The red blood cell (RBC) count

-Red blood cells make up almost 45 percent of the blood volume.
Their primary function is to carry oxygen from the lungs to every cell in the body.
-Red blood cells are composed predominantly of a protein and iron compound, called hemoglobin, that captures oxygen molecules as the blood moves through the lungs, giving blood its red color.
As blood passes through body tissues, hemoglobin then releases the oxygen to cells throughout the body.

- In human the RBC are non nucleated like large and small animals except in chickens and camel are nucleated . The red color of blood is due to the pigment of hemoglobin. The number of RBC varies greatly among species and within species it selves , also varies according to the age , sex , exercise , nutritional condition , pregnancy , lactation , egg production , estrous cycle and breed , etc .

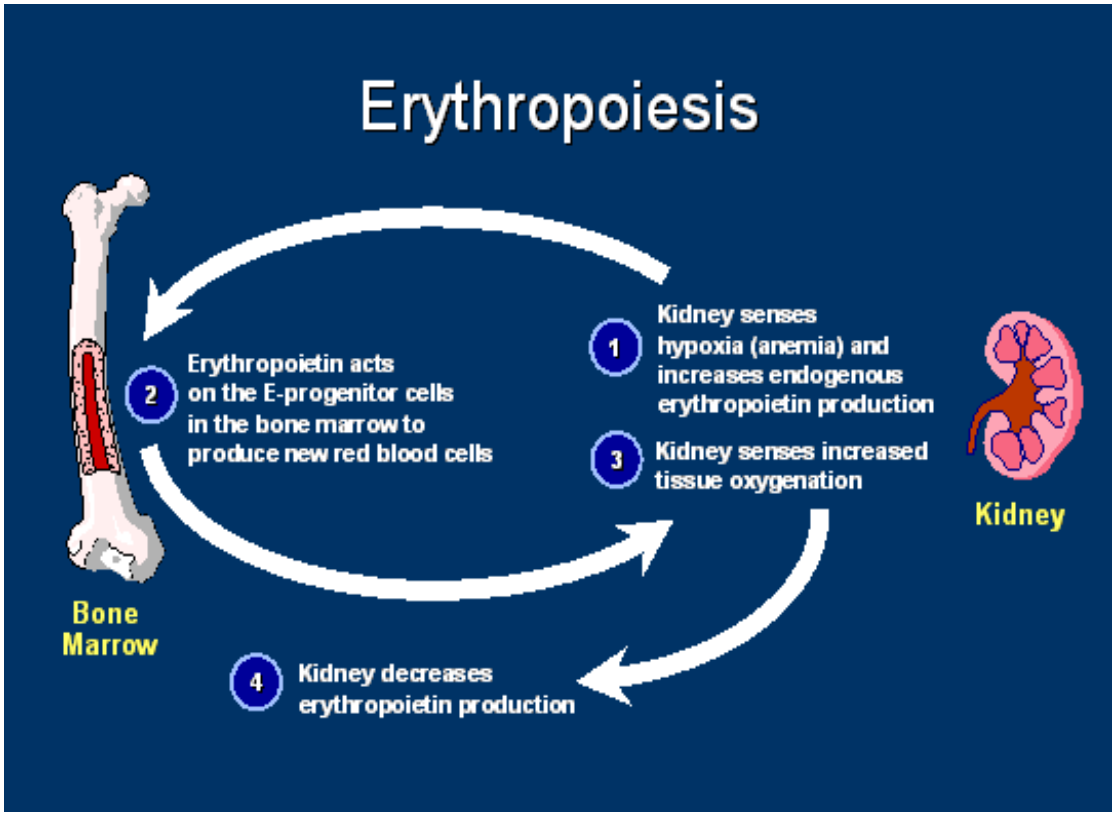
RBCs Structure

- The membrane, or outer layer, of the red blood cell is flexible, like a soap bubble, and is able to bend in many directions without breaking.
- This is important because the red blood cells must be able to pass through the tiniest blood vessels (capillaries) to deliver oxygen wherever it is needed.
- The capillaries are so narrow that the red blood cells, normally shaped like a disk with a concave top and bottom.

The mechanism of erythropoiesis:

1. The kidney monitors the level of oxygen in the blood.
2. If oxygen levels are low then the kidney secretes a hormone called *erythropoietin*.
3. Erythropoietin enters the blood stream and travels throughout the body. All cells are exposed to erythropoietin, but only red bone marrow cells, respond to the hormone.
4. Erythropoietin stimulates the production of erythrocytes in the bone marrow.
5. These erythrocytes leave the bone marrow and move into the blood stream.
6. As the erythrocyte population increases, the oxygen carrying capacity of the blood increases.
7. When the kidney senses that oxygen levels are adequate, it responds by slowing the secretion of erythropoietin.
8. This negative feedback loop ensures that the size of the erythrocyte population remains relatively constant and that the oxygen carrying

capacity of the blood is always sufficient to meet the needs of the body.

