in environment temperature (in winter) there is decrease in bleeding time (vasoconstriction) and prolongation of clotting time (enzyme reactions slow down)

Q.4. Name the condition in which clotting time is prolonged and bleeding time remains normal.

Ans. *See* text

Ans. Haemophilia.

Q.5. What happens to BT and CT in purpura/ haemophilia?

Ans. In case of purpura bleeding time increases and clotting time remains normal. In case of haemophilia bleeding time remains normal and clotting time increases.

Student's Notes

Experiment 1.9
Determine your own blood group

OBJECTIVES

At the end of the practical class student should be able to—

1. Determine the blood group.
2. Tell the principle underlying the determination of blood group.
3. Tell the clinical significance of the investigation.
4. Tell the difference between rouleaux formation and agglutination.
5. Tell the status of antigen and antibodies in a person of particular blood group?
6. Tell who are universal donor and universal recipient?
7. Tell which type of blood can be given in acute emergency if blood of same blood group is not available?
8. Tell the significance of Rh blood group in a lady of child bearing age
9. Tell can Rh+ blood of same ABO system be transferred in Rh– patient.
10. Tell the complications of Rh+ blood group foetus in a mother having Rh– blood group?
11. Tell what will be the advice to a Rh– blood group lady who has given birth to Rh+ blood group healthy baby?
12. Tell what is the crossmatching of blood.
13. Tell which blood group is most common in Indian population.
14. Tell what is Landsteiner's law. What is the exception to the law?
15. Tell the complication of mismatched blood transfusion.
16. Tell the precautions in determination of blood group of an individual.
17. Tell what is the role of Rh antibodies (anti-D) in a Rh– lady given after the birth of Rh+ baby.
18. Tell why are the antibodies in plasma of donor ignored.
19. Explain why ABO incompatibilities rarely produce haemolytic disease of newborn?
20. Tell the medicolegal significance of blood grouping.

APPARATUS

Normal saline, antisera A, antisera B, antisera D, prickling needle, porcelain tile/glass slides, dropper, application sticks, glass marking pencil, coverslips, microscope, prickling needle and spirit swab.

PRINCIPLE

Saline suspension of red cells is mixed with antisera A, antisera B, antisera D and agglutination looked for, presence or absence of agglutination may be confirmed by microscope examination of the sample.

PROCEDURE

1. Take 2 ml of normal saline in a watch glass or 1 ml in a pit of porcelain tile.
2. Add a drop of blood after prickling the finger in all aseptic conditions in saline.
3. Mark A, B, D and S near different pits on the tile or take two slides and mark A and B on one slide and D and S on other with the help of glass marking pencil as shown in Fig. 1.9.1.
4. Now put a drop of antisera A, antisera B, and antisera D in the pits or on the slide according to the respective markings from antisera vials.
5. Add one drop of saline suspension of cells with the help of dropper to different antisera without touching any antisera with dropper.
6. Add two drops of this saline suspension of cells where you have marked S on tile or on glass slide. It will act as a control to compare with agglutinated cells.
7. Mix the cells with antisera by using separate application sticks or by rocking the tile or glass slide to and fro.
8. Wait for 10 minutes and examine the mixture for agglutination (clumping of RBCs) with naked eye, if required confirm under low power of microscope.
9. With naked eye agglutination of RBCs appears as a coarse separation of red cells in isolated clumps (red precipitates of cells).
10. By rocking the tile or slide agglutinated cells do not make uniform/homogenous mixture of cells.
11. Agglutination can be compared with saline mixture of cells which is taken as control.
12. Under low power of microscope RBCs are together in clumps.
13. Blood group is determined as indicated in the table given on the next page.

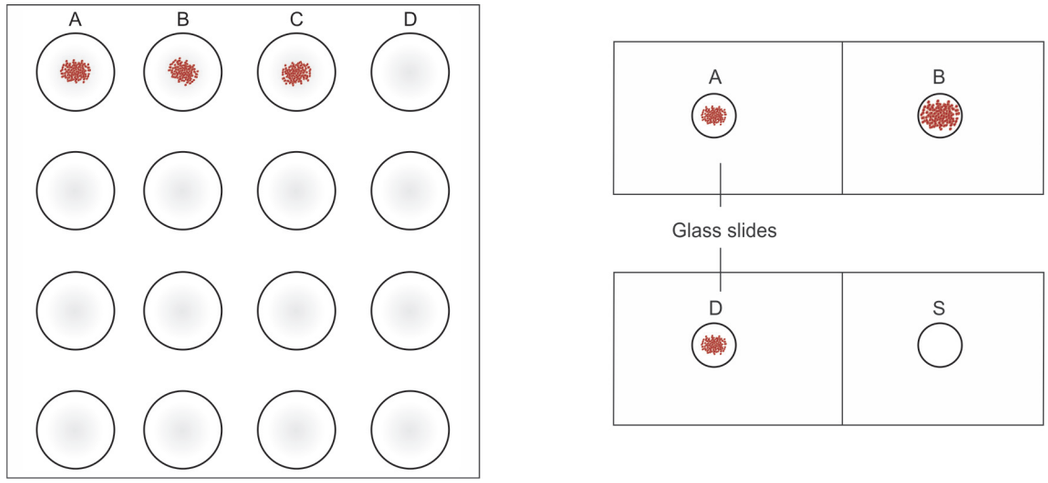

Fig. 1.9.1: Markings on porcelain tile and glass slides for determination of blood groups.

Antisera A	*Antisera B*	*Antisera D*	*Blood group*
+	–	+ / –	A + / A –
–	+	+ / –	B + / B –
+	+	+ / –	AB + / AB –
–	–	+ / –	O + / O –

+ Indicates agglutination; – Indicates no agglutination.

Result

Blood group should be expressed in ABO system and Rh system, e.g. A+ / A–.

Precautions

1. Tile or slide should be clean and dry.
2. There should be no intermixing of the mixture put on different places on tile or on slide.
3. Any doubt in agglutination must be confirmed under low power of microscope.

QUESTIONS AND ANSWERS

Q.1. What is the status of antigen and antibodies in a person of particular blood group?

Ans. *See* text in TBMP.

Q.2. What is the significance of determination of blood groups?

Ans. *See* text

Q.3. Who are universal donor and universal recipient?

Ans. *See* text in TBMP.

Q.4. Which type of blood can be given in acute emergency if blood of same blood group is not available?

Ans. *See* text in TBMP.

Q.5. What is the significance of Rh blood group in a lady of child bearing age?

Ans. In case of lady of child bearing age we have to be careful, if she has Rh –ve blood group and her husband has Rh +ve blood group. There are chances that baby may have Rh +ve blood group. These Rh +ve RBCs of foetus can enter in mother circulation, commonly in late months of pregnancy. It can lead to Ab formation against (antigen D) Rh +ve blood RBCs in mother's circulation. These antibody can cross the placental barrier and harm to foetus having Rh +ve blood. If in second pregnancy she also has foetus of Rh +ve blood, it leads to exaggerated response and complications can be of serious nature, like icterus gravis neonatorum, Hydrops fetalis, stillborn baby or baby may die just after birth.

Q.6. Can Rh+ blood of same ABO system be transferred in Rh– patient?

Ans. *See* text in TBMP.

