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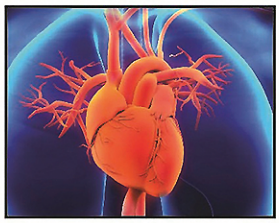
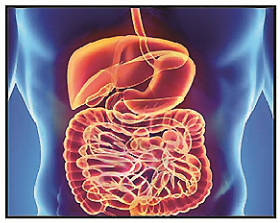
Ghai's Textbook of PRACTICAL PHYSIOLOGY

Revised & Edited by
VP Varshney
Mona Bedi

9th Edition

JAYPEE

Ghai's
Textbook of
PRACTICAL PHYSIOLOGY

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Ninth Edition

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Preface to the Ninth Edition

Ghai's Textbook of Practical Physiology, 9th edition, has been prepared for the benefit of the medical students on the basis of our long teaching experience in physiology. Even though theoretical knowledge is important for the students, it becomes more important when used in practice, hence the importance of a detailed textbook of practical physiology.

In this book, we have attempted to familiarize the students with the clinical and experimental approach to human physiology. Care has been taken to modify the practicals according to the Medical Council of India (MCI) guidelines. Well-labeled diagrams and flowcharts have been incorporated to improve understanding.

A basic format has been adhered to in each practical. Each experiment starts with STUDENT OBJECTIVES, which are basically learning objectives for the students. This is what the student is expected to learn at the end of each practical. This is followed by a brief INTRODUCTION of the practical. After explaining the PRINCIPLE, the APPARATUS required for the experiment is described. A detailed step by step PROCEDURE is then elaborated. The working instructions are simple so the average student can easily follow them. Next come the OBSERVATIONS and RESULTS. The PRECAUTIONS, which helps to minimize the error in the practicals are listed after the RESULTS. As all experiments have a physiological and clinical importance, a separate heading has been ascribed for PHYSIOCLINICAL SIGNIFICANCE in each experiment. Each chapter ends with QUESTIONS along with their answers to help the student to prepare for their examination viva. Objective Structured Practical Examination (OSPE) is a reliable, valid and important method of assessing practical skills. These have been added at the end of each practical to acquaint the student with the mode of practical assessment.

We sincerely hope that this endeavor of ours, helps the students to comprehend the scientific basis of practicals in physiology and prepare for their examinations in a systematic manner.

We would always welcome suggestions and ideas from our students and friends for the betterment of this book in future. After all "The secret in education lies in respecting the students".

VP Varshney
Mona Bedi

Contents

Section 1: Hematology

Come here! Hurry! There are little animals in this rain water. They swim! They play around! They are a thousand times smaller than any creature we can see with our eyes alone. Look! See what I have discovered.

1.1: THE COMPOUND MICROSCOPE

Student Objectives

After completing this experiment, the student should be able to:

1. Name the different parts of the microscope and explain the functions of each.
2. Explain the physical basis of microscopy and define the terms magnification, resolution, and numerical aperture.
3. Describe the mechanism of image formation and the type of image seen.
4. Explain how to use low power (LP), high power (HP), oil immersion (OI) objective to obtain different magnifications.
5. Describe the procedure (protocol) that must be followed every time while using a microscope.
6. Name the precautions that must be observed during and after using the microscope.
7. Explain the basic working of other types of microscopes.

INTRODUCTION

Antonie van Leeuwenhoek (1632–1723) was a Dutch tradesman and scientist, best known for his work on the development and improvement of the microscope and also for his subsequent contribution toward the study of microbiology.

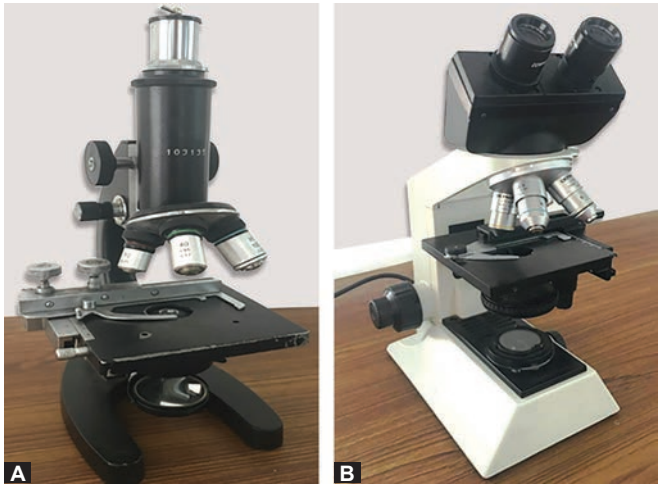
The microscope is one of the most commonly used instruments in the medical colleges and in clinical laboratories. Students of physiology use it in the study of morphology of blood cells and in counting their numbers. They will use it in histology, histopathology, and microbiology and later in various clinical disciplines.

Monocular microscope: The compound microscope is so called because, in contrast to a single magnifying convex lens, it has two such lenses—the *objective* and the *eyepiece*. It magnifies the image of an object that is not visible to the naked eye to an extent where it can be seen clearly. Monocular microscope has only one eyepiece (Fig. 1A).

Binocular microscope: It is a compound bright-field microscope but having two eyepieces instead of one so that both eyes are used simultaneously. This prevents eyestrain (Fig. 1B).

Before using a microscope, the students must familiarize themselves with its different parts and how to use it and take its care. It will be discussed under the following heads:

- **Parts of the microscope**
  - The support system
  - The focusing system
  - The optical (magnifying) system
  - The illumination system.
- **Physical basis of microscopy**
  - Visual acuity
  - Resolving power
  - Magnification
  - Calculation of total magnification
  - Numerical aperture
  - Image formation
  - Working distance.
- **Protocol (Procedure) for the use of microscope**
  - Focusing under low power (100x)
  - Focusing under high power (450x)
  - Focusing under oil immersion (1000x)
  - "Racking" the microscope.
- **Common difficulties faced by students**
- **Precautions and routine care**
- **Other types of microscopes.**



Figs. 1A and B: (A) Monocular microscope; (B) Binocular microscope.

PARTS OF THE MICROSCOPE

The Support System

The support system acts as a framework to which various functional units are attached (Fig. 2):

- **Base:** It is a heavy metallic, U-shaped or horseshoe-shaped base or foot, which supports the microscope on the worktable to provide maximum stability.
- **Pillars:** Two upright pillars project up from the base and are attached to the C-shaped handle. The hinge joint allows the microscope to be tilted at a suitable angle for comfortable viewing.

Note: The microscope is never tilted when counting cells in a chamber or when examining a blood film under oil immersion. It can be tilted for viewing histology slides.

- **Handle (the arm or limb):** The curved handle, which projects up from the hinge joint supports the focusing and magnifying systems.
- **Body tube:** Fitted at the upper end of the handle, either vertically or at an angle, the body tube is the part through which light passes to the eyepiece, thus conducting

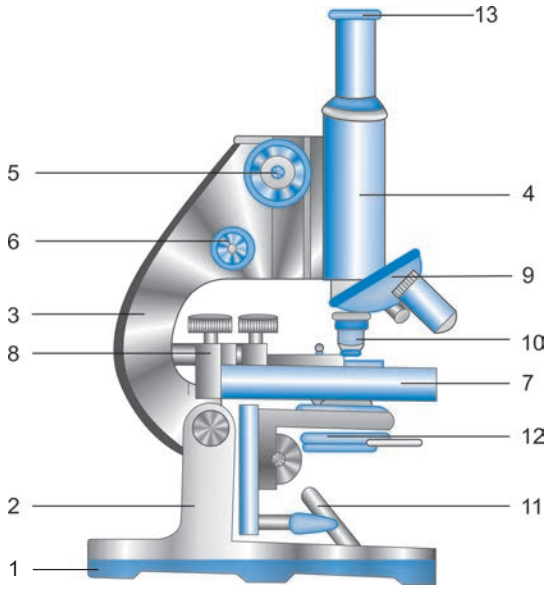


Fig. 2: Compound microscope. 1. Base, 2. Pillars, 3. Handle, 4. Body tube, 5. Coarse adjustment screw, 6. Fine adjustment screw, 7. Fixed stage, 8. Mechanical stage, 9. Fixed and revolving nose pieces, 10. Objective lenses, 11. Mirror, 12. Condenser, 13. Eyepiece.

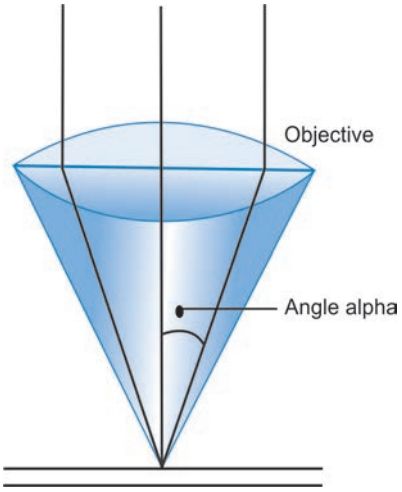


Fig. 4: Diagram to explain the numerical aperture. The angle alpha is shown.

The NA is also an index of light gathering power of a lens, i.e. the amount of light entering the objective. The NA can be decreased by decreasing the amount of light passing through the lens. Thus, as shown below, the illumination has to increase as the objectives are changed from LP to HP to OI. The magnifying power of each lens and its NA rather than its focal length, are etched on each objective lens.

Low power objective (10x; NA = 0.25; focal length = 16 mm)

High power objective (45x; NA = 0.65; focal length = 4 mm)

Oil immersion objective (100x; NA = 1.30; focal length = 2 mm).

