20 ESTIMATION OF HAEMOGLOBIN, TOTAL RBC AND WBC IN HUMAN BLOOD

20.1 INTRODUCTION

Blood when centrifuged, separates into two distinct portions — fluid and formed elements. The clear, straw-coloured fluid is called plasma. Plasma accounts for more than 50% of blood volume. The formed elements consist of red blood corpuscles, *erythrocytes;* white blood cells, *leucocytes;* and platelets or *thrombocytes.*

In human males, there are 5 million red blood cells per cubic millimeter, and in females, 4.5 to 5 million. Erythrocytes are packed with haemoglobin molecules to transport oxygen to the tissues. In a single red blood corpuscle nearly 280 million (2.8×10^8) molecules of haemoglobin are packaged. Haemoglobin concentrations in humans range from 14 to 16% in males and 12 to 14% in females. Concentrations less than this amount are indicative of anaemia. Anaemia essentially results due to iron deficiency in the body. You may recall that iron or heme is the prosthetic group of the haemoglobin molecule.

White blood cells, leucocytes, are cells with various shaped nuclei. Leucocytes average 5-9 thousand per cubic millimeter in normal blood. The count in children is higher. White blood cells are one of the body's major defence mechanisms against bacteria, virus, and other foreign substances that have entered the blood stream. During infections, the number of WBCs increases, a parameter used to diagnose infections.

In this lab exercise, you will estimate level of haemoglobin in blood using a haemometer and determine red and white blood cells count with the help of a haemocytometer.

Objectives

After completing this exercise you should be able to

- determine haemoglobin levels in a blood sample and
- estimate the total count of red blood corpuscles and white blood corpuscles in blood.

A Estimation of Haemoglobin

20.2 MATERIALS REQUIRED

Haemometer (Haldene's haemoglobinometer),

0.1 N HCl (1.2 ml of concentrated HCl made upto 100 ml. with distilled water),

distilled water

sterile needle (haemolets)

alcohol

cotton

Haemometer

The haemometer (Fig. 20.1) consists of two sealed lateral comparison tubes containing a suspension of acid haematin. These are held in a black frame against a white ground glass. Besides, a graduated test-tube of the same diameter is also provided which can fit in the haemometer in between the two side tubes. The graduations on the experimental tube refer to percentage of haemoglobin in blood that is haemoglobin in g/100 ml. of blood. The other accessories provided are a micropipette of 20 mm^3, a small glass rod, a small bottle brush, a dropper and a small bottle containing 0.1 N acid solution.

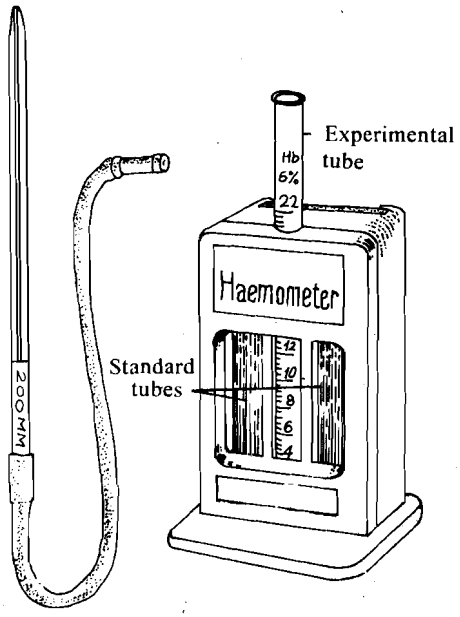


Fig. 20.1 : Haemometer.

20.3 PROCEDURE

In this method an acid haematin solution of blood is made in the graduated tube and the colour of this solution is compared with the acid haematin solution provided in the standard tubes.