Q.7. What are the complications of Rh+ blood group foetus in a mother having Rh– blood group?

Ans. *See* text in TBMP.

Q.8. What will be your advice to a Rh– blood group lady who has given birth to Rh+ blood group healthy baby?

Ans. Various advices given to such lady are as follows:

i. She should not have another pregnancy.
ii. She should be given a bolus dose of anti-D to destroy Rh+ cells just after delivery.
iii. She should come regularly for obstetrical check up if she still wants next pregnancy.

Q.9. What is the crossmatching of blood?

Ans. *See* text in TBMP.

Q.10. Which blood group is most common in Indian population?

Ans. 'B' blood group is most common in Indian population.

Q.11. What is Landsteiner's law? What is the exception to the law?

Ans. *See* text in T.B.M.P.

Q.12. What are the complications of mismatched blood transfusion?

Ans. These are of different types:

i. Inapparent haemolysis.
ii. Post-transfusion jaundice.
iii. Severe reaction leading to haematuria and renal failure.

Q.13. What are the other complications of blood transfusion?

Ans. Other complications of blood transfusion are:

i. Pyrexia (post-transfusion fever).
ii. Transfer of infections, e.g. AIDS, malaria, hepatitis.
iii. Alkalosis (when large quantity of blood is transfused).
iv. Hyperkalemia.
v. Overloading of circulation.

Q.14. What is the role of high dose of Rh antibodies (anti-D) in a Rh– lady given after the birth of Rh+ baby?

Ans. They destroy Rh+ cells entered in mother's circulation from the placenta at the time of delivery so the further sensitization and production of antibody will stop. Some anti 'D' antibodies left after destroying antigin 'D' in circulation will be metabolized slowly.

Q.15. Why we should wait for 10 minutes before looking for agglutination?

Ans. This time is given to complete the agglutination reaction.

Q.16. Why are the antibodies in plasma of donor ignored?

Ans. Because antibodies of the donor get diluted in the recipient's blood but in case of repeated blood transfusion they may create problem.

Q.17. Explain why ABO incompatibilities rarely produce haemolytic disease of newborn.

Ans. ABO incompatibilities are rare because a and b antibodies are of IgM type and cannot cross the placenta.

Q.18. What is the medicolegal significance of blood grouping?

Ans. With the help of blood group of child and a father, we can say that he may not be the father of the child.

Q.19. Can O –ve blood be transfused repeatedly in an individual has A or B or AB blood group?

Ans. No because O –ve blood has anti-A or anti-B or both, with repeated transfusion titre of these antibodies may rise to harmful level and can cause agglutination in recipient leading to the serious complication.

Q.20. What are the differences between agglutination and coagulation?

Ans. *See* text in TBMP.

Note: TBMP means *Textbook of Medical Physiology.*

Student's Notes

Student's Notes

Experiment 1.10 (Demonstration)
Determination of erythrocyte sedimentation rate (ESR)

OBJECTIVES

At the end of the practical class student should be able to—

1. Determine ESR.
2. Tell normal value of ESR.
3. Tell the significance of the investigation.
4. Tell why cells settle down in anticoagulant mixed blood.
5. Tell what is rouleaux formation.
6. Tell why ESR is less in infancy.
7. Tell why ESR is higher in pregnancy.
8. Tell why blood sample is taken in fasting for ESR.
9. Tell why ESR is higher in woman.
10. Tell why double oxalate is used in Wintrobe's method.
11. Tell the various factors and conditions in which ESR is decreased or increased.
12. Draw well-labeled diagram of Westergran's and Wintrobe's tube.

APPARATUS

Westergren's tube with stand, Wintrobe's tube with stand, long nozzled capillary pipette (Pasture pipette), 3.8% sodium citrate solution, mixture of double oxalate, 2 ml disposable syringe with needle and spirit swab.

Westergren's Tube with Stand

i. It is a thick walled glass tube of length 30 cm, open at both the ends with internal bore 2.5 mm.
ii. Tube is graduated along the lower 2/3 portion, 0–200 mm from up to down.
iii. Westergren's stand: Tall stand for holding the Westergren's tube vertically with rubber pad at the bottom and screw at the upper end.

Wintrobe's Tube with Stand

i. It is a thick walled glass tube of length 11 cm, closed at one end with internal bore of 2.5 mm.
ii. Lower 10 cm of the tube is graduated from 0–100 mm from top to bottom and bottom to upper end.
iii. Markings from 0–100 mm from up to down are used for reading ESR and markings from bottom to upward are used for reading PCV (haematocrit).
iv. Wintrobe's stand: It is a small stand as compared to Westergren's stand. It is used to keep the Wintrobe's tube vertically.

ERYTHROCYTE SEDIMENTATION RATE (ESR)

It is the rate at which RBCs settle down when anticoagulated blood is allowed to stand in a narrow tube for one hour. It is measured in terms of clear plasma above the settling RBCs.

There are two methods for estimation of ESR.

I. Westergren's Method

Westergren's tube is used to measure ESR in this method.

Procedure

1. Take 0.5 ml of 3.8% sodium citrate solution in a clean and dry glass vial.
2. Withdraw 2 ml of blood from anticubital vein in all aseptic conditions. Remove the needle of the syringe and transfer the blood in vial containing sodium citrate solution. Immediately mix the content.
3. Suck citrated blood in Westergren's tube up to mark '0' and immediately close its upper opening with the help of index finger or thumb.
4. Press the tube after placing its lower end on rubber pad in the stand and fix the tube vertically in the stand with the help of screw.
5. Note the time and take the reading for ESR at the end of one hour of clean plasma column.

Normal Value

i. Man: 0–4 mm at the end of one hour
ii. Woman: 0–8 mm at the end of one hour

II. Wintrobe's Method

Wintrobe's tube is used to determine ESR by this method.

Procedure

1. Take powder mixture of double oxalate (ammonium and potassium in ratio of 3:2) in a clean and dry glass vial.
2. Draw 2 ml of blood from anticubital vein in all aseptic conditions and remove the needle of the syringe and eject the blood in glass vial.
3. Mix the blood with anticoagulant gently.
4. Fill this blood in pasture pipette and introduce the capillary tube of pipette up to the bottom of Wintrobe's tube.

Diagrams

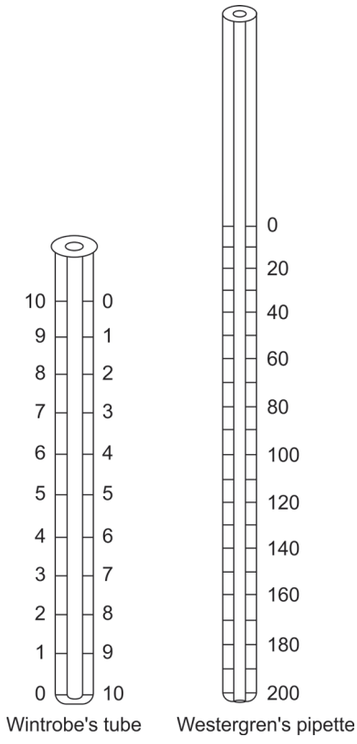

Fig. 1.10.1

5. Now apply the pressure on rubber teat and slowly take the pipette nozzle out so that tube will be filled up with the blood up to mark zero.
6. Note down the time and put the tube in the stand for one hour. Take the reading at the end of one hour of clean plasma column.