Image Formation in the Compound Microscope

It is the objective that starts the process of magnification. It forms a real, inverted, and enlarged image (primary image: A'-B') (Fig. 5) in the upper part of the body tube (A real image is that which can be received on screen). The field lens of the eyepiece collects the divergent rays of light of the primary image and passes these through the eye lens, which therefore the image seen by the eye is—virtual, inverted, and magnified, and appears to be further magnified the image. The light rays reaching the observer's eye are divergent and about 25 cm in front of the eye. Figure 5 shows the ray diagram of a compound microscope.

Working Distance

The working distance is the distance between the objective and the slide under study. This distance decreases with increasing magnification. It is 8–13 mm in LP, 1–3 mm in HP,

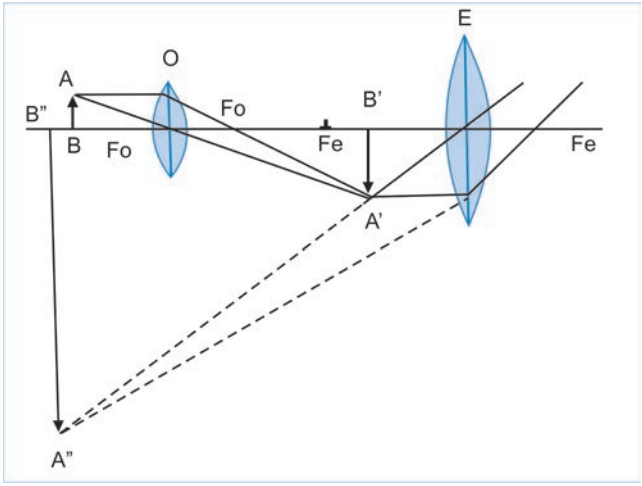


Fig. 5: The ray diagram of a compound microscope. AB = object; A' B' = real, inverted, magnified image; A" B" = virtual, inverted, magnified image; O = objective lens; E = Eyepiece; Fo = Focus of objective; Fe = Focus of eyepiece

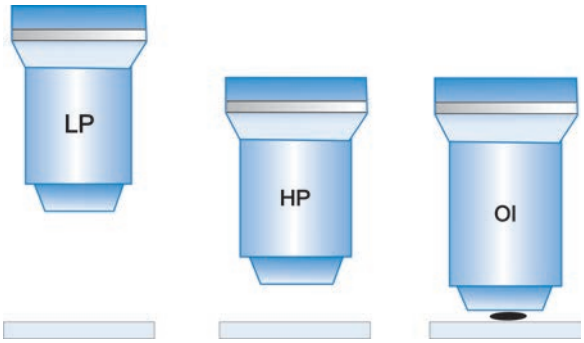


Fig. 6: Diagram to show the working distances of low power (LP), high power (HP) and oil immersion (OI) lenses.

and 0.5–1.5 mm in OI lenses, respectively. Figure 6 shows the approximate working distances for each lens.

Note that the OI lens has to be immersed in a drop of oil.

PROTOCOL/PROCEDURES FOLLOWED WHILE USING THE MICROSCOPE

Principle

A focused beam of light passes through the material under study into the microscope. Parts of the specimen that are optically dense and having a high refractive index or are colored with a stain (dye), cast a potential shadow which is magnified in two main stages as it passes into the observer's eye.

Procedure

The student must avoid the bad habit of using objective lenses in a haphazard manner, starting with any lens at

***For a sample of serum*:** No anticoagulant is used. The blood is allowed to clot in the container and serum is collected as described later. Obviously, capillary blood does not require a container or anticoagulant.

Collection of Blood Samples

Collection of Capillary Blood (Skin-prick Method)

A single bold prick is given on finger under aseptic conditions (Fig. 7). **Never squeeze the pricked finger** as this will expel tissue fluid along with blood to come out of the puncture site. The dilution of blood will, thus, nullify the results. *Hence for clinical work, venous blood is always preferred.* Skin-prick may be used on the bedside of a patient, or in an emergency when it is not convenient to take a venous sample.

Note: The thumb and little finger are never pricked because the underlying palmar fasciae (venous bursae) from these digits are continuous with those of the forearms. Any accidental injury to these fasciae may cause the infection to spread into the forearm. Thus finger prick is given on the distal digit on the palmar surface of the 3rd/4th finger.

- ❑ Remember that one deep puncture, which will give you free-flowing blood, is less painful than 3 or 4 superficial pricks.

Apparatus

- ❖ **Blood Lancet/Pricking needle:** Disposable, sterile, one-time use, blood lancets (flat, thin metal pieces with 3–4 mm deep penetrating sharp points) are commercially available and should be preferred.

  Ordinary, narrow-bore **injection needles** are useless since they only make shallow cuts rather than deep punctures. However, wide-bore (22 gauge) needles may be used in an emergency or if blood lancets are not available.

  Cutting needle with three-sided cutting point (used by surgeons) can serve the purpose well.

  Pricking gun: A spring-loaded pricking gun that has a disposable, three-sided sharp point, and a loading and releasing mechanism, is ideal because the depth of the puncture can be preselected.
- ❖ Sterile gauze/cotton, moist with 70% alcohol/methylated spirit.
- ❖ Glass slides, pipettes, etc. according to requirements.

Fig. 7: Finger prick method.

Note: The students should bring their own lancets. These may be reused two to three times, if required, after passing their points through a flame. Spirit does not kill the hepatitis virus which can be killed on heating. However, too much heating, however, is likely to blunt the pricking points.

Procedures

All aseptic precautions must be taken. The person giving the prick should wash his/her hands with soap and water, and wear gloves if possible.

Note: Keep all the equipment ready before getting a prick. If the finger to be pricked appears cold and bloodless, especially in winter, immerse it in warm water for 2–3 minutes.

- ❖ Clean and vigorously rub the ball of the finger with the spirit swab, followed by a final cleaning with dry gauze. (Scrubbing increases local blood flow).
- ❖ Allow the alcohol to dry by evaporation for the following reasons:
  - ■ Sterilization with alcohol/spirit is effective only after it has dried by evaporation.
  - ■ The thin film of alcohol can cause the blood drop to spread sideways along with alcohol so that it will not form a satisfactory round drop.
  - ■ The alcohol may cause hemolysis of blood.
- ❖ Steadying the finger to be pricked in your left hand, apply a gentle pressure on the sides of the ball of the finger with your thumb and forefinger to raise a thick, broad ridge of skin (Do not touch the pricking area).
- ❖ Hold the lancet between the thumb and fingers of your right hand, and keeping it directed along the axis of the finger, but slightly "off" center so as to miss the tip of the phalanx (i.e. not too far down or too far near the top of the nail bed), prick the skin with a sharp and quick vertical stab to a depth of 3–4 mm and release the pressure. The blood should start to flow slowly, spontaneously and freely (without any squeezing)—if a good prick has been given.

Counting Chamber: Improved Neubauer Chamber

The counting chamber was introduced by Crammer in 1805. Its modification by Thoma, and later by Neubauer remained in use for a long time. Improved Neubauer chamber is in current use.

The counting chamber (Figures 9A and B) is a single, solid, and heavy glass slide. Extending across its middle third are three parallel platforms (pillars, or flanges) separated from each other by shallow trenches (moats, gutters, or troughs). The central platform or "floorpiece" (sometimes also called the plateau) is wider, and exactly 0.1 mm (one-tenth of a mm) lower than the two lateral pillars. The floorpiece is divided into two equal parts by a short transverse trench in its middle as shown in Figures 9A and B. Thus, there is an H-shaped trench or trough enclosing the two floorpieces. The two lateral platforms can support a coverslip which, when in position, will span the trenches and provide a capillary space 0.1 mm deep between the undersurface of the coverslip and the upper surface of the floorpieces. Identically ruled areas, called "counting grids", consisting of squares of different sizes, are etched on each floorpiece. The two counting grids allow RBC and WBC counts to be made simultaneously if needed, or duplicate samples can be run.

Note: Hemocytometers with silver-coated floorpieces show the grids beautifully, which makes them easier to use by the students.

Thoma's Chamber

In this counting chamber, not used now, the central depressed platform is circular. The grid is only 1 mm^2, consisting of 25 groups of 16 smallest squares each. The dimensions of the smallest squares are the same, i.e. 1/20 mm × 1/20 mm. The 1 mm squares at the corners are absent.

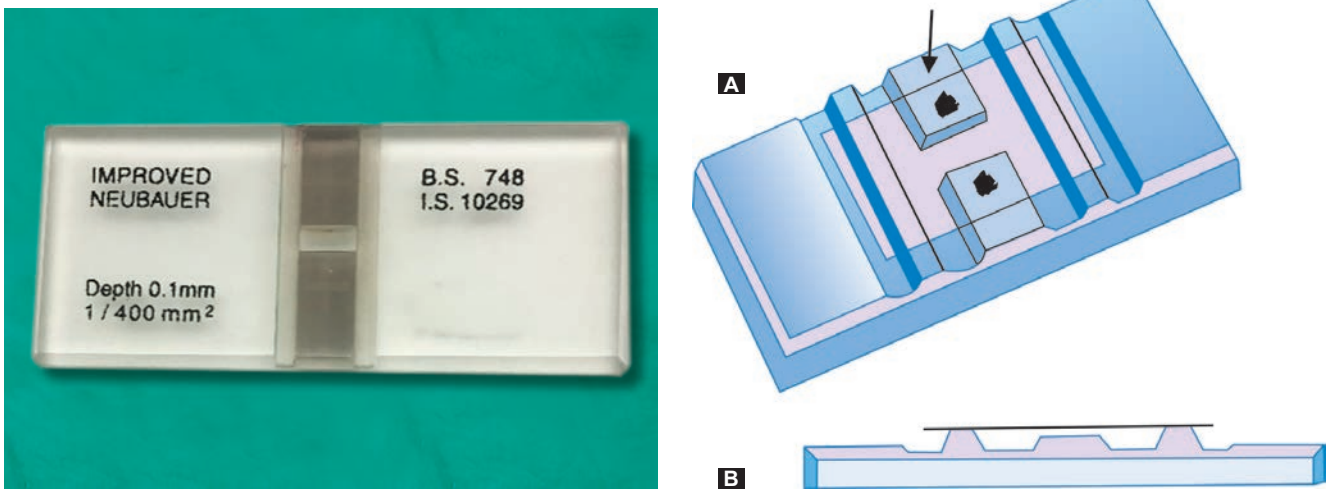
Old Neubauer Chamber

There are nine 1 mm squares. The four corner groups of 16 squares each are for WBC counting, while the central 1 mm^2 area has 16 groups of 16 squares each for RBC counting (rather than 25 groups of 16 smallest squares as in improved Neubauer chamber). The medium squares are separated by triple lines.

