JHARKHAND Rai University
RANCHI

Practical Laboratory Manual

HAEMATOLOGY – I

B.Sc. MLT (IInd Semester)

INDEX

EXPERIMENT- 1

Aim: To study and investigate the concentration of hemoglobin in the blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin or double oxalate anticoagulants are used.

Method: Sahlis Acid Haematin Method

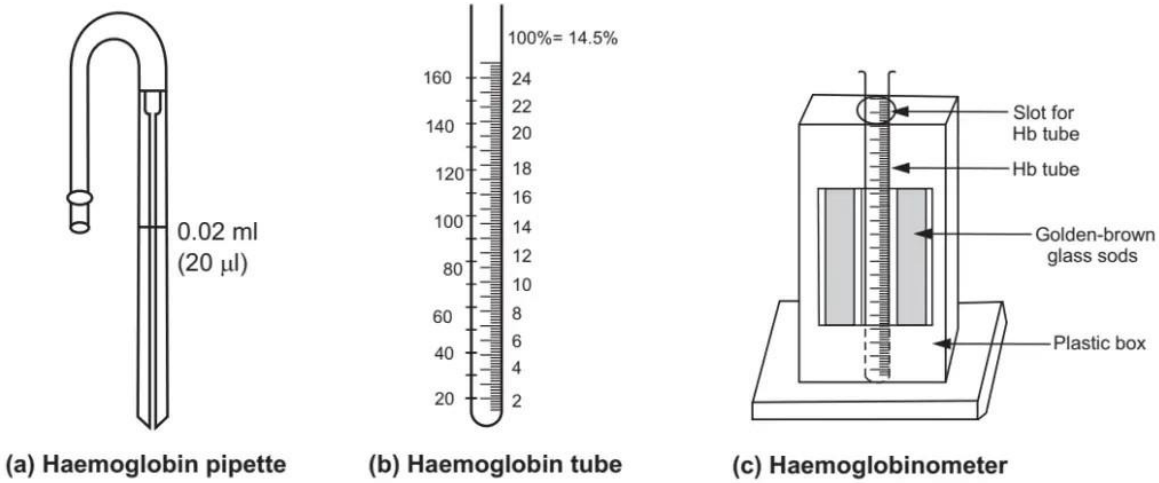
Principal: Hemoglobin (Hb) is converted to acid haematin by the action of Hydrochloric Acid (HCl). The acid haematin solution is further diluted until its colour matches exactly to that of permanent standard comparable tube. The Hb is read directly from the graduated tube.

Requirement:

1. Sahlis tube (Haemoglobin tube)
2. Sahlis pipette (Haemoglobin pipette)
3. N/10 HCl Solution
4. Stirring glass rod and dropper
5. Standard comparable tube (Haemoglobinometer)

Procedure:

1. Fill the Sahlis tube up to the lowest mark with 0.1 normal (N/10) HCl solution.
2. Add 0.02 ml (20 µ) of blood with the help of Sahlis pipette.
3. Make sure that there is no air bubble inside the pipette.
4. Mix the blood with HCl, which is already placed in the pipette.
5. Take care that there is no blood left to the sides wall of the pipette.
6. Rinse the pipette twice in the blood solution.
7. Allow it to react for about 10 min. till the solution become dark brown in colour.
8. Acid haematin is produced after the combination of Hb and acid.
9. Dilute the solution by adding drop by drop of distill water, until perfect match is obtained with standard comparable tube.
10. Read the Hb concentration directly from the level of diluted solution.
11. The reading may be in percentage (%) or grm/lit.



(a) Haemoglobin pipette (b) Haemoglobin tube (c) Haemoglobinometer

Normal Values:

1. **Male (Adult):** 13.5 to 18 gm
2. **Female (Adult):** 11.5 to 16.5 gm
3. **Infants:** 13.6 to 19.6 gm
4. **Children:** 11 to 33 gm (1 year) and 11.5 to 14.8 gm (10 – 12 years)

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 2

Aim: To study and investigate the concentration of hemoglobin in the blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin or double oxalate anticoagulants are used.

Method: Colourimetric Method

It is also known as cyanmethemoglobin method.

Principal: A small quantity of blood is taken and allowed to react with potassium cyanide and potassium ferricyanide (Drabkin's solution). The chemical reaction gives product of stable colour. This product is called as cyanmethemoglobin. The intensity of colour is directly proportional to Hb concentration.