Normal Value

i. Man: 0–4 mm at the end of one hour
ii. Woman: 0–6 mm at the end of one hour

ESR value more than 20 mm at the end of one hour is pathological.

Precautions

1. ESR tube should be clean and dry.
2. Blood sample must be collected in fasting state.
3. There should be no air bubble in ESR tube.
4. Single oxalate should not be used as anticoagulant in Wintrobe's method.
5. Do not disturb the tube once kept in the stand for ESR.

Variations in ESR

Increase in ESR is seen in physiological and pathological conditions.

a. ***Physiological:***
   1. Pregnancy
   2. Menstruation
   3. Increased temperature
   4. Higher in female
b. ***Pathological: Any destructive process***
   1. Acute infection—pneumonia process
   2. Chronic infections—tuberculosis, rheumatic fever
   3. Anaemia
   4. Malignancy
   5. Severe trauma.

Decrease in ESR is seen in:
1. Infancy
2. Polycythemia
3. Afibrinogenemia
4. Spherocytosis

Clinical Significance

a. **Diagnostic significance:** It is non-specific investigation. ESR increases in the various pathological conditions when there is destruction of cells in the body.
b. **Prognostic significance:** It is a very important prognostic investigation. Repeated estimation is done during treatment period. Progressive decrease in ESR shows patient is improving and treatment is effective.

QUESTIONS AND ANSWERS

Q.1. What is the normal value of ESR in adult male and female?

Ans. *See* text

Q.2. Why cells settle down in anticoagulant mixed blood?

Ans. Because specific gravity of cells is more as compared to plasma and rouleaux formation further enhances this process.

Q.3. What is rouleaux formation?

Ans. *See* text in *Textbook of Medical Physiology.*

Q.4. Why ESR is less in infancy?

Ans. Because of high RBC count in infancy ESR is less.

Q.5. Why ESR is higher in pregnancy?

Ans. Because of haemodilution ESR is higher in pregnancy and in later months of pregnancy there is increase in fibrinogen level in the blood which also increases ESR.

Q.6. Why blood sample is taken in fasting for ESR?

Ans. Because water and food intake affect erythrocyte sedimentation rate.

Q.7. Why ESR is higher in woman?

Ans. Because RBC count is less in woman.

Q.8. Why double oxalate is used in Wintrobe's method?

Ans. Because double oxalate is not affecting the size of the blood cells as this method is used to measure PCV.

Q.9. Name the pathological conditions in which ESR is raised.

Ans. *See* text

Q.10. Name the pathological conditions in which ESR is decreased.

Ans. *See* text

Q.11. Name the physiological conditions when ESR is decreased.

Ans. *See* text

Q.12. What are the various factors which effect ESR?

Ans. *See* text

Note: Students are supposed to draw well-labelled diagram of Westergren's and Wintrobe's tubes.

Student's Notes

Student's Notes

Experiment 1.11
Determination of packed cell volume (PCV) and calculation of blood indices

OBJECTIVES

At the end of the practical class student should be able to—

1. Estimate PCV.
2. Tell significance.
3. Enumerate factors affecting PCV.
4. Tell why double oxalate is used as an anticoagulant in this test.
5. Tell what is the buffy coat. What is its normal thickness.
6. Tell when does buffy coat size increase.
7. Tell is there any difference between PCV of arterial and venous blood.
8. Define MCV, MCH, MCHC and colour index.
9. Tell the clinical significance of MCV.
10. Tell the clinical significance of MCH and MCHC.
11. To name the cause of microcytic hypochromic anaemia.
12. Tell in which condition there is macrocytic anaemia seen.
13. Tell the cause of normocytic normochromic anaemia.
14. Tell is there any possibility of hyperchromic anaemia.

APPARATUS

Wintrobe's tube with stand, 2 ml disposable syringe with needle, pasture pipette, mixture of double oxalate, centrifuge machine and spirit swab.

PRINCIPLE

Anticoagulant mixed blood is filled in a tube and centrifuged. Cells settle down towards the bottom because of their greater density leaving the clear plasma on upper side. Percentage of volume of cells in total blood is known as packed cell volume (PCV).

PROCEDURE

1. Take powdered mixture of double oxalate (ammonium oxalate 3 mg and potassium oxalate 2 mg) in a clean and dry glass vial.
2. Draw 2 ml of blood from the vein in all aseptic condition and eject the blood in the vial after removing the needle from the syringe. Mix the blood with anticoagulant gently.
3. Fill the Wintrobe tube with the help of pasture pipette up to the mark 100 mm.
4. Centrifuge the blood in centrifuge machine at 3000 revolutions per min for 30 minutes.
5. Three layers are formed:
   i. Clear plasma in upper part of tube.
   ii. Buffy coat (layer) (in between two layers)
   iii. Red cells (red column) in lower part of tube.
6. Packed cell volume is read directly from the calibration of tube from bottom to upper end.
7. Buffy layer: Grayish white layer about 1 mm size on the upper end of red layer. It consists of WBC and platelets.

Normal Value

Adult man: 45% (40–50%)
Adult woman: 42% (37–47%)

Precautions

1. There should be no air bubble or froth of blood in the tube.
2. Always use double oxalate as anticoagulant.
3. Wintrobe's tube should be clean and dry.

Variation in PCV

a. Increase in PCV

Physiological
1. Newborn and infant
2. High attitude
3. Higher in man as compared to woman

Pathological
1. Hypoxia
2. Polycythemia
3. Spherocytosis
4. Dehydration

b. Decrease in PCV

Physiological
1. Less in woman than man
2. Pregnancy

Pathological
1. Anaemia
2. Bone marrow depression

CALCULATION OF BLOOD INDICES

1. Mean Corpuscular Volume (MCV)

It is the average volume of a single red blood cell.

$$\text{MCV} = \frac{\text{MCV/100 ml of blood}}{\text{RBC count in million/cu mm}} \times 10$$

Normal: 78–94 cu μ

MCV Variation

Increased in:
1. Megaloblastic anaemia
2. Spherocytosis

Decreased in: Iron deficiency anaemia.

Clinical Significance

Useful in laboratory classification of anaemia.
i. Microcytic anaemia MCV < 78 cu μ
ii. Normocytic anaemia MCV = 78–94 cu μ
iii. Macrocytic anaemia MCV > 94 cu μ

2. Mean Corpuscular Haemoglobin (MCH)

It is an average quantity of Hb of a single RBC.

$$\text{MCH} = \frac{\text{Hb gm/100 ml}}{\text{RBC count in million/cu mm}} \times 10$$

Normal: 27–32 pg (picogram)/mmg.
MCH decreases in iron deficiency anaemia.

Clinical Significance

Useful in laboratory classification of anaemia.
i. Normochromic anaemia: MCH = 27–32 pg.
ii. Hypochromic anaemia: MCH < 27 pg

3. Mean Corpuscular Haemoglobin Concentration (MCHC)

It is a percentage of Hb in relation to packed cell volume.

$$\text{MCHC} = \frac{\text{Hb gm/100 ml}}{\text{PCV/100 ml}} \times 100$$

Normal: 32–38%

Clinical Significance

Normochromic anaemia: MCHC = 32–38%
Hypochromic anaemia: MCHC < 32%

4. Colour Index (CI)

It is the ratio of Hb% with RBC%.

$$\text{CI} = \frac{\text{Hb\%}}{\text{RBC count \%}}$$

Normal: 0.85–1.15

It is clinically insignificant index, because of wide variation in 100% Hb and 100% RBC count.