The Counting Grid

The ruled area on each floorpiece, the counting grid, has the following dimensions:

- Each counting grid (Fig. 10) measures 9 mm^2 (3 mm × 3 mm). It is divided into nine large squares, each 1 mm^2 (1 mm × 1 mm).
- Of these nine squares, the four large corner squares are lightly etched, and each is divided by single lines into 16 medium-sized squares each of which has a side of 1/4 mm, and an area of 1/16 mm^2 (1/4 mm × 1/4 mm). These four large corner squares are employed for counting leukocytes and are, therefore, called WBC squares (Fig. 10).



Figs. 9A and B: Hemocytometer, or counting chamber with improved Neubauer's ruling. (A) Surface view, with the coverslip in position. The locations of the counting grids on the two platforms (*floorpieces*) are indicated. The arrow indicates the place where the tip of the pipette should be placed for *charging* the chamber; and (B) Side view with the coverslip in position. The space between the underside of the coverslip and the surface of the platform is 0.100 mm in depth. The depth and the area of the smallest square are etched on the surface of the chamber.

millimeter, but we are not concerned with these but only the relative volumes or parts in relation to each other. It can be seen that the capillary bore in WBC pipette is wider than that in RBC pipette, and therefore, will hold more blood though the volume of the stem in both cases is 1.0 (one).

Differences between Red Blood Cell Pipette and White Blood Cell Pipette

The differences between RBC pipette and WBC pipette are described in Table 3.

Note: Though the dilution obtained with the RBC pipette is 10 times that obtained with WBC pipette, its bulb is not 10 times bigger. The reason is the much finer bore in the red cell pipette.

Filling the Pipette (Fig. 12)

- ❖ Place a drop of anticoagulated blood on a glass slide or get a fingerprick under aseptic conditions.
- ❖ Holding the mouthpiece of the pipette between your lips and keeping the pipette (with its graduations facing you) at an angle of about 40° to the horizontal, place its tip within the edge of the drop. Gently suck on the mouthpiece and draw blood until it is just above the mark 0.5 (capillary action cannot fill the pipette at this angle).
  - The blood drop should be of adequate size (say 3–4 mm in diameter). If it is too small or if the tip is lifted out of the drop, air will enter the pipette along with blood. If the tip presses against the skin, the bore at the tip will get blocked and no blood will enter the stem even if you suck hard at the mouthpiece.
  - Alternately, the pipette (after removing the rubber tube) may be filled with blood (without sucking/ by lowering its bulb end below the horizontal) and allowing the blood to flow down the stem by gravity.
- ❖ Remove the pipette from the blood drop and clean its outer surface with a cotton swab by wiping it toward the tip. Do not touch the bore at the tip otherwise some blood will be pulled out.
- ❖ Keeping the pipette horizontal all the time, bring the blood in the stem to the exact mark 0.5 by wiping the tip on your palm (or on a paper) a couple of times till the blood recedes to the exact mark.

Note: Do not use filter paper for this purpose as it will absorb a large amount of blood, and neither should you try to blow out the extra blood.

- ❖ Holding the pipette nearly vertical, immerse its tip in the diluting fluid taken in a watch glass, and suck the diluent to the mark 11 (WBC) or 101 (RBC). As the fluid is sucked up, the blood is swept before it into the bulb of 10 volumes (WBC) or 100 volumes (RBC pipette).
  - The sucking up of diluting fluid should not be done very quickly because blood being viscous, if a sufficient time is not allowed, a thick film of blood will remain sticking to the inside of the capillary bore, thus introducing a significant error.
  - The dilution of blood should not be delayed otherwise it is likely to clot in the stem.
  - Once the diluent has been taken to the appropriate mark, keep the pipette horizontal so that the fluid does not run out by gravity.
  - Do not place the pipette on the table, or delay the mixing because it becomes impossible to dislodge the cells from the walls of the bulb once they settle down.
- ❖ **Mixing the blood with the diluting fluid**: Once the diluting fluid has been sucked up, remove the rubber tube. Holding the short stem above the bulb between your thumb and first two fingers, and pressing the tip of the pipette against the palm of the other hand, rotate it to and fro for 3–4 minutes so that the blood and diluent get thoroughly mixed.

Table 3: Differences between RBC pipette and WBC pipette.

RBC pipette	*WBC pipette*
Calibrations are 0.5 and 1.0 below the bulb, and 101 above the bulb	Calibrations are 0.5 and 1.0 below the bulb, and 11 above it
The capillary bore is narrow, thus it is a **slow-speed pipette**	The capillary bore is wider, hence it is a **fast-speed pipette**
Bulb is larger and has a red bead	Bulb is smaller and has a white bead
The volume of the bulb is 100 times the volume contained in stem	The volume of the bulb is 10 times the volume of the stem

(RBC: red blood cell; WBC: white blood cell)

Fig. 12: Filling the pipette.

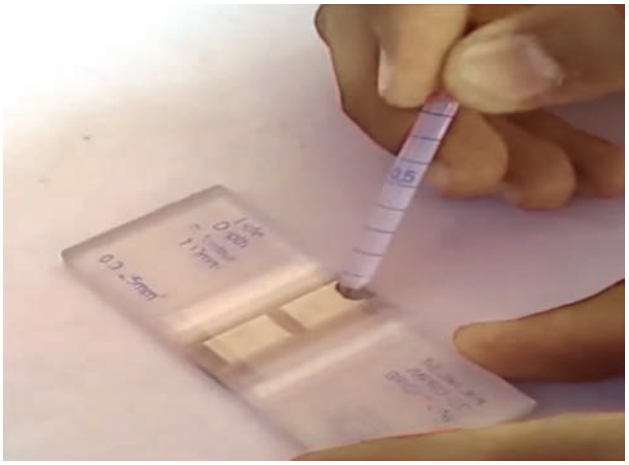


Fig. 13: Charging a chamber.

Alternately, remove the rubber tube, close the pipette ends with thumb and forefinger of your right hand, and shake it vigorously with a figure of eight motion.

Do not shake the pipette with an endwise motion as this will force the cells out of the bulb into the stem.

- **Charging the chamber:** Once the blood and the diluent have been mixed well, "charge" the chamber (Fig. 13). Charging the chamber requires patience, practice, and understanding of how to correctly judge the size of the drop, the angle at which the pipette should be held on the floorpiece, and the time needed for filling (charging) the chamber. This is called the "speed of the pipette". Obviously, it varies with the size of the capillary bore in the stem of the pipette.
  - **High-speed pipette:** Since the bore of the WBC pipette is wider, a drop will form more quickly at its tip, and it will be larger, as compared to the RBC pipette. This requires that this pipette should be held more horizontally say, at an angle of 10–20° and for a shorter time.
  - **Slow-speed pipette:** The bore of the RBC pipette being narrow, it will take a longer time for a suitable drop to form. It should, therefore, be held at a steeper angle—say, 60–70°.

It is for this reason that the students should first practice charging a chamber with the RBC pipette and then with the WBC pipette.

Calculation of Dilution Obtained (Dilution Factor)

When blood is sucked up to the mark 0.5 (half part or volume) and is followed by the diluting fluid, the blood enters the bulb first and is followed by the diluent to the mark 101 (RBC pipette), or mark 11 (WBC pipette). The stem in both pipettes contains only the diluent. Thus, *the dilution of the blood occurs in the bulb only.*

- **Red blood cell pipette:** Since the volume of the bulb is 100 (101 – 1.0 = 100), it means that 100 volumes (or parts) of diluted blood contain 0.5 (half) part of blood and 99.5 (100 – 0.5 = 99.5) parts or volumes of diluents.
- **White blood cell pipette:** In this case, the volume of the bulb is 10 (11 – 1 = 10). When blood is taken to the mark 0.5 (half part or volume) followed by diluent to the mark 11, the volume of the diluted blood is now 10, which contains 0.5 part of blood and 9.5 parts or volumes of the diluting fluid. This gives a dilution of 0.5 in 10 (half in ten), or 1 in 20 (one in 20), the dilution factor being 20 (the blood will be diluted 20 times). Similarly, if blood is taken to the mark 1.0 followed by diluted to mark 11, the dilution now would be 1 in 10.

For Red Blood Cell Counting

The red cells are counted in four corner groups and one central group of medium squares, each of which has 16 smallest squares, i.e. in a total of 80 smallest squares.

Area of smallest square = 1/20 mm × 1/20 mm = 1/400 mm^2.

Since the depth of the chamber is 1/10 mm, the volume of the smallest square = 1/400 × 1/10 = 1/4,000 mm^3.

For White Blood Cell (Total Leukocyte) Counting

This count is done in the four corner groups of large squares, each of which has 16 medium squares.

Area of one medium = 1/4 mm × 1/4 mm square = 1/16 mm^2.

Volume of this square = 1/16 mm^2 × 1/10 mm = 1/160 mm^3.

Focusing the Counting Grid

Examine the grid on each floorpiece, without the coverslip, under low and high magnifications. Rack the condenser up and down, closing/adjusting the diaphragm at the same time. Find out the best combination of these two that shows the grid lines and squares clearly. When properly focused, the rulings (lines) appear as translucent darkish lines.