1) Rinse the graduated tube with distilled water and then 90% alcohol. Dry the tube well before use.
2) With the help of a dropper add 0.1 N HCl solution to the graduated tube upto 2 g mark.
3) Cleanse your third or fourth finger with a piece of cotton soaked with alcohol and obtain a drop of fresh blood using a sterile needle. You may use a sewing needle or a ball pin for this purpose but make sure that they are well sterilized. For sterilizing, show the needle or pin or the lancet on a flame and wipe it well with alcohol. Under no circumstance, the pin or needle or lancet used by one student, be used by another student.
4) Wipe off the first drop of blood and then suck the micropipette by fresh blood upto the mark of 20 mm^3.
5) Wipe off the small amount of blood adhering to the outside of micropipette by sterilized cotton.
6) Transfer the blood from the micropipette to the graduated tube containing HCl solution. Introduce the pipette carefully into the tube and allow the tip of the pipette to reach the bottom of the tube into HCl solution. Blow into the opening of the rubber tubing to transfer the blood.
7) After the blood has been expelled into the tube rinse the pipette in distilled water and transfer the contents into the graduated tube. You may repeat this step twice or thrice so that no blood is sticking to the sides of the pipette and all of it is transfered to the graduated tube.
8) Stir the acid haematin solution thoroughly with the help of a glass rod and then allow it to stand for at least ten minutes.
9) In the next step dilute the acid haematin solution gradually by adding distilled water drop by drop.
10) After the addition of each drop of distilled water stir the solution with the glass rod and match the colour with that of the solution in the standard sealed tubes. You will

continue to do this step until the colour of acid haematin solution just fades away as compared to that of the standard comparison tubes. Note the reading on the graduated tube after the addition of each drop of water.

11) The reading on the graduated tube just before the colour fades away is taken as the correct and final reading. This reading is the amount of haemoglobin in grams per 100 ml of blood.

Haematin: When the blood is treated with a dilute acid, the heme in the haemoglobin is dissociated from the globin. Heme has iron in the ferrous state and in the reaction with acid is oxidised to haematic which has the iron in the ferric state. Addition of dilute acid (decinormal solution of HCl) to haemoglobin produces acid haematin which is dark brown in colour.

20.4 RESULTS

Tabulate your readings as shown below

No. of drops of water added	Haemoglobin concentration g/100 ml.
1	11.5
2	11.6
3	11.7
4	11.8
5	11.9
6	The colour of haematin fades away
Final reading	11.9 grams/100 ml.

Results: Haemoglobin content = 11.9 grams/100 ml.

20.5 PRECAUTIONS

1) Give a light prick to the finger tip while taking blood.
2) The finger and the prick (needle) should be disinfected.
3) Do not use uncleaned tubes, pipettes etc.
4) Fill the micropipette accurately.
5) Avoid inclusion of blood sticking at the outer surface of the mouth of the pipette.
6) Experiment should be performed quickly so that fresh blood does not clot before transfer to 0.1 N HCl.

B Estimation of Number of Erythrocytes in Blood

In this exercise you will determine the number of red blood corpuscles present in per cubics mm of blood.

20.6 MATERIALS REQUIRED

Haemocytometer

Hayem's solution

Microscope

Haemocytometer

The apparatus used for the enumeration of blood cells is called haemocytometer (Fig. 20.2). Examine and become familiar with the equipment which consists of a counting slide, a coverslip and pipettes. The counting chamber has a stage on which are

ruled two sets of fine lines at right angles to each other. The whole ruled area occupies 1 sq. mm and consists of 25 large (0.04 sq. mm) and 400 small squares (0.0025 sq. mm). On each side of the counting chamber is a ridge of glass 0.1 mm high. When a coverslip is placed on the two ridges covering the counting chamber a cubic space is enclosed. Each of the smallest cuboid has a volume of 0.00035 c. mm. The volume of the entire ruled counting chamber is 0.1 c mm.

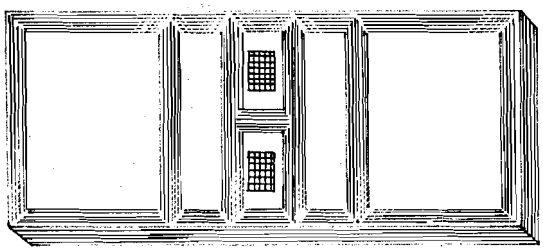


Fig. 20.2 : A haemocytometer.

There are two pipettes provided along with the counting chamber for specific purpose of accurately diluting the blood sample. One pipette with a red bead in the bulb of the pipette is used for drawing blood for the counting of RBCs. The other pipette with a white bead in it is used for drawing the blood for the counting of WBCs.