Observation and Calculations

Observation and Calculations (*Contd.*)

QUESTIONS AND ANSWERS

Q.1. Why double oxalate is used as an anticoagulant in this test?

Ans. Ammonium oxalate responsible for increase in size of the cells and potassium oxalate decreases the size of the cells, this is why only mixture of these two oxalates (double oxalate) is used as an anticoagulant in this test.

Q.2. What is the buffy coat? What is its normal thickness?

Ans. There is a white layer on the column of the blood cells in the Wintrobe's tube which is called buffy coat. This contains WBCs and platelets. It is about one mm in thickness.

Q.3. When does buffy coat size increase?

Ans. Its thickness increases in leucocytosis, leukaemia and marked thrombocytosis.

Q.4. Is there any difference between PCV of arterial and venous blood?

Ans. Because of the phenomenon of chloride shift venous blood cells are slightly larger in size as compared to arterial blood. This is why the PCV of venous blood is about 3% higher than that of arterial blood.

Q.5. What is the significance of PCV?

Ans. *See* text

Q.6. Enumerate physiological and pathological conditions which cause decrease or increase in PCV.

Ans. *See* text

Q.7. What do you mean by MCV, MCH, MCHC and colour index?

Ans. *See* text

Q.8. What is the clinical significance of MCV?

Ans. *See* text

Q.9. What is the clinical significance of MCH and MCHC?

Ans. Both are used to classify the anaemia. If the MCH and MCHC are decreased it is called hypochromic anaemia. The MCHC is a better indicator as compared to MCH because there are less chances of error in MCHC.

Q.10. Name the common cause of microcytic hypochromic anaemia.

Ans. Most common cause of microcytic hypochromic anaemia is iron deficiency.

Q.11. In which condition there is macrocytic anaemia seen?

Ans. Deficiency of cyanocobalamin (vitamin B12) or folic acid (vitamin B6).

Q.12. What is the cause of normocytic normochromic anaemia?

Ans. Haemorrhage induced anaemia.

Q.13. Is there possibility of hyperchromic anaemia?

Ans. No, because RBC cannot accommodate more Hb than its capacity.

Q.14. What is the laboratory classification of anaemia?

Ans. This is the classification of anaemia on the basis of size and Hb concentration in the RBCs.

i. Normocytic normochromic anaemia:
MCV 78–94 cu μ, MCH 27–32 pg

ii. Microcytic hypochromic anaemia:
MCV <78 cu μ, MCH <27 pg

iii. Macrocytic normochromic anaemia:
MCV >94 cu μ, MCH 27–32 pg

iv. Macrocytic hypochromic:
MCV >94 cu μ, MCH <27 pg

Student's Notes

Experiment 1.12a (Demonstration)
Determination of specific gravity of a given sample of blood by copper sulphate method

At the end of the practical class student should be able to—

1. Tell what is the range of specific gravity of blood.
2. Tell what are the physiological and pathological factors affecting SG of blood.
3. Tell what determines the SG of blood.
4. Tell what change will be there in SG of blood in anaemia.
5. Explain why the blood remains in the shape of drop.

APPARATUS

Stock solution of copper sulphate ($CuSO_4$), distilled water, wide mouth glass bottle, blood sample and dropper.

PRINCIPLE

The specific gravity of blood is compared with copper sulphate solution of known specific gravity.

PROCEDURE

1. Prepare one liter of stock solution by adding 159 gm of $CuSO_4.5H_2O$ in distilled water. Specific gravity of this solution will be 1.100.
2. Take 9 wide mouth bottles or beakers of capacity about 110–150 ml. Now label the bottles 1 to 9. Now add stock solution in each bottle of volume 49, 51, 53, 55, 57, 59, 61, 63, 65 ml in bottle No. 1 to 9 respectively. Now add distilled water in each bottle of volume 51, 49, 47, 45, 43, 41, 39, 37, 35 from bottle number 1 to 9. Specific gravity in each bottle from 1 to 9 will be from 1.050 to 1.066 (Table 1.12a.1).
3. Add a drop of blood in each bottle by keeping the dropper 1 cm above the surface of the solution. Blood remains in the form of drop because a layer of copper proteinate is formed around blood.
4. Observe the behaviour of the drop in solution within 15 seconds. There are three possibilities (Fig. 1.12a.1).
   i. Blood drop shrinks in the solution and goes towards the bottom. Because specific gravity of solution is less.
   ii. Blood drop goes in and comes out towards the surface of solution. It shows specific gravity of solution is higher than blood.
   iii. It goes in the solution and remains suspended in the solution. It is because specific gravity of solution is equal to that of blood. Note down the specific gravity of this solution. It will be the specific gravity of blood.

After 20 seconds all the drops in different bottles go towards the bottom in the solution because of formation of more copper proteinate and the drops become heavier.

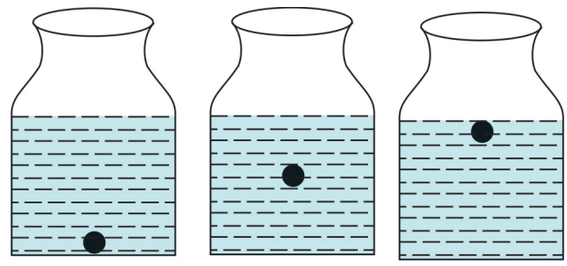

Fig. 1.12a.1: Position of blood drop in copper sulfate solution of different specific gravity.

Table 1.12a.1: Procedure to prepare $CuSO_4$ solution of different specific gravity

Bottle No.	1	2	3	4	5	6	7	8	9
$CuSO_4$ solution (ml)	49	51	53	55	57	59	61	63	65
DW (ml)	51	49	47	45	43	41	39	37	35
SG solution	1.050	1.052	1.054	1.056	1.058	1.060	1.062	1.064	1.066

Normal Value

Specific gravity of blood: 1.048–1.066
Specific gravity of plasma: 1.026–1.035
Specific gravity of RBCs: 1.092–1.095

Result

Precautions

1. Same size of blood drop should be added in each bottle. It can be done by using the same dropper.
2. Blood drop should be added from same height in each bottle.
3. Regarding position of blood drops observation should be made within 15 seconds.

QUESTIONS AND ANSWERS

Q.1. What is the range of specific gravity of blood?

Ans. *See* text

Q.2. What are the physiological and pathological factors affecting SG of blood?

Ans. Factors affecting the specific gravity are –

A. Physiological factors:
   i. Sex: Less in female.
   ii. Pregnancy: Less during pregnancy.
   iii. Increase with high water intake.

B. Pathological factors: Specific gravity increases when there is a loss of water from the body, e.g. vomiting and diarrhea.

Q3. What determines the SG of blood?

Ans. RBC count, Hb concentration, plasma protein and water content are measure determinants of specific gravity of blood.

Q.4. What change will be there in SG of blood in anaemia?

Ans. It will decrease.