- With low magnification of 100 times, one large square, 1 mm × 1 mm is visible in one field, i.e. a group of 16 medium squares (for WBC counting), or a groups of 25 medium squares (for RBC counting).
- Examine the squares under high magnification.

PROCEDURES

1. Assuming that the blood and the diluent have been properly mixed, the next step is to charge the chamber. Place a coverslip on the chamber so that it spans the floorpieces and the trenches around them—a process called "centering" the coverslip.

mix the contents of the bulb for 3–4 minutes as described earlier.

- **Charging the chamber:** Observing all the precautions, fill the chamber with diluted blood. Since the RBC pipette is a slow-speed pipette, it will need to be kept at an angle of 70–80° while charging the chamber. Move the chamber to the microscope and focus the grid once again to see the central 1 mm square with the red cells distributed all over. Wait for 3–4 minutes for the cells to settle down because they cannot be counted when they are moving and changing their positions due to currents in the fluid. During this time draw a diagram once again showing the RBC square. Then draw five groups of 16 square each, showing their relative positions—the four corner groups and one central group for entering your counts.
- **Counting the cells:** Switch over to high magnification (HP lens) and check the distribution of cells. If they are unevenly distributed, i.e. bunched at some places and scanty at others, the chamber has to be washed, dried, and recharged.
- Move the chamber carefully and bring the left upper corner block of 16 smallest squares in the field of view (There are no smallest squares above and to its left).

Rules for Counting (Figs. 17 and 18A and B)

***Note*: As discussed in Experiment 1.3**

An occasional WBC (may be 1 in 600–700 RBCs) may be seen appearing grayish and granular but it is not to be counted with the red cells.

The counting will have been done in 80 smallest squares, i.e. in 5 blocks of 16 squares each.

Observations and Results

RBC in the RBC square under high power (Fig. 19)

Add up the number of cells in each of the 5 blocks of 16 smallest squares. A difference of more than 20 between any 2 blocks indicates uneven distribution.

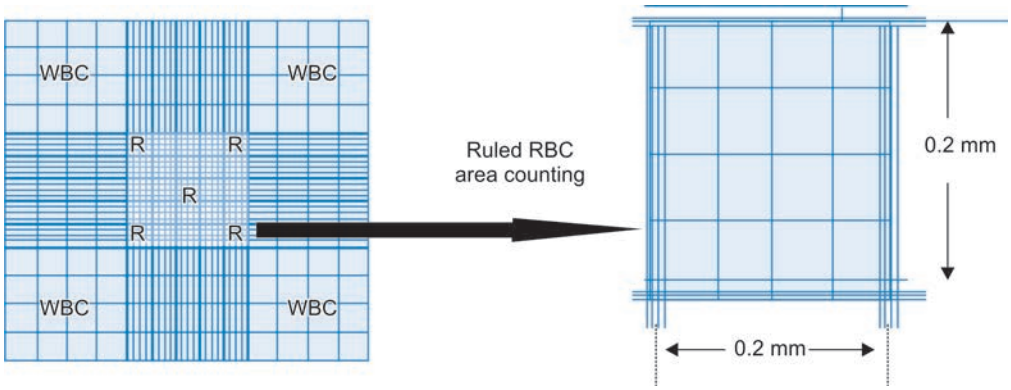
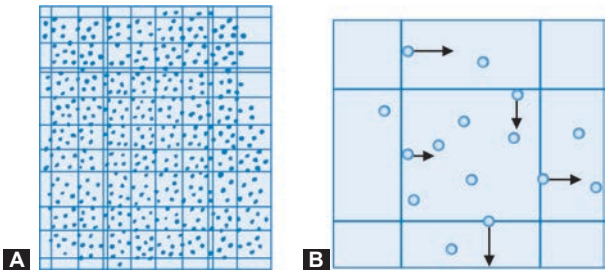


Fig. 17: Rules of counting cells.



Figs. 18A and B: (A) Microscopic view of a charged chamber showing even distribution of red cells. A group of 16 smallest squares is shown in the middle; (B) Rules of counting: Count the cells lying within a square and those lying on or touching its upper horizontal and left vertical line cells lying or touching its lower horizontal and right vertical lines are to be omitted as they will be counted in the adjacent squares. Arrows indicate the squares to which the red cells belong.

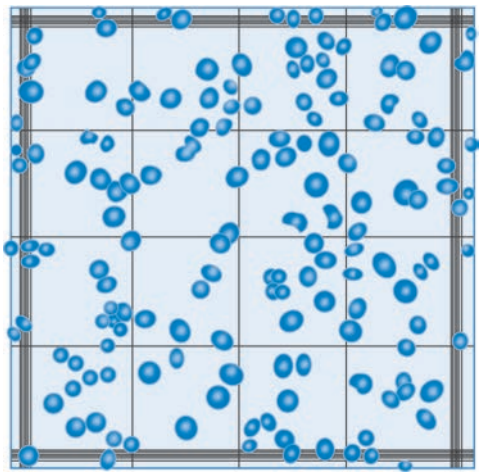
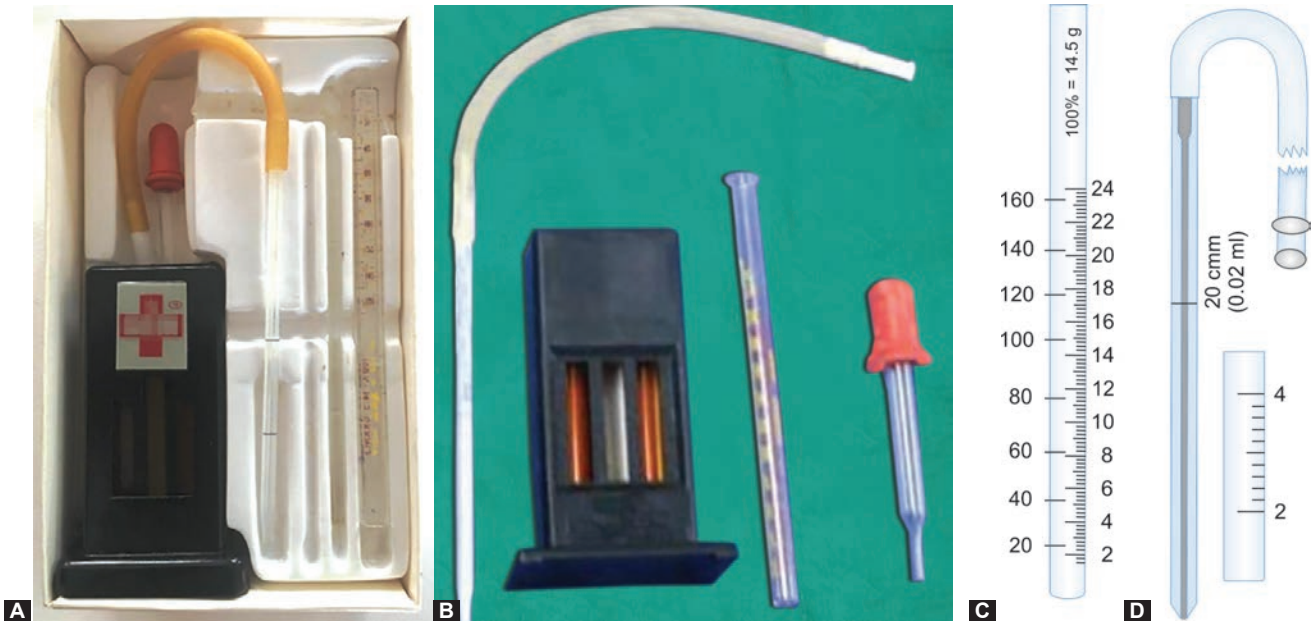


Fig. 19: Red blood cells in the RBC square.



Figs. 23A to D: (A and B) Sahli-Adams hemoglobinometer; (C) Hemoglobin tube. It has graduations in g% on one side, and in percentage on the other. In this tube, 100% is equal to 14.5 g Hb/100 mL blood; (D) Hemoglobin pipette. It has only one marking, indicating 20 mm^3 (0.02 mL, or 20 µL). Each division represents 0.2 g of Hb).

1. **Comparator:** It is a rectangular plastic box with a slot in the middle which accommodates the calibrated Hb tube. Nonfading, standardized, golden brown glass rods are fitted on each side of the slot for matching the color. An opaque white glass (or plastic) is fitted behind the slot to provide uniform illumination during direct visual color matching.
2. **Hemoglobin tube:** The square or round glass tube is calibrated in grams percent (2–24 g%) in yellow color on one side, and in percentage Hb (20–160%) in red color on the other side. There is a brush to clean the tube (Fig. 23C).
3. **Hemoglobin pipette:** It is a glass capillary pipette with only a single calibration mark—0.02 mL (20 mm^3, or 20 µL) (Fig. 23D). There is no bulb in this pipette (as compared to cell pipettes) as no dilution of blood is done.

Note: The calibration mark 20 mm^3 indicates a definite, measured volume and not an arbitrary volume, as is the case with diluting pipettes.

4. **Stirrer:** It is a thin glass rod with a flattened end which is used for stirring and mixing the blood and dilute acid.
5. **Pasteur pipette:** It is an 8–10 inch glass tube drawn to a long thin nozzle, and has a rubber teat. Ordinary glass dropper with a rubber teat also serves the purpose.
6. Distilled water.

B. Decinormal (N/10) hydrochloric acid (0.1 N HCl) solution: Mixing 36 g HCl in distilled water to 1 L gives "Normal" HCl; and diluting it 10 times will give N/10 HCl solution.

C. Materials for skin prick:

- Sterile lancet/needle
- Sterile gauze and cotton swabs
- Methylated spirit/70% alcohol.

Procedure

Review the instructions for obtaining skinprick blood, and filling a pipette as described in Experiments 1.2 and 1.3.