Requirement:

1. Colourimeter with green filter (540 nm)
2. Sahlis pipette (Haemoglobin pipette)
3. Test tube
4. Colourimetric tube

Reagents:

1. Drabkin's solution
2. Cyanmethemoglobin – standard solution

Composition:

Potassium cyanide –	50 mg
Potassium ferricyanide –	200 mg
Distill water –	1000 ml

Store in dark bottle at room temperature.
Standard solution with Hb content of 5gm, 10 gm, 15 gm is recommended.

Procedure:

1. Take two test tube and label it as 'B' (Blank) and 'T' (Test solution).
2. Add 5 ml of Drabkin's solution in each test tube.
3. Avoid mouth pipetting as Drabkin's solution in poison.
4. Stopper the tube with rubber cap, add 0.02 ml of blood specimen into the tube marked with 'T'.
5. The specimen is taken with the help of Shalis pipette.
6. Wipe off the tip of the pipette before adding blood into test tube.
7. Mix the content of the tube and wait for 10 min. with the help of blank and standard solution.
8. Find out absorption of test solution in Colourimeter at 540 nm.

Calculation:

Hb conc.= Absorbance of test solution x Conc. Of std / Absorbance of std.

Normal Values:

1. **Male (Adult):** 13.5 to 18 gm
2. **Female (Adult):** 11.5 to 16.5 gm
3. **Infants:** 13.6 to 19.6 gm
4. **Children:** 11 to 33 gm (1 year) and 11.5 to 14.8 gm (10 – 12 years)

Reference: Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 3

Aim: To study and investigate the sedimentation rate of RBC in blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin anticoagulants are used.

Principal: Anticoagulated blood is taken in a tube and kept undisturbed in vertical position in a rack. This will allow the sedimentation of erythrocytes. After a specific time, generally one hour, the level of red cell is noted. The distance travelled by erythrocytes in one hour is called as Erythrocyte Sedimentation Rate (ESR).

Method: Westergren Method

Requirement:

1. Westergren Pipette
2. Westergren Stand
3. Anticoagulant

Procedure:

1. Fill the pipette by sucking blood upto 0 mark.
2. Fix it vertically in Westergren stand.
3. Read the upper level of RBC column exactly after one hour.

posable ESR Pippette

Normal Values:

1. **Male (Adult):** 3 to 10 mm/hr.
2. **Female (Adult):** 5 to 15 mm/hr.

Reference:

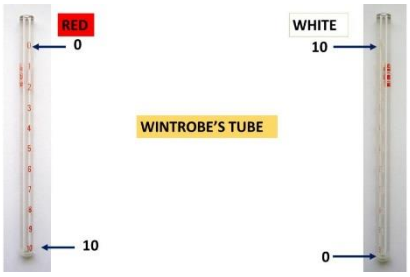
Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 4

Aim: To study and investigate the sedimentation rate of RBC in blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin anticoagulants are used.

Principal: Anticoagulated blood is taken in a tube and kept undisturbed in vertical position in a rack. This will allow the sedimentation of erythrocytes. After a specific time, generally one hour, the level of red cell is noted. The distance travelled by erythrocytes in one hour is called as Erythrocyte Sedimentation Rate (ESR).



Method: Wintrobe Method

Requirement:

1. Wintrobe tube
2. Wintrobe Stand
3. Anticoagulant

Procedure:

1. With the help of long necked pasture pipette or a special syringe, fill the Wintrobe tube upto '0' mark.
2. Place the tube in an exactly vertical position in a Wintrobe stand.
3. Read the upper level of RBC column exactly after one hour.

Normal Values:

4. **Male (Adult):** 0 to 9 mm/hr.
5. **Female (Adult):** 0 to 20 mm/hr.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 5

Aim: To study and investigate the total Leukocyte (White cell) count in blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin anticoagulants are used.

Method: Haemocytometric method

Principal: The whole blood is used for total white cell count. With the WBC diluting fluid, it is diluted 20 times and placed in haemocytometer. The cells are counted under proper magnification over specified area. With the help of known factors, the number of WBC/cu. mm. of undiluted blood can be calculated.