20.7 PROCEDURE

Clean and dry the haemocytometer and place the cover slip on it.

1) Sterilize your finger tips and the needle, with alcohol and obtain a large drop of blood as instructed in the previous experiment. Using the pipette suck the blood accurately upto 0.5 mark.

Hayem's solution has the following composition

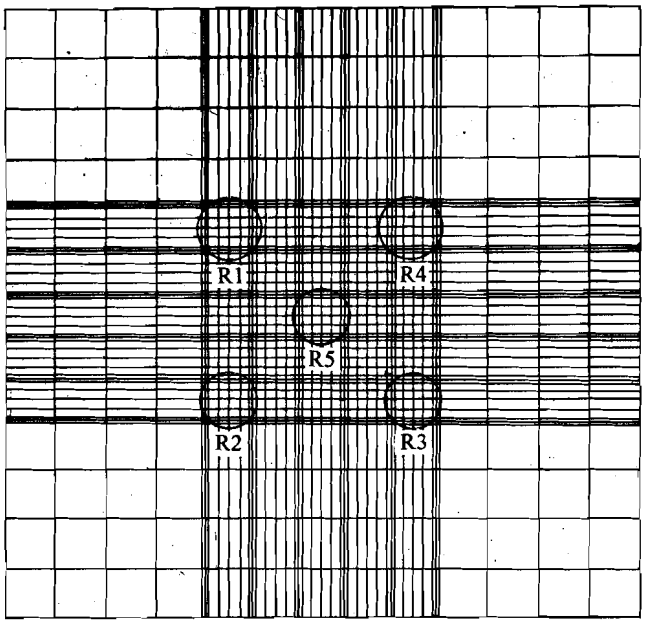
i) Mercuric chloride ($HgCl_2$) = 0.5 g

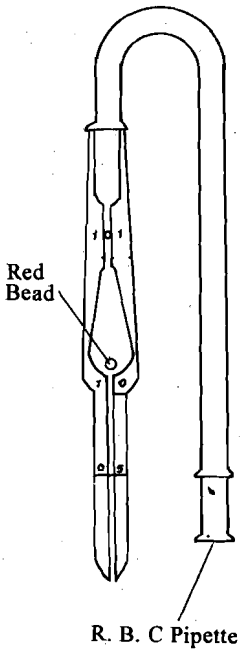
ii) Sodium chloride (NaCl) = 1.0 g

iii) Sodium sulphate (Na_2SO_4) = 5.0 g

iv) Distilled water (H_2O) = 200 ml

2) Now draw carefully into this pipette HAYEM'S solution upto 101 mark. Hold the pipette horizontally and rotate many times so that blood thoroughly gets mixed with **Hayem's** fluid. The red bead in the micropipette helps in mixing of blood fluid. Now the blood is dilutated 200 times.





R. B. C Pipette

Fig. 20.3 : RBC pipette and small squares in the counting chamber. R denotes small squares for red corpuscles count.

3) Dicard the first 3 or 4 drops of the diluted blood from the pipette by releasing your hand from the tube. Now apply the tip of the pipette between the coverslip and the platform and allow a few drops of blood mixture to flow in the narrow space between the coverslip and the counting chamber. Blood mixture remains filled up between the coverslip and the counting chambers because of capillary action. Make sure that air is not introduced into the counting chamber. Do not pour excess of blood mixture to avoid blood entering into H-shaped groove.

4) When the counting chambers are properly flooded the slide may be kept aside for a few minutes so that the RBCs settle down to the bottom of the counting chamber.

5) Now place the slide gently and carefully under the microscope. Focus the red corpuscles under the high power of microscope for the purpose of counting (Fig. 20.3).

6) Count the RBC in 80 small squares or 5 large squares randomly. The RBCs lying on the middle of the line of square to your side or to the right are also to be counted in the total, while those lying on the upper and left sides of the line of square are not to be counted.

Record your counts in the Table provided below.

20.8 RESULTS

No. of squares	No. of RBCs
1	83
2	
3	
4	
5	

Total RBCs =

Number of RBCs counted in 80 small squares × 200 × 50.

[where 200 is the dilution factor. The volume of fluid in 80 small squares or 5 large squares = 1/50 c mm. Therefore to calculate the numbers of RBCs in one ml. of blood, you need to multiply it by 50.]