Student's Notes

Experiment 1.12b
To study the effect of hypotonic, hypertonic, isotonic saline, HCl and tanic acid

OBJECTIVES

At the end of the practical class student should be able to—

1. Tell what are isotonic, hypotonic and hypertonic fluids.
2. Tell what is the effect of HCl and alkali on RBCs.

APPARATUS

Compound microscope, watch glass, few glass slides, 0.4% saline, 0.9% saline, 2% saline, N/10 HCl, tannic acid, pricking needle and spirit swab.

PROCEDURE

1. Take 2 ml of 0.9% saline in a water glass.
2. Add a drop of blood after pricking the finger in all aseptic conditions.
3. Take a drop of suspended cells on a clean dry glass slide and cover it with coverslip.
4. Examine the cells first under low power and then in high power.
5. Take a drop of hypotonic saline (0.4%) on a glass slide and add a drop of cell suspension from watch glass. Wait for 5 minutes and examined under microscope after placing coverslip on it.
6. Similarly study the effect of hypertonic saline, 0.1 N hydrochloric acid, tannic acid and alkali.

OBSERVATIONS

1. Effect of normal saline: Shape of RBCs is not disturbed (Fig. 1.12b.1).
2. Effect of hypotonic saline: Size of RBCs increases and burst RBCs can be seen (Fig. 1.12b.1).
3. Effect of hypertonic saline: Size of RBCs decreases and margin becomes crenated (Fig. 1.12b.1).
4. Effect of N/10 HCl: Haemolysed red cells are seen with brown acid haematin in the surrounding.
5. Effect of alkali: Lysed RBCs are seen.
6. Effect of tannic acid: Condensed Hb in the cell is seen.

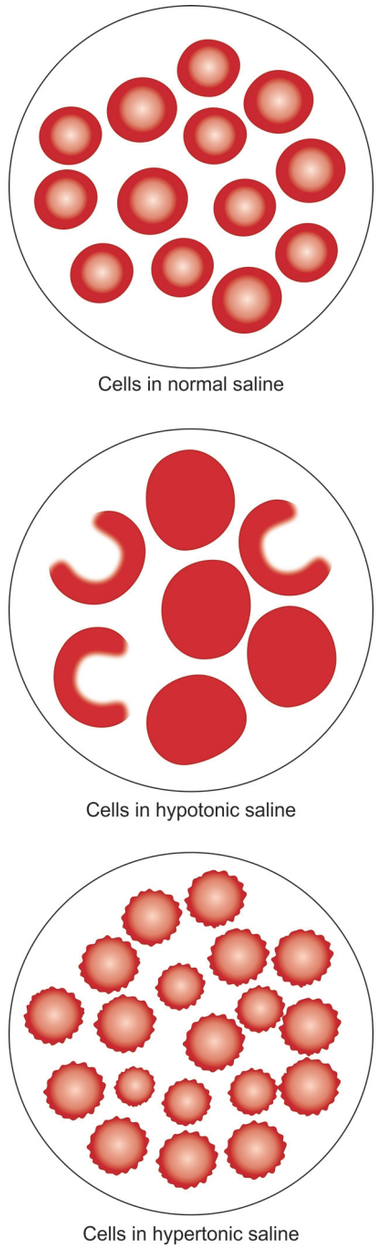

Fig. 1.12b.1: RBCs under high power in normal saline, hyptoinic saline and hypertonic saline (diagrammatic).

Experiment 1.12c
Determination of osmotic fragility of erythrocytes

At the end of the practical class student should be able to—

1. Define osmotic fragility of erythrocytes.
2. Tell what are isotonic, hypotonic and hypertonic solutions.
3. Tell what is haemolysis.
4. Tell the cause for haemolysis at lower concentration of saline solution.
5. Tell the clinical significance of test.
6. Name some haemolytic agents.
7. Name the conditions in which fragility of erythrocytes is increased.

APPARATUS

12 small test tubes, rack for holding tubes, long nozzle dropper, freshly prepared 1.0% NaCl solution and distilled water.

Osmotic Fragility

Osmotic fragility is the tendency of RBC to breakdown. Isotonic solution which is used for RBC contains 0.9% NaCl, 5% glucose, 10% mannitol and 20% urea solution for hours without rupturing or any change in the size or shape. Haemolysis begins at 0.46% and completes at 36%.

It is designed as the ease with which the cells are broken down in hypotonic solutions. It is expressed in terms of concentration of saline solutions in which cells are haemolysed.

Haemolysis begins at 0.42% saline solution and completes at 0.35% saline solution.

Indicator of haemolysis is appearance of red colour in mixture and when colour becomes saturated shows completion of haemolysis.

PROCEDURE

1. Take 12 small test tubes and keep them in rack.
2. Mark the test tubes S, 2, 3, 4 .... 11 and W from 1st test tube to 15th.
3. Now add number of drops of 1.0% NaCl solution and distilled water as shown in Table 1.12c.1.
4. Add a drop of anticoagulant mixed blood with the help of a dropper in each test tube.
5. Shake each test tube gently to mix the blood and keep them for one hour.
6. After one hour check for haemolysis by observing the colour of mixture.
7. Note down the concentration of saline in which red colour just appears and concentration of saline in which intensity of colour becomes maximum (saturation of colour).
8. Just appearance of colour indicates beginning of haemolysis and saturation of colour indicates completion of haemolysis.
9. Residue at the bottom of tube can be used to see haemolysed cell under microscope.

Table 1.12c.1: Procedure for preparation on increasing hypotonicity of NaCl solution

No. of test tubes	*Labelling of tube*	*No. of drops of 1.0% NaCl*	*No. of drops of distilled water*	*Concentration of saline*
1	S	22	3	0.88
2	2	16	9	0.64
3	3	15	10	0.60
4	4	14	11	0.56
5	5	13	12	0.52
6	6	12	13	0.48
7	7	11	14	0.44
8	8	10	15	0.40
9	9	9	16	0.36
10	10	8	17	0.32
11	11	7	18	0.28
12	W	0	25	0

OBSERVATIONS

Haemolysis starts at ..... % saline solution.
Haemolysis completes at ..... % saline solution.

RESULT

Osmotic fragility of RBCs ranges from ..... % saline to ..... % saline solution.

Precautions

1. Use the same dropper for adding 0.5% saline, distilled water and blood in all the test tubes.
2. Do not shake the tube vigorously for mixing blood drop in saline.
3. Take the reading exactly after one hour.

Clinical Significance

Test detects increased red cell fragility in patient with intrinsic or acquired red cells abnormality.

QUESTIONS AND ANSWERS

Q.1. Define osmotic fragility of erythrocytes.
Ans. *See* text

Q.2. What are isotonic, hypotonic and hypertonic solutions?
Ans. *See* text

Q.3. What is haemolysis?
Ans. The haemolysis is the release of haemoglobin after breakdown of red blood cells.

Q.4. What is the cause for haemolysis at lower concentration of saline solution?
Ans. *See* text

Q.5. What is the clinical significance of test?
Ans. *See* text

Q.6. Name some haemolytic agents.
Ans.
i. Hypotonic saline.
ii. Acids and alkalies.
iii. Agglutinins.
iv. Snake venoms.

Q.7. Name the conditions in which fragility of erythrocytes are increased.
Ans. Fragility of erythrocytes increases in hereditary spherocytosis and glucose-6-phosphate dehydrogenase (G6PD) deficiency.