1. Using a dropper, place 8–10 drops of N/10 HCl in the Hb tube, or up to the mark 20% or 3 g, or a little more till the tip of the pipette will submerge, and set it aside.
2. Get a fingerprick under aseptic conditions, wipe away the first 2 drops of blood. When a large drop of free-flowing blood has formed again, draw blood up to the 20 mm^3 mark (0.02 mL). Carefully wipe the blood sticking to the tip of the pipette with a cotton swab, but avoid touching the bore or else blood will be drawn out by capillarity.

Note: If any blood remains sticking to the outside of the pipette, it will be that much extra blood in addition to 20 mm^3.

3. Without any waiting, immerse the tip of the pipette to the bottom of the acid solution and expel the blood gently. Rinse the pipette three to four times by drawing

up and blowing out the clear upper part of the acid solution till all the blood has been washed out from it. Avoid frothing of the mixture. Note the time.

4. Withdraw the pipette from the tube, touching it to the side of the tube, thus ensuring that no mixture is carried out of the tube. Mix the blood with the acid solution with the flat end of the stirrer by rotating and gently moving it up and down.
5. Put the Hb tube back in the comparator and let it stand for 6–8 minutes (or as advised by the manufacturer). During this time, the acid ruptures the red cells, releasing their Hbs into the solution (hemolysis). The acid acts on the Hb and converts it into acid hematin which is deep golden brown in color.
   - The color of acid hematin does not develop fully immediately, but its intensity increases with time, reaching a maximum, after which it starts to decrease. An adequate time usually 6–8 minutes must be allowed before its dilution is started. Too little time and all Hbs may not be converted into acid hematin. And, waiting too long may result in fading of color. In either case, the result will be falsely low.
6. **Diluting and matching the color:** The next step is to dilute the acid hematin solution with distilled water (preferably buffered water, if available) till its color matches the color of the standard tinted glass rods in the comparator (Fig. 24).

Note: Each time you compare the color, lift and hold the glass stirrer against the side of the Hb tube above the solution (rather than taking it out completely) thus allowing it to drain fully back into the tube. (If the stirrer is left in the solution when comparing the color, the solution will appear lighter).

Fig. 24: Comparator.

7. Take the Hb tube out of the comparator and add distilled water drop by drop (or larger amounts depending on the experience), stirring the mixture each time and comparing the color with the standard.
8. Hold the comparator at eye level, away from your face, against bright but diffused light. Read the lower meniscus (lower meniscus is read in colored transparent solutions).

Note: If the stirrer is left in the solution, it will lighten the color (since it is translucent) and thus matching will occur earlier. This will give a false low value. If, however, it is taken out every time the color is matched, it is bound to take away some of the solution out of the tube, thus, again giving a low value.

Note: The tip of the pipette should not be lifted out of the blood drop during pipetting. This is done to prevent air bubbles from entering the pipette.

Sources and Degree of Error

False results with this method may be due to:

1. ***Technical error*:** It may be due to: Not taking exactly 20 mm^3 blood, or not giving enough time for formation of acid hematin, or using an old comparator that has faded glass rods.
2. ***Personal error*:** Generally, it is not difficult to match color but since it is a visual method, color matching may vary from person to person.

For example, you may think that the color is matching, while your work-partner may consider it lighter or darker than the standard. The **Degree of Error** may be as high as 10–15%. However, it can be reduced to about 5% by taking three readings on the same test solution as described here.

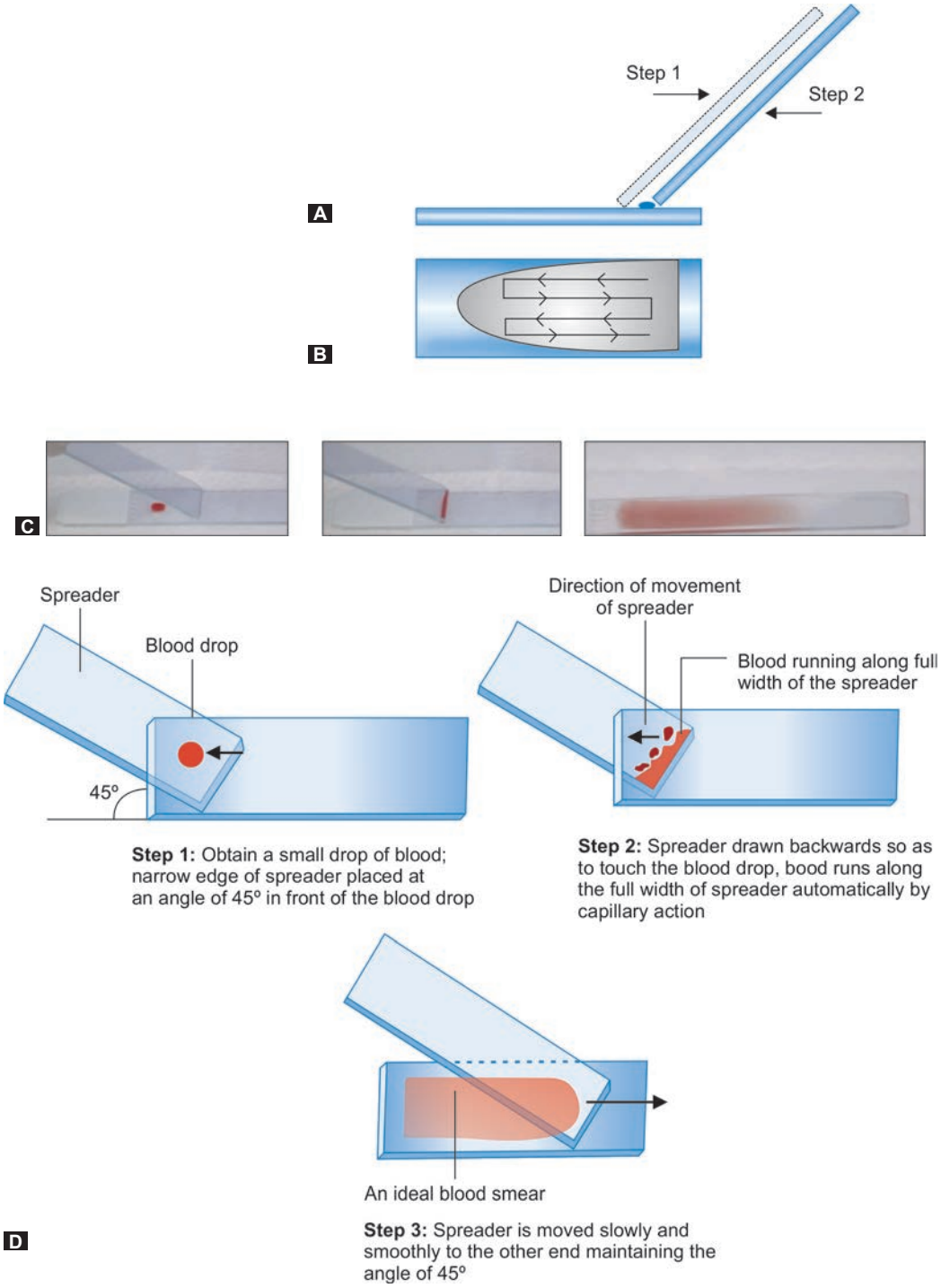
Observations and Results

Compare the color of the solution in the tube with that of the standard and record the observations in your workbook. Take the average of three readings as shown here, and report your result as: Hb = ...g/dL.

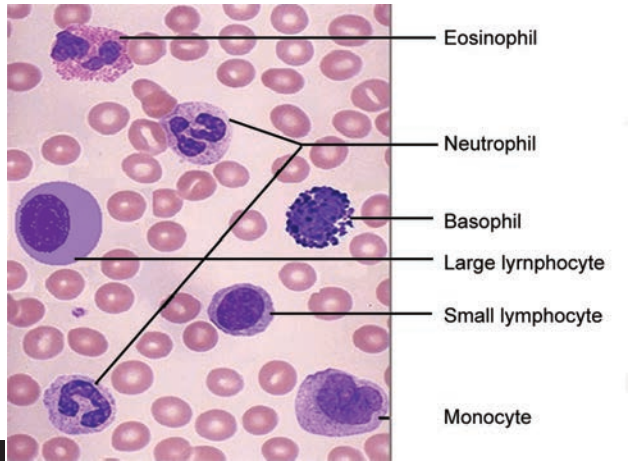
- ***1st reading,*** when the color is slightly darker than the standard: .....g/dL.
- ***2nd reading,*** when, after adding a few drops of distilled water, the color exactly matches the standard: .....g/dL.
- ***3rd reading,*** when, after adding some more drops, the color becomes a little lighter than the standard: .....g/dL.

Oxygen carrying capacity: Knowing your Hb concentration, and that 1.0 g of Hb can carry 1.34 mL of O_2, calculate its oxygen-carrying capacity as .....mL O_2/dL.

- **100% saturation:** When blood is equilibrated with pure (100%) oxygen at a PO_2 of 120 mm Hg, the Hb gets 100% saturated, i.e. it picks up as much O_2 as it possibly can.



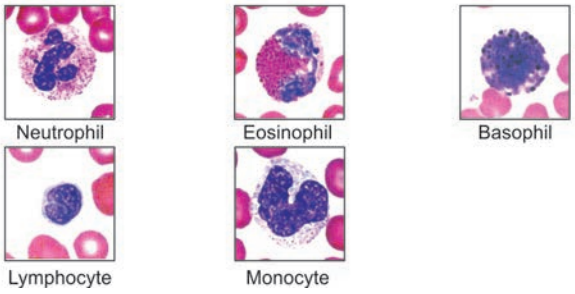
Figs. 25A to D: (A) Method of spreading a blood film. Step 1: The spreader is placed in front of the blood drop and pulled back till it touches the blood. Step 2: Spreader is pushed forward to spread the film; (B) The appearance of a well-prepared film, showing the movement of the objective over it; (C) Steps in preparation of a blood smear; (D) Schematic representation of steps in preparation of a blood smear.