Requirement:

1. WBC diluting fluid
2. WBC pipette
3. Haemocytometer
4. Microscope
5. Blood sample

Composition: (WBC diluting fluid)

Glycial Acetic Acid –	3 ml
Gention Violet –	1%
Distill water –	97 ml

Procedure:

1. Assemble all the equipment.
2. Draw the blood directly into WBC pipette upto 0.5 mark.
3. Wipe off the tip of pipette to remove extra blood present at the tip.
4. Then, immediately draw up the WBC diluting fluid upto 11 mark.
5. Now rotate the pipette gently, so that the fluid and the blood get properly mixed. This will give dilution 1:20.
6. Place coverslip in position over the ruled area.
7. Once again mix the solution thoroughly by rotating the pipette.
8. Expel one drop of fluid from the pipette. Now second drop is allowed to hang from the pipette.
9. When the drop is in hanging position, touch the tip against the edge of coverslip.
10. Leave the counting chamber as it is without disturbing for about 2 to 3 minutes for settling down the WBC in the counting chamber.
11. Place the counting chamber on the stage of microscope, adjust the light and the ruled area.
12. Now, count the WBC in all four outer squares and make the total number of WBC.

Calculation:

The known factors are:

1. The known volume of fluid inside the chamber = Area x Depth
   = 4 x 0.1
   = 0.4

2. Dilution of blood = 20
3. The number of cells counted in four squares = N

Therefore,

Total Leukocyte Count = N x 20 / Area x Depth
= N x 20/ 0.4
= N x 50

With the help of this formula, we can calculate the total number of leukocytes in blood.

Normal Values:

The normal white blood cell count in male and female is **4000** to **10000 cells/ cu. mm.** of blood.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 6

Aim: To study and investigate the total Erythrocyte (Red cell) count in blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin anticoagulants are used.

Method: Haemocytometric method

Principal: The blood specimen is diluted (usually 200 times) with red cell diluting fluid. This diluted blood is placed in haemocytometer. The cells are counted under proper magnification over specified area. With the help of known factors, the number of RBC/cu. mm. of undiluted blood can be calculated.

Requirement:

1. RBC diluting fluid
2. RBC pipette
3. Haemocytometer
4. Microscope
5. Blood sample

Composition: (RBC diluting fluid)

Trisodium Citrate –	3 gm
Formalin –	1 ml
Distill water –	99 ml

Procedure:

1. Assemble all the equipment.
2. Draw the blood directly into RBC pipette upto 0.5 mark.
3. Wipe off the tip of pipette to remove extra blood present at the tip.
4. Then, immediately draw up the RBC diluting fluid upto 101 mark.
5. Now rotate the pipette gently, so that the fluid and the blood get properly mixed. This will give dilution 1:200.
6. Place coverslip in position over the ruled area.
7. Once again mix the solution thoroughly by rotating the pipette.
8. Expel one drop of fluid from the pipette. Now second drop is allowed to hang from the pipette.
9. When the drop is in hanging position, touch the tip against the edge of coverslip.
10. Leave the counting chamber as it is without disturbing for about 2 to 3 minutes for settling down the RBC in the counting chamber.
11. Place the counting chamber on the stage of microscope, adjust the light and the ruled area.
13. Now, count the RBC in each four corner and one at center; and make the total number of RBC.

Calculation:

The known factors are:

1. Number of cells counted = N
2. The volume of fluid inside the chamber = Area x Depth

Area = Central 1 sq. mm
= 25 squares

Out of which, 5 squares are counted

25 = 1 sq. mm.
05 = ?
05/25 = 1/5

Therefore,
Total Red Cell Count = N x Dilution / Area x Depth
= N x 200 x 5 / 0.1
= N x 10000

With the help of this formula, we can calculate the total number of erythrocytes in blood.

Normal Values:

The normal red blood cell counts in:

1. Male = **5** to **5.5 million cells/ cu. mm.** of blood.
2. Female = **4** to **4.5 million cells/ cu. mm.** of blood.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 7

Aim: To study and investigate the total Thrombocyte (Platelet) count in blood.

Specimen: Capillary blood can be used directly. If veins blood is used, EDTA or heparin anticoagulants are used.

Method: Haemocytometric (Direct) method

Principal: The blood specimen is diluted (usually 200 times) with Platelet diluting fluid. This diluted blood is placed in haemocytometer. The cells are counted under proper magnification over specified area. With the help of known factors, the number of Platelets/cu. mm. of undiluted blood can be calculated.

Requirement:

1. Platelet diluting fluid
6. RBC pipette
7. Haemocytometer
8. Microscope
9. Blood sample

Composition: (RBC diluting fluid)

Procaine hydrochloride – 3 gm
Sodium chloride – 1 gm
Distill water – 100 ml

Procedure:

1. Assemble all the equipment.
2. Draw the blood directly into RBC pipette upto 0.5 mark.
3. Wipe off the tip of pipette to remove extra blood present at the tip.
4. Then, immediately draw up the RBC diluting fluid upto 101 mark.
5. Now rotate the pipette gently, so that the fluid and the blood get properly mixed. This will give dilution 1:200.
6. Place coverslip in position over the ruled area.
7. Once again mix the solution thoroughly by rotating the pipette.
8. Expel one drop of fluid from the pipette. Now second drop is allowed to hang from the pipette.
9. When the drop is in hanging position, touch the tip against the edge of coverslip.
10. Leave the counting chamber as it is without disturbing for about 10 to 15 minutes for settling down the Improved Neubauer chamber in the counting chamber.
11. Place the counting chamber on the stage of microscope, adjust the light and the ruled area.
14. Now, count the Platelet in all 25 squares and make the total number of Platelets.