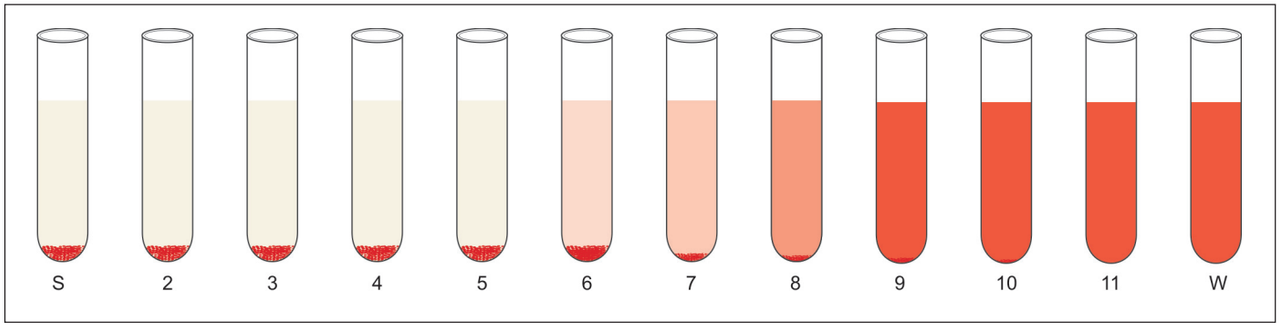

Fig. 1.12c.1: Fragility test (haemolysis starts at tube number 6 and complete at tube number 9)

Diagrams

Student's Notes

Experiment 1.13 (Demonstration)
Determination of reticulocyte count

OBJECTIVES

At the end of the practical class student should be able to—

1. Perform vital staining of reticulocytes with new methylene blue.
2. Prepare a slide with stained blood.
3. Identify the reticulocytes in the film.
4. Perform and interpret relative reticulocyte count.
5. Tell what is reticulocyte. What is its normal count in newborn and adult?
6. Tell what are the physiological and pathological causes of reticulocytosis.
7. Tell what is reticulocyte response.
8. Tell the identifying features of reticulocytes.
9. Tell indications of doing reticulocyte count.
10. Tell, what is vital staining. How does it differ from supravital staining?

APPARATUS

Glass slides, new methylene blue, 3% sodium citrate, 0.9% NaCl, incubator, compound microscope, cedar-wood oil and pricking needle.

Vital stains used are:

1. 1 gm new methylene blue in
   100 ml citrate saline solution.
   (Citrate saline solution—1 volume of 3% sodium citrate solution + 4 volumes of 0.9% NaCl)
2. i. Brilliant cresyl blue 1.0 gm
   ii. Sodium citrate 400 mg
   iii. 0.85% NaCl 100 ml

Reticulocytes

These are juvenile red cells. They contain ribosomes and ribonucleic acid (RNA) in their cytoplasm. Ribosomal material has the property of reacting with new methylene blue or brilliant cresyl blue to form a blue dots. This reaction takes place only in vitally stained unfixed preparations. Vital staining means the staining of cells after their somatic death but before their molecular death, i.e. after their removal from the living body but before all cellular activity stops.

PRINCIPLE

Relative count of reticulocytes is done in relation to number of red cells after their identification among RBC. Reticulocytes are expressed as 1% age of RBCs.

PROCEDURE

1. Preparation of staining solution: Fresh mixture of new methylene blue is prepared citrate saline and filtered.
2. Preparation of blood film:
   - Take 3–4 drops of new methylene blue solution in a glass tube.
   - Add 4–8 drops of blood sucked in heparinised pipette on syringe.
   - Keep the tube in water bottle at 37°C for 15–20 min.
   - The red cells are then resuspended by gentle mixing and films are made on glass slides as usual way.
   - When dry films are examined under oil immersion objective.
3. Identification of reticulocytes and counting.
   - Reticulocutes contain blue coloured reticulum or blue dots in pale greenish blue cytoplasm (Fig. 1.13.1).
   - RBCs are containing pale greenish blue cytoplasm without reticulum or dots.
   - Fix a piece of paper having pinhole in its center in eyepiece.
   - For counting an area of film should be chosen where the cells are undistorted and staining is good. The reticulocytes are counted in 1000 RBCs in different fields and entered in the table under observations.

Divide the total number of reticulocyte counted in 1000 cells by 10 to find percentage reticulocyte.

Absolute count (reticulocyte/cu mm) can be calculated as follows:

Absolute count = % Reticulocyte × RBC count/cu mm

Normal Value

Adult: 0.2 to 2%
Absolute count: 24000–84000/cu mm
Newborn: 30–50%

Increased reticulocyte count (reticulocytosis) in:

1. Anaemia (during recovery).
2. After haemorrhage
3. Proliferation of bone marrow.

Decrease reticulocyte count in bone marrow depression

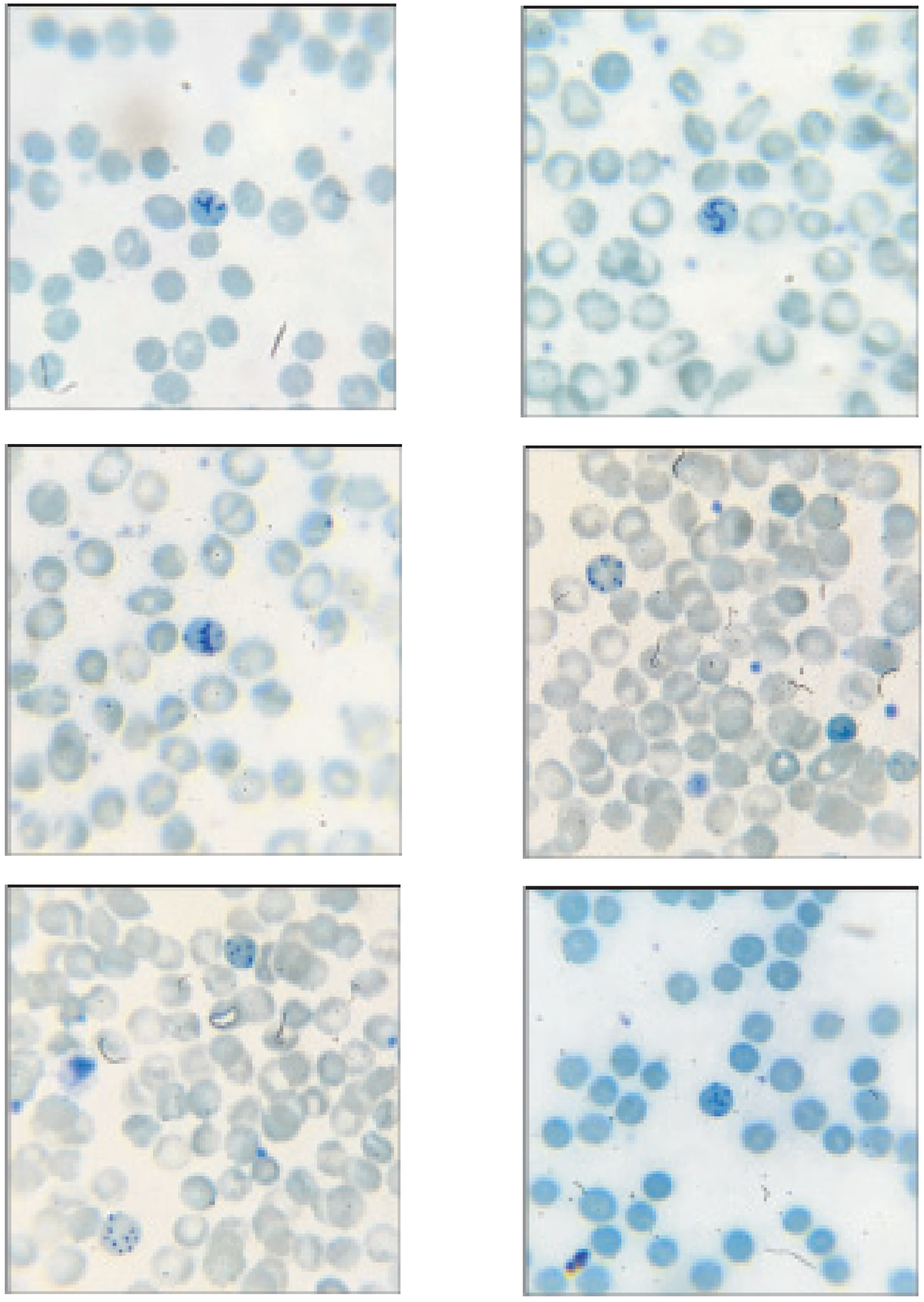

Fig. 1.13.1: RBCs and reticulocytes under oil immersion lens (100X)

Observations

Field No.	Number of reticulocytes (1)	Number of RBCs (2)	Total cells (1) + (2)
1.			$n1$ = ...
2.			$n2$ = ...
3.			$n3$ = ...
4.			$n4$ = ...
5.			$n5$ = ...

Total = $n1 + n2 + n3 + n4 + n5 = 1000$

QUESTIONS AND ANSWERS

Q.1. What is reticulocyte? What is its normal count in newborn and adult?

Ans. *See* text

Q.2. What are the physiological and pathological causes of reticulocytosis?

Ans. *See* text

Q.3. What is reticulocyte response?

Ans. When anaemia is treated with iron or folic acid reticulocyte count increases during recovery. This increase in reticulocyte count is called reticulocyte response.

Q.4. What are the identifying features of reticulocytes?

Ans. *See* text

Q.5. What are the indications of doing reticulocyte count?

Ans. Indications of reticulocyte count are:

i. All conditions where reticulocytosis are suspected.
ii. Aplastic anaemia.
iii. Haemolytic anaemia.

Q.6. What is vital staining? How does it differ from supravital staining?

Ans. Vital staining means the staining of cells after their somatic death but before their molecular death, i.e. after their removal from the living body but before all cellular activity stops. Supravital staining is the process *in vitro*, where stain reacts with the cells, when it is partly injured.

Student's Notes

Experiment 1.14
Determination of platelets count

OBJECTIVES

At the end of the practical class student should be able to—

1. Fill the pipette with blood and diluting fluid.
2. Charge the Neubauer's chamber.
3. Identify the squares required for platelets count.
4. Identify and count the platelets.
5. Tell the composition of platelets diluting fluid, and the functions of ingredient of platelets diluting fluid.
6. Tell the normal range of platelet count.
7. Tell, what are thrombocytosis and thrombocytopenia.
8. Enumerate the functions of platelets.
9. Tell what is purpura.
10. Tell the causes of thrombocytosis.
11. Tell the causes of thrombocytopenia.
12. Tell how platelets appear in Leishman's stain in peripheral blood smear.
13. Name any other diluting fluid for platelet count.
14. Explain that dilution factor is 200 in platelet count.

APPARATUS

Neubauer's chamber, RBC pipette, platelets diluting fluid (1.0% ammonium oxalate), compound microscope, coverslips, pricking needle and spirit swab.

Function of platelets diluting fluid (1.0% ammonium oxalate):

1. Acts as anticoagulant.
2. Preserves the platelets.
3. Destroys RBCs and WBCs.

PRINCIPLE

Platelets are counted in diluted blood in Neubauer's chamber. Number of platelets counted then multiplied by dilution factor to calculate platelet count in undiluted blood.

PROCEDURE

1. Prick the finger under all septic conditions and suck the blood up to mark 0.5 in the RBC pipette.
2. After wiping off tip of the pipette, suck platelet diluting fluid up to mark 101.
3. Mix the solution and blood for 10 minutes so that there will be complete haemolysis of RBCs.
4. Discard first two drops of mixture and charge the Neubauer's chamber.
5. Wait for 15–20 min so that platelets will settle down.
6. Identify platelets and count them in 25 medium size RBC squares.

Platelets are 2–4 µm size nonnucleated cells. They are highly refractile in nature.

OBSERVATIONS

Enter the number of platelets counted in all RBC squares like *n*1, *n* 2, ...*n* 24, *n* 25.

Total number of cells in all 25 RBC squares

$$= n\,1 + n\,2 + ...+ n\,24 + n\,25 = N$$

n 1	*n* 2			
			n 24	*n* 25

Calculation

Volume of fluid in which cells are counted

$$= 1 \text{ mm} \times 1 \text{ mm} \times 0.1 \text{ mm} = 0.1 \text{ cu mm}$$

Total number of cells in 0.1 cu mm

Undiluted blood = N

Number of platelets in 1 cu mm

Undiluted blood = N × 10

Dilution factor = 200

Total number of platelets in undiluted blood

$$= N \times 10 \times 200 = N \times 2000$$

Result

Normal platelets count: 1.5– 4 lac/cu mm.

Variations in count:

1. Thrombocytosis: When platelet count is more than 4 lac/cu mm.
2. Thrombocytopenia: When platelet count is less than 1.5 lac/cu mm

Precautions

Same as in RBC count.

QUESTIONS AND ANSWERS

Q.1. What are identifying features of platelets?

Ans. *See* text

Q.2. What is the composition of platelets diluting fluid?

Ans. *See* text

Q.3. What are the functions of ingredient of platelets diluting fluid?

Ans. *See* text

Q.4. What is the normal range of platelet count?

Ans. *See* text

Q.5. What are thrombocytosis and thrombocytopenia?

Ans. *See* text

Q.6. Enumerate the functions of platelets.

Ans. Platelets are playing role in:

i. Haemostasis (arrest of bleeding).
ii. Coagulation.
iii. Clot retraction.
iv. Storage of certain chemical substances, e.g. histamine and 5-HT.
v. Phagocytosis of very small micro organisms (viruses) and particles.

Q.7. What is purpura?

Ans. It is a condition in which there is a tendency to spontaneous bleeding usually beneath the skin, from the various mucous membranes and in internal organs. Purpura may be thrombocytopenic (when reduction in platelets count is decreased) or athrombocytopenic (platelets count normal).

Q.8. What are the causes of thrombocytosis?

Ans. Common causes of thrombocytosis are:

i. After trauma, e.g. surgery and injury.
ii. Splenectomy.
iii. Haemorrhage.
iv. Injection of epinephrine (because of contraction of spleen).

Q.9. What are the causes of thrombocytopenia?