A

Type	Microscopic appearance	Diagram	Diameter (μm)	Percentage of TLC
Neutrophil			10–14	40–70
Eosinophil			1–6	10–15
Basophil			0–1	10–15
Lymphocyte			20–40 (S) 5–10 (L)	7–9 10–15
Monocyte			5–10	12–20

B



C

Figs. 27A to C: (A) Different types of blood cells in a blood film stained with Leishman stain. The size, shape of the nucleus, and staining features of the cytoplasmic granules distinguish them from one another; (B) White blood cells, their microscopic appearance, diagrams, diameter, and their percentage of TLC are shown (400x); (C) White blood cells (1,000x).

Differential Counting of Leukocytes

1. Draw 100 squares in your workbook for recording various WBCs as they are encountered and identified one after another. Enter these cells by using the letters "N" for neutrophils, "M" for monocytes, "LL" for large lymphocytes, "SL" for small lymphocytes, "E" for eosinophils, and "B" for basophils as shown in Figure 28.
   - You can indicate these cells in a column and as you identify a cell, put a short vertical stroke against that cell. In this way, you can place different types of cells in groups of 5, a horizontal stroke representing the 5th cell (e.g. Neutrophils = **III**).
2. Place a drop of cedar wood oil on the right upper corner of the film, a few millimeters away from the head end. Bring the oil-immersion lens into position till it enters the oil drop. Adjust the focus.
   - Do not flood the entire surface of the slide with oil; as you move the slide, the oil will move with the objective lens.
3. Move the slide slowly to the right (the image will move to the left) and as you encounter a leukocyte, identify it, and enter it in the Figure 28. As you approach the end of the smear, move two fields down and scan the film in the opposite direction. As you near the head, again move two fields down and scan the film toward the tail. Traverse the film in this to and fro fashion till you have examined 100 cells (Fig. 29). This "battlement" procedure, as shown in Figures 25A to D, ensures that you do not count a leukocyte more than once.
4. **Recount:** After you have, counted 100 cells, count the leukocytes once more, starting from the lower left corner of the film, and going up in the "battlement" procedure.

N	N	SL	N	L	L	N	N	N	L
LL	E	N	N	N	N	L	M	N	L

Fig. 28: Differential counting of leukocytes.

Differential leukocyte count: When counting has been done, calculate the percentage of each type of cell in your count of 100 white cells. The neutrophils are the prominent cells of the blood and constitute about 50–60% of the WBCs.

2. Clean, dry microscope slides. (A special porcelain tile with 12 depressions is available for this purpose and may be used in place of glass slides).
3. 1% sodium citrate in normal saline (or normal saline alone).
4. **Anti-A serum:** Contains monoclonal anti-A antibodies (against human); these antibodies are also called anti-A or alpha (α) agglutinins. The anti-A serum can also be obtained from a person with blood group B. (see Q/A 6).
5. **Anti-B serum:** Contains monoclonal anti-B antibodies (against human); these antibodies are also called anti-B or beta (β) agglutinins. The anti-B serum can also be obtained from a person with blood group A. (see Q/A 6).
6. **Anti-D (anti-Rh) serum:** Contains monoclonal anti-Rh (D) antibodies (against human). These antibodies are also called anti-D agglutinins.

Note: The antibodies against Rh factor do not occur naturally (see below).

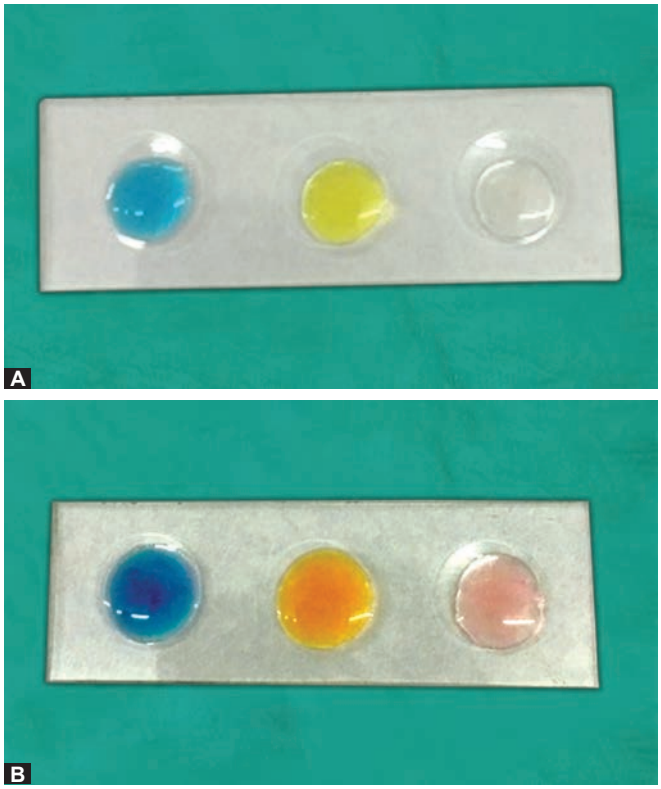
Note: These antisera are available commercially. For a quick identification, the anti-A serum is tinted blue, anti-B serum yellow, while the anti-D serum is colorless (Fig. 34).

Caution: Do not interchange the droppers provided with antisera bottles.

PROCEDURE

1. Using a glass-marking pencil, divide three slides, each into two halves by a line drawn down the middle (the left sides will act as "test sides" and right sides as the "control sides"). Mark the left corner of 1st slide "anti-A", left corner of 2nd slide "anti-B", and the left corner of 3rd slide "anti-D" (Figs. 35A and B). Mark the right corners of these 3 slides "C" (for control).
2. Mark another slide (4th) "S" (for only red cell suspension in saline, i.e. no antiserum will be added on this slide).
3. Place 8–10 drops of saline in the center of slide "S".
4. **Preparation of red cell suspension:** A suspension of red cells in saline should preferably be prepared and used instead of adding blood drops directly from the fingerpick to the antisera for the following reasons (Fig. 36):
   - Dilution of blood permits easy detection of agglutination and hemolysis, if present. (Red cells in undiluted blood tend to form large rouleaux and masses. These may be difficult to disperse and may be mistaken for agglutination).
   - Plasma factors likely to interfere with agglutination are eliminated.
5. Get a fingerprick under aseptic conditions, and add two drops of blood to the saline on the slide marked "S". Mix the saline and blood with a clean glass dropper to get a suspension of red cells. You may use a toothpick for this purpose.
   - A better method is to place 2 mL of saline in a small (5 mL) test tube. Then get a finger pricked and allow

Fig. 34: Commercially available Anti-A, anti-B, and anti-D serum.



Figs. 35A and B: Marking of serum anti-A, anti-B, and anti-C.

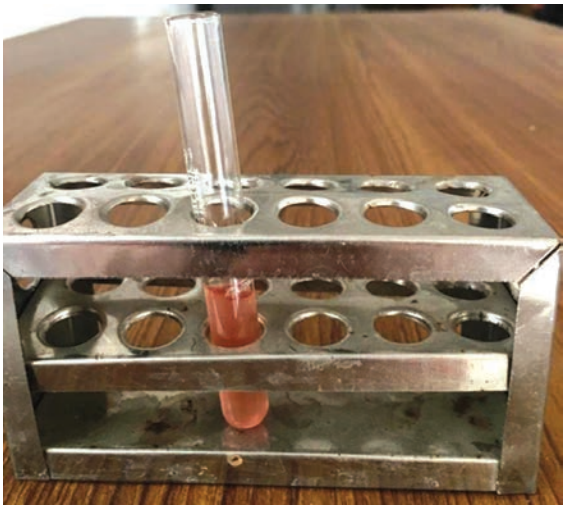


Fig. 36: Preparation of red cell suspension.

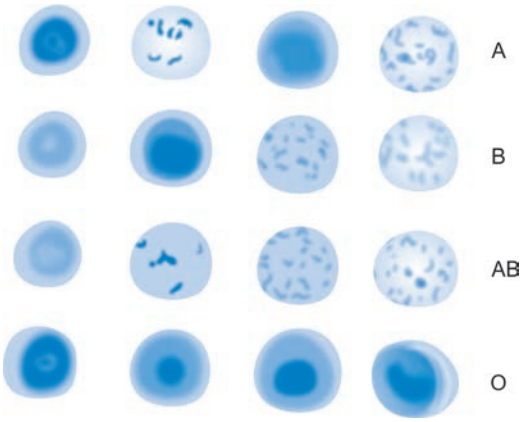


Fig. 37: Determination of blood groups (types) showing agglutination (hemolysis and clumping of red cells) and no agglutination (cells remain uniformly distributed). The reaction between anti-D serum and red cells is not shown.

a blood drop to form. Now place the pricked fingertip on top of the test tube and invert it. Mix the blood and saline by inverting the tube two or three times. A suspension of red cells is now ready.

- Washed red cell suspension gives the best results. The red cells are "washed" in saline by centrifuging the diluted blood, removing the supernatant, and adding fresh saline to get a suspension of "washed" red cells.

6. **Determination of blood group:** Put one drop of anti-A serum on the left half ("test side") of 1st slide (marked anti-A), one drop of anti-B serum on the left half of 2nd slide (marked anti-B), and one drop of anti-D serum on the left half of 3rd slide (marked anti-D).
7. Put one drop each of normal saline on the "control" sides (right halves) of the three slides (i.e. areas marked "C").
8. Add a drop each of red cell suspension (from the slide "S", or from the test tube of red cell suspension) on anti-A, one drop on anti-B and one drop on anti-D sera, and one drop each on the normal saline taken on the "control" sides of the three slides.