Calculation:

The known factors are:

1. Number of cells counted = N
2. The volume of fluid inside the chamber = Area x Depth
   = 1 x 0.1
   = 0.1 cu. mm.

Therefore,
Total Platelet Count = N x Dilution / Area x Depth
= N x 200 / 0.1
= N x 2000

With the help of this formula, we can calculate the total number of thrombocytes in blood.

Normal Values:

The normal Platelet cell count in male and female is **1.5** to **4 lakh/ cu. mm.** of blood.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 8

Aim: To study and investigate the duration of the bleeding in haemorrhagic disorder.

Specimen: Capillary blood is used by ear lobe or finger puncture.

Method: Dukes method

Principal: A standard cut is made in the skin of patient and the length of time required to cease (to stop) the blood is noted.

Requirement:

1. Cotton swab
2. Lancet/ sterile needle
3. Filter paper
4. Stop watch
5. Spirit/ Alcohol

Procedure:

1. Clean the ear lobe or fingertip with spirit by using a cotton swab.
2. For ear lobe, a glass slide is placed behind the ear lobe to hold it firmly in place.
3. Puncture the ear lobe deeply, about 1 mm by using sterile lancet/ needle.
4. Start the stopwatch.
5. The blood should flow freely without pressing the ear lobe.
6. Now remove the glass slide from ear lobe after pricking.
7. The blood is allowed to drop on filter paper.
8. The filter paper should be moved so that each drop will fall on fresh area.
9. The bleeding of wound should be allowed without pressing.
10. When bleeding slows, the wound is touched gently with a fresh area of filter paper at every 30 sec. of interval.
11. When blood stain disappears from the from to filter paper, stop the watch and note the time.

Normal value: 1 to 5 minutes.

Precaution:

1. In case of children heel should be used.
2. In suspected cases of bleeding disorder, puncture the fingertip.
3. The area to be punctured should not be standard.
4. The size and depth of wound should be standard.
5. If bleeding continues for more than 15 min., it should be stopped by placing dry sterilized cotton and applying little pressure on the wound.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 9

Aim: To study and investigate the duration of the bleeding in haemorrhagic disorder.

Specimen: Capillary blood is used by arm puncture.

Method: Ivy's Method

Principal: A standard puncture is made in the arm of patient and the length of time required to cease (to stop) the blood is noted.

Requirement:

1. Cotton swab
2. Lancet/ sterile needle
3. Filter paper
4. Stop watch
5. Spirit/ Alcohol

Procedure:

1. Ivy's method requires sphygmomanometer cuff, which is applied on the patient's arm above the elbow.
2. The puncture site is above the forearm. As soon as bleeding starts, touch the filter paper and start the stopwatch.
3. When the blood drop on filter paper disappears, record the time.

Normal value: 1 to 5 minutes.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition

EXPERIMENT- 10

Aim: To study and investigate the duration of the coagulation in haemorrhagic disorder.

Specimen: Capillary blood is used by ear lobe or finger puncture.

Method: Capillary Method (Wright Method)

Principal: Whole blood when removed from vascular system and exposed to foreign surface will form a solid clot. In Wright method, the blood is collected in capillary tube by finger puncture and in Lee- White method the blood is collected from vein in a test tube.

Requirement:

1. Cotton swab
2. Lancet/ sterile needle
3. Capillary tube
4. Stop watch
5. Spirit/ Alcohol

Procedure:

1. By using spirit, disinfect the tip of finger of patient and make about 1 mm deep cut with sterile lancet.
2. Start the stopwatch, wipe off the first drop of blood and collect blood in capillary tube uto $2/3^{rd}$ of its length.
3. After every 30 sec. break off about 1 cm of capillary to find out whether fibrin string/ thread has formed.
4. When fibrin string appears, stop the stopwatch and note the time.

Normal value: 5 to 10 minutes.

Reference:

Clinical Pathology and Haematology, Nanda Maheshwari, Jaypee Brothers, Third Edition