Ans. Common causes of thrombocytopenia are:

i. Bone marrow depression.
ii. Hypersplenism.
iii. Leukaemia.
iv. Drug administration, e.g. aspirin.

Q.10. How platelets appear in Leishman's stain in peripheral blood smear?

Ans. In Leishman's stain platelets are seen in groups with light blue cytoplasm and reddish purple granules looking like nucleus, but no nucleus is present.

Q.11. Name any other diluting fluid for platelet count.

Ans. Rees-Ecker fluid may also be used as a diluting fluid. It contains sodium citrate (anticoagulant), brilliant cresyl blue (staining platelets) and formaldehyde (fixative and lyses RBCs).

Q.12. Explain that dilution factor is 200 in platelet count.

Ans. *See* text

Student's Notes

Student's Notes

Experiment 1.15 (Demonstration)
Determination of absolute eosinophil count

OBJECTIVES

At the end of the practical class student should be able to—

1. Identify the eosinophils.
2. Perform absolute eosinophil count.
3. Tell the clinical significance of the investigation.
4. Charge Neubauer's
5. Tell what is the normal absolute eosinophil count.
6. Enumerate the conditions causing increase in eosinophil count.
7. Mention any other diluting fluid for eosinophil count.
8. Tell the functions of eosinophils.
9. Tell the clinical significance of test.
10. Explain why the diluting factor is 10 in eosinophil count.

APPARATUS

Eosinophil diluting fluid, WBC pipette, Neubauer's chamber, compound microscope, coverslips, pricking needle and spirit swab.

EOSINOPHIL DILUTING FLUID

1.0% solution of eosin in water = 5 ml
Acetone = 5 ml
Distilled water = 90 ml
Functions:
Eosin: Stains granules (red or pink)
Acetone: Lyses RBCs and other white cells

PRINCIPLE

Eosinophils are counted in diluted blood in Neubauer's chamber. Count is then multiplied by dilution factor to calculate eosinophil count in undiluted blood.

PROCEDURE

1. Get a prick in the finger under all aseptic conditions.
2. Discard first drop of blood and fill the WBC pipette up to mark 1.0 with blood from second drop.
3. Clean the tip of the pipette and suck eosinophil diluting fluid up to mark 11.
4. Mix the fluid and blood by rotating the pipette in between palms. Wait for 15–20 min for lyses of other cells.
5. Mix the fluid once again and discard two drops of fluid.
6. Charge the Neubauer's chamber.
7. Count the eosinophil in 4 WBC squares under low power. The cells which are visible under low power are mainly eosinophils because these are the only cells which are stained by eosin. If still any doubt, that cell may be confirmed under high power.
   Note: Eosinophils should be counted under low power (10X).

OBSERVATION

Enter the cells in squares (Fig. 1.15.1) as shown below:
Total number of cells counted in WBC squares = x1 + x2 + x3 + x4 = x
No. of cells = x1
No. of cells = x2
No. of cells = x3
No. of cells = x4

CALCULATION

Dilution factor = 10
Volume of fluid in which eosinophils are counted
= (1 mm × 1 mm × 0.4 mm) 4 = 0.4 cu mm
Number of eosinophils in 0.4 cu mm volume = x
No. of eosinophils in 0.1 cu mm of diluted blood
= x ÷ 4
No. of eosinophils in 1.0 cu mm of diluted blood
= (x × 10) ÷ 4
No. of eosinophils in 1.0 cu mm of undiluted blood
= (x × 10 × 10) ÷ 4
= x × 25
(*Note:* Calculations same as in TLC but dilution factor is 10.)

Result

Absolute eosinophil count = x × 25/cu mm
Normal eosinophil count: 10 – 400/cu mm
Variation: Eosinophil count increases in allergic conditions and parasitic manifestations.

Precautions

Same as in total leucocytic count.

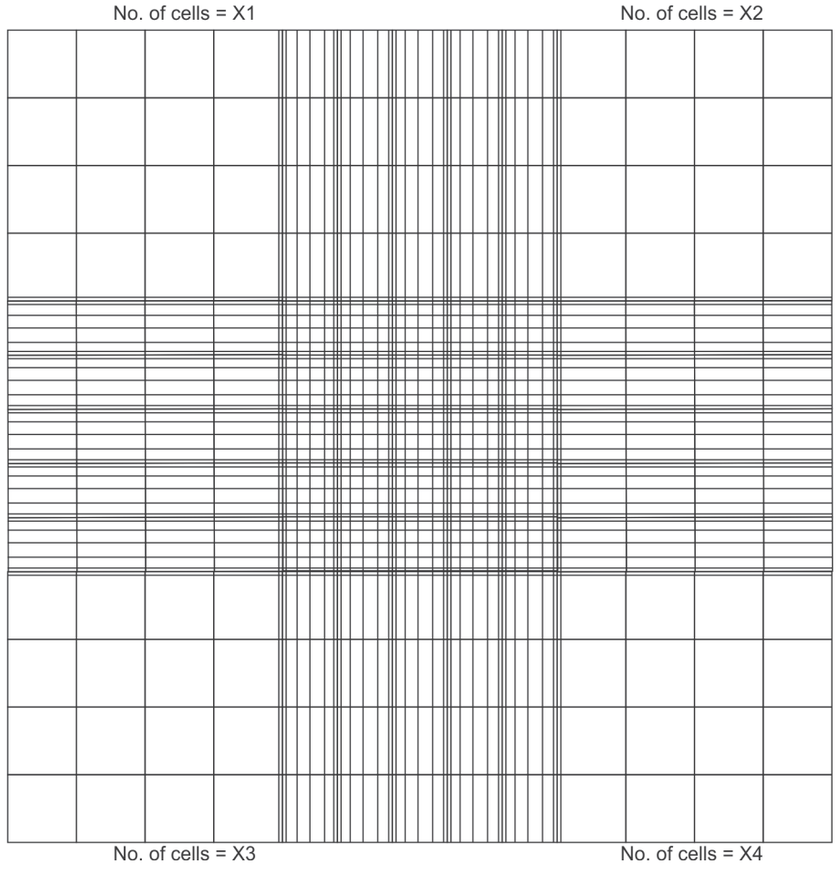

Fig. 1.15.1: Eosinophil counting squares (WBC squares).

QUESTIONS AND ANSWERS

Q.1. What is the normal absolute eosinophil count?

Ans. *See* text

Q.2. Enumerate the conditions causing increase in eosinophil count.

Ans. *See* text

Q.3. Mention any other diluting fluid for eosinophil count.

Ans. Pilot's solution. It contains phloxine, propylene glycol, sodium carbonate and heparin.

Q.4. What are the functions of eosinophils?

Ans. Functions of eosinophils are:

i. They are phagocytic but less motile.
ii. Their granules are lysosomal in character.
iii. There is a very high content of peroxidase enzyme (parasiticidal).
iv. It limits the effects of mediators of allergic reactions.

Q.5. What is the clinical significance of test?

Ans. To find out whether patient is suffering from eosinophila. Eosinophil count is increased in different allergic conditions, worm infestations and in pulmonary eosinophilia.

Q.6. Why the diluting factor is 10 in eosinophil count?

Ans. *See* text

Student's Notes

Any other Experiment

Any other Experiment

Any other Experiment

Any other Experiment

Any other Experiment

Any other Experiment

Any other Experiment