ENUMERATION OF TOTAL RBC

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(B.SC PART III PAPER VIII)

Red Blood Cells (Erythrocytes):

(Erythrocytes)

Erythros – Red

Cyte – Cell

The cell which is red in appearance is generally known as Red blood cell. The colour of RBC is due to presence of haemoglobin in which haem is a colour pigment and globin is a protein.

The red blood cell count ranges between 4 to 5.5 million per cubic millimeter of blood.

In adult males it is 5 millions/cu mm of blood and in adult females it is 4.5 millions cu mm of blood.

Morphology or Red Blood Cells:

Normal Size

Diameter – 7.2 microns (µ) (6.9-7.4 m)

Thickness – At the peripheral it is thicker with 2.2 m % at the centre it is thinner with 1 micron

Normal Shape:

Normally the shape of R.B.C. is biconcave disc or dumb bell shaped.

Total RBC Count by Hemocytometry:

Specimen:

i. Double oxalated or EDTA blood or

ii. Capillary blood.

Principle:

The blood specimen is diluted 1: 200 with the RBC diluting fluid cell are counted under high power (10 X objective) by using a counting chamber. The number of cells in undiluted blood calculated and reported as the number of red cells per cum (µl) of whole blood.

Requirement:

1. Microscope

2. Improved neubaeur chamber

3. RBC pipette

4. **RBC diluting fluid preparation as follows:**

a. Sodium Citrate: 3.09gm

b. Formalin: 1.0ml

c. Distilled water: 100ml.

This solutions is stable at room temperature (25°c ± 5°C) for at least one year.

Composition of RBC diluting fluid hayem's fluid:

(1) Mercuric chloride — 0.5gm

(2) Sodium Chloride — 5gm

(3) Sodium Sulphate — 5gm

To prevent bacterial or fungal growth

(4) D.W. —200ml

Note:

1. RBC diluting fluid is isotonic with blood hence haemolysis does not take place. Normal saline also can be used but it causes slight crenation of red blood cells and allows rouleaux formation.

2. Formalin acts as a preservative and checks bacterial and fungal growth.

3. Sodium citrate prevents Coagulation of blood and provides correct osmotic pressure.

Procedure:

1. Mix the anti-coagulated blood carefully by swirling the bulb.

2. In the presence of capillary blood lancet stab should be sufficiently deep to allow free flow of blood; it is drawn quickly in the RBC pipette.

3. Draw blood up to 0.5 mark.

4. Carefully wipe the excess blood outside the pipette by using cotton or a gauze.

5. Draw diluting fluid up to 101 mark.

6. The pipette is rotated rapidly by keeping it horizontal during mixing.

7. After five minutes, by discarding few drops from the pipette and holding it slightly inclined small volume of the fluid is introduced under the cover slip which is placed on the counting chamber.

8. Allow the cell to settle for 2 to 3 minutes.

9. Place the counting chamber on the stage of the microscope.

10. Switch to lower power (10 x objective). Adjust light and locate the large square in the centre with 25 small squares.

11. Now switch to high power (40 x objective).

12. The red blood cells in the four corner squares and in the centre square are counted.

13. Use the following formula for the calculation of red blood cells.

Total red blood cell/cu mm (µl). = Number of red cells counted × dilution factor/Area counted × depth

Where (i) Dilution = 1:200 (i.e. 200)

(ii) Area Counted = $\frac{80}{400} = \frac{1}{5}$ Sq. mm.

Since cells are counted in 5 bigger squares and such square is further divided into 16

Small squares. Each small square = $\frac{1}{400}$ sq. mm.

Hence, area of (5 X 16) = 80 such areas = $\frac{80}{400} = \frac{1}{5}$

(iii) Depth of fluid $\frac{1}{10}$ mm.

(iv) Number or red cells counted = N.
Hence, total red blood cells/ cumm

$$M = \frac{N \times 200}{\frac{1}{5} \times \frac{1}{10}} \; N \times 200 \times 50 = N \times 10{,}000$$

Normal Value:

Male: 4.5 to 6.0 x 10^6 cells/cu.mm (µl)

Female: 4.0 to 44.5 x 10^6 cells/cu.mm (µl)

Source of Error:

1. Diluting fluid should not be contaminated with RBC.

2. Keep the counting chamber and cover slip free from dust, lint and dried blood.

3. Use mild detergent (1% Sodium Bicarbonate) followed by washing with tap water and rinsing with deionzed water.

4. Some major source of technical error includes improper volume measurement (blood & diluent). Improper charging of the neubauer's chamber, use of defective pipette improper counting, disturbance of the chamber during the switching of the objective, don't allow the objective to touch the coverslip, failure to clean the blood sampling pipette, failure to clean the blood sampling pipette, wrong calculation and clinical mistake in recording.

Falsely High Count:

1. Inadequate wiping of the pipette.

2. Improper mixing.

3. Improper pipetting of blood as well as the fluid.

4. Error in calculations.

Falsely Low Count:

1. Blood dilution with tissues fluid