The Enumeration Of Red Blood Cells (The Erythrocyte Count):

- Erythrocyte :is a cell biconcave ,diameter is 7.8 m ,volume 90-95 mm3 is formed by stem cell.
- Function of RBC: Transport ( Hb ) which transport O2 from the lung to tissue and it is contain large amount of carbonic anhydrase which act the reaction between (CO2 + O2) which increase speed of reaction multithousands.
- RBC formed in yolk sac in embryo ,in liver during pregnant after labor in bone marrow.

Normal range of RBC :

- In Men 4.5-6.5×10⁶ cell\mm³
- In Women 3.9-5.6 ×10⁶ cell\mm³
- In Infant 4.5-6.6 ×10⁶ cell \mm³
- In Children (10 years age) 4.2-5.2×10⁶ cell\mm³
- Decrease of erythrocyte in Anemia
- Increase of RBC Thalassemia

Types of Anemia:

- Hereditary anemia, Sickle cell anemia, Pernicious anemia,Megaloplastic anemia,Aplastic anemia.
- The red blood cell is the determination of the number of erythrocytes in 1 mm3 of blood.

Apparatus and Reagents:

1-Neubauers chamber with cover slip
2-Red cell pipette.
3-Microscope.
4-Diluting fluid.

5-The center secondary square in the surface of hemocytometer chamber has an area of 1 square mm, that is divided into 25 tertiary squares each of the 25 squares is further divided into 16 smaller quarter nary squares (as described above).so the total area of the center secondary square is divided into 400 very small squares (25×16).
6-The RBC pipette like WBC pipette except that contain a red bead and making dilutions of 1:200.

7-For RBC counts ,the solution that is used is isotonic with erythrocytes ,the diluting fluids used to not lyses the leukocytes .normally the leukocytes are too few to interfere with the RBC count .(incases of leukocytosis,however,the leukocytes are easily identified and are not counted).

A-Trisodium citrate solution:

- Trisodium citrate       3.8 gm
- Formalin                  1 ml
- Distilled water            99 ml

B-Hayems fluid:

- Mercuric chloride        0.5 gm
- Sodium chloride          1 gm
- Sodium sulphate         5 gm
- D.W.                      200 ml

C-Normal Saline:

- Sodium chloride        9 gm
- D.W.                  1000 ml

Procedure of Erythrocyte:

1-Draw blood to the 0.5 mark in the RBC pipette without letting an any bubbles into the pipette by holding the pipette almost horizontally the pipette must be clean and dry.

2-Wipe tip clean and draw in the diluting fluid up to the mark 101 (dilution 1 in 200) while fulling the bulb the pipette should be gently rotated to obtain good mixing .

3-The cover slip is placed over the Neubauers chamber so as to cover both ruled platforms evently.

4-Now loud the chamber ,this is done in 3 steps :

a-Mix the contents of pipette for 3 minutes .
b- Expel 6 drops from the pipette to remove the fluid in the stem which has not been mixed with blood.

e- By holding the pipette at an angle of 45 degree and touching the space between the cover slip and the chamber by the point of the pipette, an appropriate drops of the mixture is allowed to run under the cover slip by capillary action: it must be sufficiently large to cover the whole ruled plate –form yet not large enough to fill the moat. Also there must be no air bubbles.

5- Allow 2 minutes for setting of the cells and then count.

6- The count is done as follow:

The erythrocytes count, the central double ruled square is used, red cells lying in 80 very small squares have to be counted, these 80 small squares, comprise 5 medium sized squares each of which is bound by a triple line, it is recommended that the 5 medium sized squares chosen for counting cells should consist of 4 corners and one central; this is to secure a never distribution of cells which touch the left hand lines or the upper lines of the square are taken to be within that square and those which touch the lower or right hand lines are omitted as outside the square.

**Calculation:**

RBC count = Dilution \times 1 \times Volume \times Number of cells counted (N)

= 200 \times 5 \times N

= 10000 \times \text{No. of cells} \text{\,cu.mm.}