   In this way, the red cells–saline mixture on the "control" sides of each slide will act as a control to confirm agglutination or no agglutination on the corresponding test side (Fig. 37).
9. Mix the antisera and red cells, and saline and red cells on each slide by gently tilting it first one way and then the other a few times. Take utmost care that the "test" mixtures and "control" mixtures do not flow into each other and get mixed up. The red cells and sera can also be mixed by gently blowing on them. You may use three separate toothpicks to transfer red cell suspension to the three antisera, and for mixing them, and three toothpicks to transfer red cells to saline drops taken on the "control" sides of the three slides.

   "+": **Agglutination** RBC is massed together in clumps and loses their outline

   "–": **No agglutination** RBCs remain separate and evenly distributed.

Important: Do this one at a time and then discard each toothpick.

10. Wait for 8–10 minutes then inspect the three antisera–red cell mixtures ("test" mixtures) and "control" mixtures, first with the naked eye to see whether agglutination (clumping and hemolysis of red cells) has taken place or not. Then confirm under low magnification microscope, comparing each "test mixture" with its corresponding "control mixture".

OBSERVATIONS AND RESULTS

It is essential that you should be able to distinguish between ***"agglutination"*** and ***"no agglutination".*** The features of each are as follows:

Agglutination

- If agglutination occurs, it is usually visible to the naked eye. The hemolyzed red cells appear as isolated (separate), dark-red masses (clumps) of different sizes and shapes.
- There is brick-red coloring of the serum by the hemoglobin (Hb) released from ruptured red cells.
- Tilting or rocking the slide a few times, or blowing on it does not break or disperse the clumps.
- Under lichen planus (LP) objective, the clumps are visible as dark masses and the outline of the red cells cannot be seen.

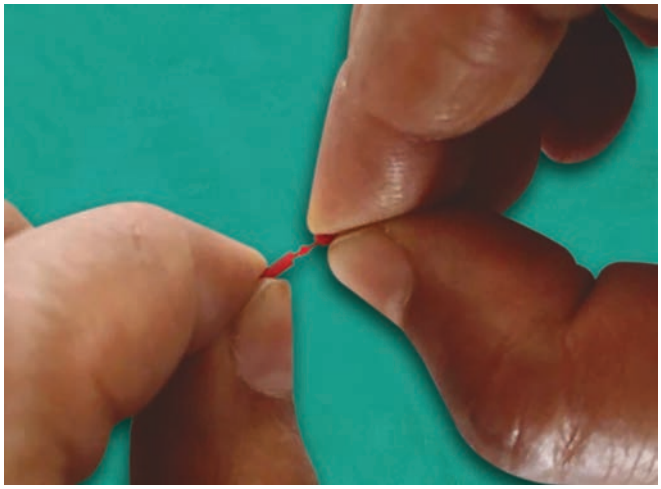


Fig. 40: Formation of fibrin thread.

Lee and White Test Tube Method

Single Test Tube Method

This method needs more arrangements than capillary tube method but is more sensitive and reliable method for the determination of CT.

1. Draw 5 mL venous blood by a clean, non-traumatic venepuncture. Note the time when blood starts to enter the syringe. This is the zero time. Transfer the blood to a chemically clean and dry test tube.
2. Holding the test tube in a water bath at 37°C, take it out at 30 second intervals and tilt it. The end point is when the tube can be tilted without spilling the blood.

Normal CT with this method is 5–10 minutes.

Multiple Test Tube Method

The CT can be determined more accurately by using 3 test tubes rather than 1 only.

1. Rinse 3 test tubes of 8 mm diameter with normal saline, drain them and place them in a metal rack kept in water at 37°C. Transfer 1.5 mL blood into each test tube.
2. Take out the first tube after 1 minute, tilt it to 45° and return it to the rack. Repeat every 30 seconds until clotting occurs, i.e. where the test tube can be tilted without spilling the blood. Note the time.
3. Repeat the tilting on the second test tube and note the time when clotting occurs (this happens a few seconds later because tilting the tube hastens clotting). The third tube acts as a control and a check on the end point in the second test tube.

Note: If a siliconized test tube is used at the same time, a delayed CT (40–70 minutes) can be shown.

Normal CT with this method is 5–10 minutes.

The CT depends on the condition of the glass itself, and even on the size of the test tube. Therefore, a high degree of standardization is needed.

Comments: This method is more reliable than the capillary blood CT method, because there is no admixture of blood with tissue fluid which contains tissue thromboplastin (extrinsic system). Thus, this method tests only the intrinsic system of blood clotting. However, this method is nonspecific because the CT can theoretically increase due to deficiency of any of the factors in the intrinsic system. But, in actual practice, a prolonged CT nearly always means hemophilia in which the CT may exceed 1 hour in severe cases.

Another method (drop method): This method is less accurate than the above method. Place a large drop of blood from a skin puncture on a clean and dry glass slide. Draw a pin through the drop every 30 seconds, and note the time when fibrin threads adhere to the pin and move with it out of the blood drop. The time elapse between placing the blood drop on the slide and the formation of fibrin threads is the CT.

Normal CT is 2–4 minutes.

- In the original Duke's drop method for CT, two drops of 4–5 mm diameter are placed on a glass slide. The slide is tilted at 30 second intervals. The end point is absence of change in the previous shape when the slide is held vertical.

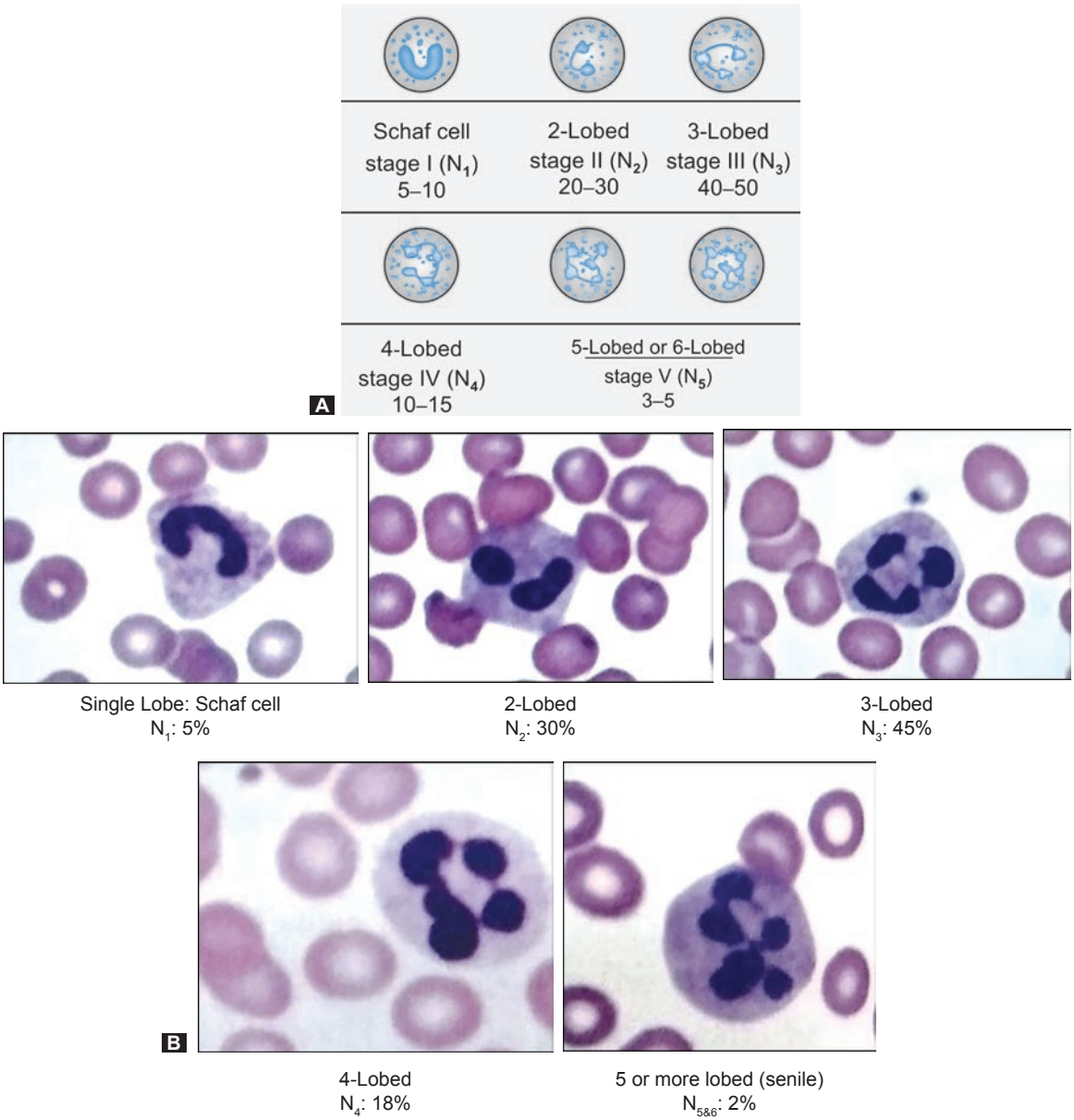
Physioclinical Significance

- The clotting of blood with this method involves both the intrinsic and extrinsic systems of clotting. There is injury to the blood (coming in contact with glass, intrinsic pathway), and the injury to the tissues (extrinsic pathway).
- The CT is prolonged in hemophilia and other clotting disorders, because thrombin cannot normally be generated. Yet, the BT which reflects platelet plug formation and vasoconstriction, independently of clot formation is normal.

CLOT RETRACTION TIME

Transfer the larger test tube containing clotted blood to an incubator at 30°C. Normally, the clot starts to shrink (retract) in about 30 minutes (leaving behind straw-colored serum), becomes half its size in 2–3 hours, and complete in 24 hours. Note if there is any digestion of the clot or discoloration of serum.