C Estimation of White Blood Cells in Blood

20.9 MATERIALS REQUIRED

Haemocytometer

Thoma's fluid

water

distilled water.

20.10 PROCEDURE

The procedure of counting of WBCs is the same as that of the RBCs. But use the WBC pipette and Thoma's fluid as the diluting fluid. The WBCs are counted in the four corners of 1 square millimeter in the central ruled area on both the sides of the counting chambers of the haemocytometer (Fig. 20.4). The WBCs are recognized by the refractile appearance and by the slight violet colour. The cells touching the boundary lines are not counted.

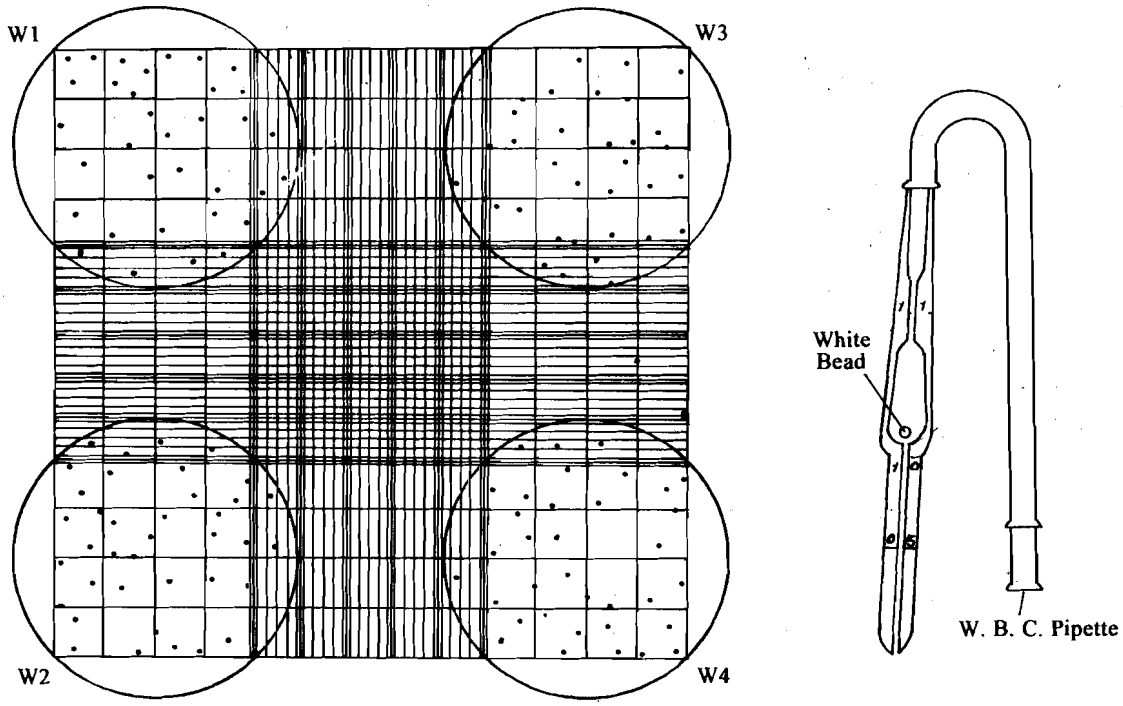


Fig. 20.4 : WBC pipette. Big squares in the counting chamber. W denotes big squares for white blood cell count.

Record your results in the table below.

20.11 RESULTS

No. of Squares	No. of WBC
1	
2	
3	
4	
5	

$$\text{Number of WBCs per cubic mm} = \frac{\text{Number of cells counted } 20 \times 10}{\text{Number of 1 sq. mm counted}}$$

Since the dilution is 20 times and the cubic capacity of the area counted is 1/10 cubic millimeter, the total volume is 1/200 cubic millimeter. Say for example, the number of WBCs counted 25. In other words number of WBCs in 1/200 cubic millimeter = 25. Therefore, the number of WBCs in 1 cubic millimeter = 25 × 200 = 5000 WBCs. Normally healthy man has 4000 to 6000 WBCs per cubic millimeter of blood.