Comments: Clot retraction (tightening or consolidation) depends on the release of many factors from the platelets, and so the CRT depends on the platelet count. The fibrin-stabilizing factor causes more and more cross-linking bonds between nearby fibrin fibers. Their spicules also release contractile proteins—actin, myosin, and thrombosthenin. The contraction (retraction) of the clot is activated by thrombin, and calcium ions stored in the endoplasmic reticulum, Golgi apparatus, and mitochondria.



Figs. 43A and B: Cooke-Arneth count. The percent distribution of neutrophils in the circulation based on the number of their lobes is shown.

Table 13: Percentages of each stage with description.

Stage		*Description*	*Percentage*
Stage I	(N1)	Nucleus is C- or U-shaped, the two limbs being connected by a thick band of chromatin	5–10
Stage II	(N2)	The two lobes are connected by a narrow band of chromatin	20–30
Stage III	(N3)	Three lobes connected by chromatin filaments. (Actively motile and functionally most effective)	40–50
Stage IV	(N4)	Four lobes connected by chromatin filaments	10–15
Stage V	(N5, N6)	❑ Five lobes or more (N6 = >6 lobes) ❑ Outline may be irregular ❑ Cytoplasmic granules poorly stained ❑ Functionally least motile and effective	3–5

during World War II for assessing battle casualties requiring blood transfusions.

Use of specific gravity test in blood banks: A working solution (specific gravity = 1.053) is prepared by adding 48 mL of distilled water to 52 mL of stock solution of $CuSO_4$ (described above). A drop of blood is allowed to fall into it from a height of 1 cm. If the drop sinks, the Hb in that sample is over 12.5 g%, and the potential donor is bled for blood donation. If the drop floats for more than 15 seconds, the Hb level is less than 12.5 g% and the donor is considered unsuitable for blood donation.

Q.5. What are the physiological and pathological conditions in which the specific gravity of blood is increased and decreased?

The specific gravity of blood is affected by:

1. Red cell count
2. Hemoglobin concentration
3. Plasma (or serum) protein concentration
4. Water content of blood.

Physiological conditions: The specific gravity of blood is high in newborns, and at high altitude due to polycythemia. It is decreased during pregnancy (due to hemodilution) and after excess water intake.

Pathological conditions: The specific gravity is **increased** in polycythemia due to any disease (e.g. congenital heart disease, cardiac failure), polycythemia vera, severe dehydration (diarrhea, vomiting) and hemoconcentration (loss of plasma due to burns).

The specific gravity **decreases** in anemias, hemodilution (excessive secretion or prolonged treatment with glucocorticoids), and kidney disease (loss of albumin and water retention).

Q.6. Name the various fractions of plasma proteins. What are their functions?

The normal plasma protein concentration is 6–8 g%. Some of the proteins present in plasma are also found elsewhere in the body. Because of the large size of their molecules, plasma proteins tend to remain within the bloodstream. The various fractions of plasma proteins are:

- Albumin = 4.0–5.5 g%
- Fibrinogen = 0.3–0.5 g%
- Globulins = 1.5–3.0 g%
- Prothrombin = 30–40 mg%.

Using filter paper electrophoresis, the patterns of serum proteins are:

- Albumin = 57%
- Alpha-1 globulin = 4.7%
- Alpha-2 globulin = 8.45%
- Beta-1 and beta-2 globulins = 11.33%
- Gamma globulins = 18.52%.

With the exception of gamma globulins which are synthesized in the plasma cells in lymphoid tissue, all the other proteins (albumin, fibrinogen, and some globulins) are synthesized in the liver.

Functions of plasma proteins: The proteins perform the following functions:

- **Osmotic pressure:** The osmotic pressure of plasma proteins called oncotic pressure is involved in tissue fluid exchanges (see Experiment 1.16).
- **Protein metabolic pool:** Though these proteins form part of the protein metabolic pool, they are not ordinarily used for providing energy.
- **Buffering function:** They exert about 15% of the buffering action of the blood (all proteins, including Hb are buffers). They function to convert strong acids or bases into weak acids or bases. Strong acid or bases ionize easily and can contribute many H^+ or OH^- ions, which can affect the pH to a great extent. Weak acids or bases do not ionize as much and thus contribute fewer H^+ or OH^- ions.
- **Viscosity of blood:** The plasma proteins contribute to the viscosity of blood and so affect the blood pressure (see Experiment 1.18).
- **Coagulation of blood:** Fibrinogen, prothrombin, and many clotting factors form part of the clotting mechanism.
- **Role as carriers:** They act as carriers in the transport of many substances, such as hormones, metals, calcium, ions, amino acids, bilirubin, vitamin B_{12}, drugs, etc. Binding these substances prevents their rapid clearance from the body by the kidneys. Major lipids do not circulate in the free form but in combination with plasma proteins.

Q.7. What are lipoproteins? What are their functions and clinical significance?

The major lipids in plasma (cholesterol, triglycerides—TGs) do not circulate in the blood in free form. Since they are nonpolar and thus hydrophobic molecules, they must first be made water-soluble in order to be transported in watery plasma. This is achieved by binding them to proteins formed in liver. Free fatty acids are bound to albumin, while others are transported in the form of round particles called lipoproteins that are made up of hundreds of molecules.

In a lipoprotein particle, there is an outer coat of proteins, amphipathic phospholipids, and cholesterol that surrounds the inner core of hydrophobic TGs and cholesterol ester molecules. The proteins in the outer shell are called **apoproteins** (or simply **APO).** The APOs are designated by letters (A, B, C, D, and E) plus a number. **APO B** has two forms—a low mw form, APO B-48 that transports ingested lipids, and a high mw form, APO B-100, which transports endogenous lipids. Their levels have clinical significance in atherosclerosis. APO E concentration greatly

increases in nerve injuries and is concerned with the repair process.

Functions of lipoproteins: There are several types of lipoproteins each having a different function. However, all are mainly transport vehicles functioning as a sort of pick-up and delivery service. They are classified on the basis of density that varies with the ratio of lipids (they have low density) to proteins (they have a high density). Thus, the lipoproteins are grouped in, from largest and lightest to smallest and heaviest, the following groups—**very low, low, intermediate, and high-density lipoproteins.**

Clinical significance: Their clinical significance lies in relation to coronary artery disease, and atherosclerosis (a form of arteriosclerosis).

The **low-density lipoproteins (LDLs)** carry about 50% of cholesterol. They transport cholesterol from the liver to body cells for use in repair of cell membranes and production of steroid hormones and bile salts. However, excess of LDLs promotes atherosclerosis, so that their cholesterol is called "bad cholesterol".

The **high-density lipoproteins (HDLs)** contain about 20% cholesterol. They remove and carry excess of cholesterol from body cells to liver for elimination. Since, they lower blood cholesterol level, their cholesterol is called "good cholesterol".

The desirable levels of various lipoproteins are:
Triglycerides:

- Males = Up to 165 mg/dL
- Females = 40–140 mg/dL
- Very low-density lipoprotein (VLDL) cholesterol = Less than 40 mg/dL
- LDL cholesterol = Less than 130 mg/dL
- Intermediate-density lipoprotein (IDL) cholesterol = 5–50 mg/dL
- HDL cholesterol = More than 40 mg/dL
- **Total cholesterol = Less than 200 mg/dL.**

1.18: DETERMINATION OF VISCOSITY OF BLOOD

Viscosimeter (Viscometer)

The viscosimeter is a U-shaped glass tube, one limb of which is wider with a bulb near its lower end. The other limb has a bulb near its upper end and a narrow capillary bore below it. There are two markings, 1 and 2, above and below the bulb in this limb.

PROCEDURE

The limb with the wide tube is filled with anticoagulated blood; the blood is then sucked up into the narrow limb to above the mark 1. The time taken by the blood to fall from mark 1 to mark 2 is noted. The procedure is then repeated with water and is compared with that of blood. The apparatus must be kept vertical throughout the experiment.
Normal viscosity of blood = About 3–4 times that of water, i.e. its relative viscosity is 3–4.

VISCOSITY OF BLOOD

Viscosity which represents the mutual attraction between the particles of a fluid is the internal friction or "lack of slipperiness" between the adjacent layers especially between the outermost layer of the flowing blood and the walls. The shape of the molecules rather than their size determines the viscosity.

The viscosity of blood depends mostly on the ratio of red cells to plasma (fluid) volume and to a lesser extent on the plasma protein concentration. The viscosity of blood in vivo especially in the microvessels is about 1.2 (water = 0.695; see here) because of axial streaming of red cells (Fahraeus–Lindqvist effect).

Effect of Temperature on Viscosity

Temperature has an important effect on viscosity. Water has a viscosity of 1 cP [centipoise (after Poiseuille)—the unit of viscosity] at 20.3°C and about 1.8 cP at 0°C. The viscosity of plasma and blood is even more sensitive to changes in temperature. Thus, the temperature of skin and subcutaneous tissues exerts an important effect on the viscosity of blood.

Significance of Viscosity of Blood

The **viscosity** of blood is one of the three factors on which the resistance (i.e. the opposition) to flow of blood in the blood vessels depends, the other two being—**average radius of the blood vessels** and the **total blood vessel length.** Since resistance, in fact, peripheral resistance to blood flow is directly proportional to the viscosity of blood, any factor that affects viscosity will increase or decrease the blood pressure. In this way, variations in viscosity of blood influence the load to which the heart is subjected during contraction.

Increased viscosity is seen in polycythemia due to any cause, congestive heart failure, diabetes mellitus, multiple myeloma, icterus, profuse sweating when water intake is limited, severe vomiting and diarrhea, and leukemias.

Decreased viscosity is seen in anemias, edematous states, and sometimes in malaria.