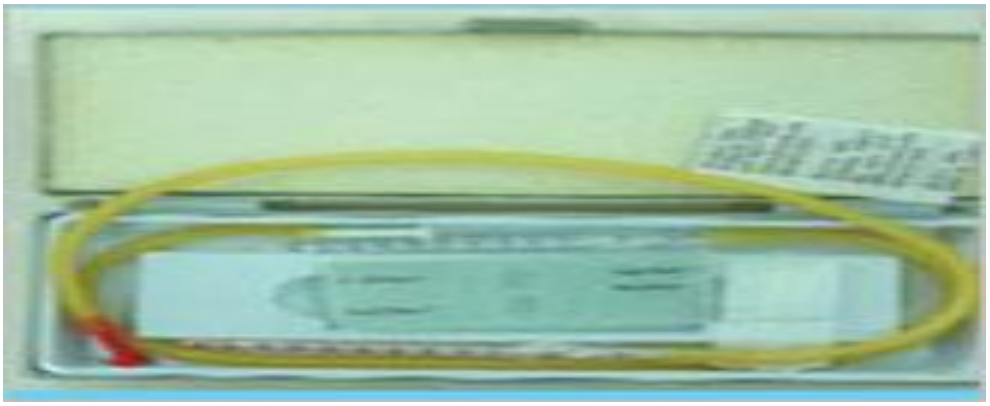


-Is the number of erythrocytes per micro litter of blood.

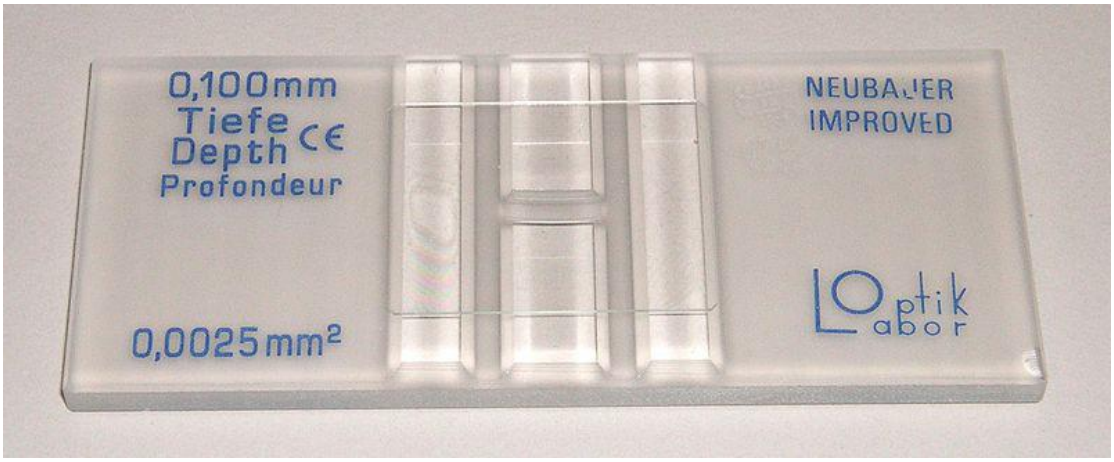
-Normal ranges:

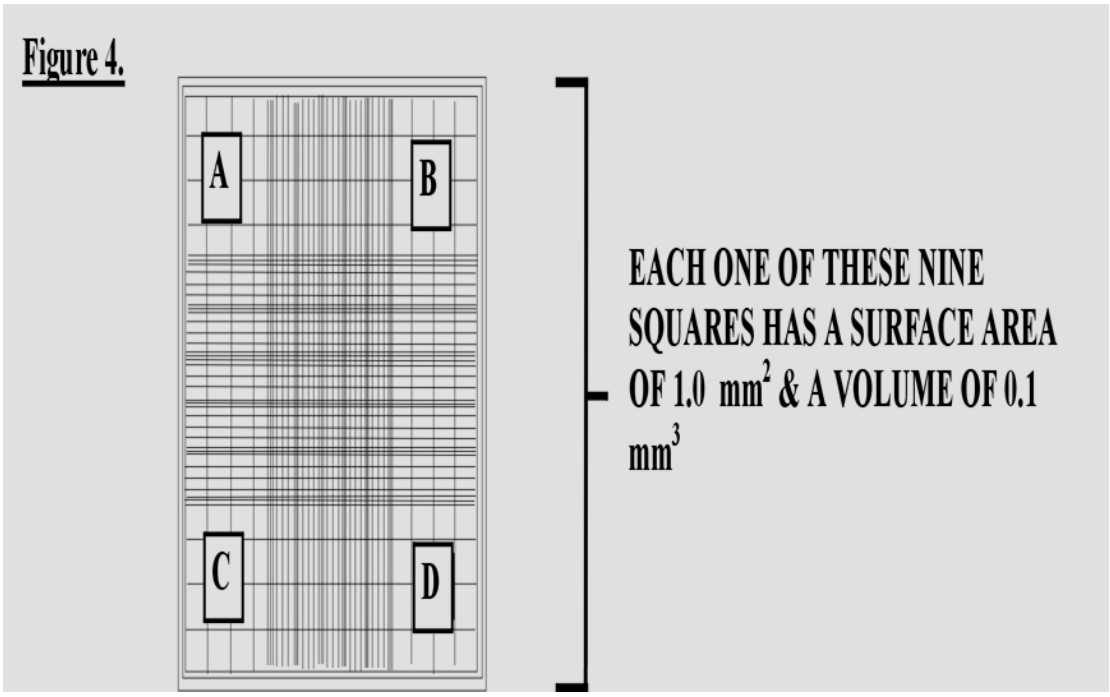
Animal	Cat	Cattle	Chicken	Dog	Goat	Horse	Pigeon	Rabbit	Sheep	Man	woman
Millions\cu.mm	6-8	6-8	2.5-3.2	6-8	13-14	9-12	3.5-4.5	5.5-6.5	10-13	5-6	4-5

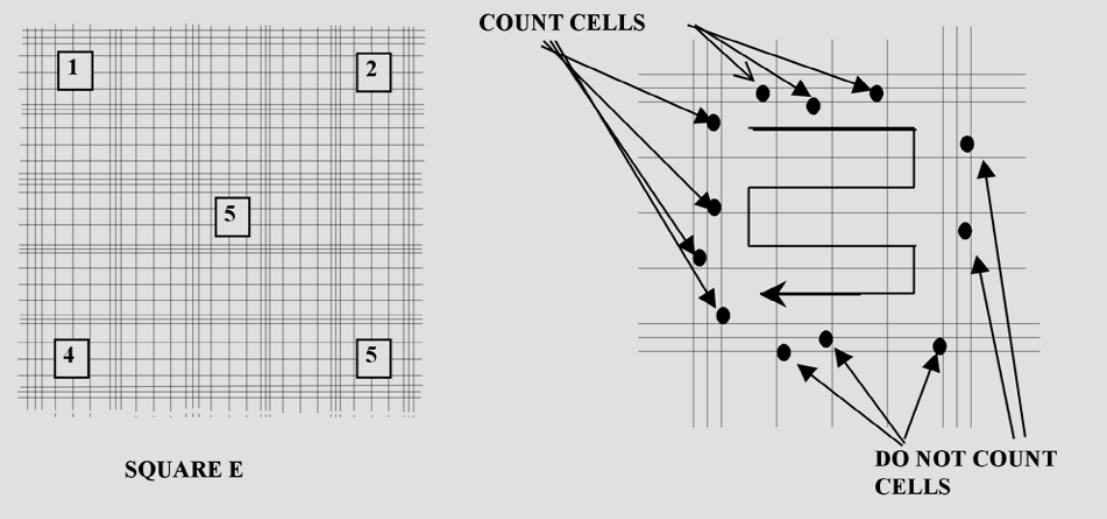
►The instrument use for RBCs count is called (Haemocytometer).



Haemocytometer : it consist of 1. counting chamber (Neubauer ruling chamber). It is a heavy glass slide in the center of which is a ruled platforms (some types have 2 ruled platform). a. The platform is lower than slide about 0.1 mm. (depth of platform 0.1mm). b. The graduated area in the slide is ruled both horizontally and vertically forming 9 large squares . c. Central large squares is subdivided into 16 medium squares , each of which in turn is divided into 16 small squares , each small square is 1/400 mm2 . Since the depth of fluid between ruled surface and cover slide is 0.1 mm the volume of fluid covering , the smallest squares is 1/400×1/10 =1/4000 cu mm.







2. Special pipette (red cell pipette) . graduated pipette indicating 0.5 and 1.0 and has a bulb contain a red glass bead for mixing blood with diluting fluid and then the bulb narrow again and at this point is marked to 101.

3. cover slip .

Hayem's solution consist of $HgCl_2$ 0.05 g, Na_2 So_4 2.5 g , NaCl 0.5 g in 1oo ml water.

- ***Principle:***

➢ In order to facilitate RBCs count a specified volume of blood is diluted with a specified volume of isotonic fluid.

➢ Red cell diluting fluid must be:

- anti-coagulant anti-hemolysis.
- anti-aggregation.
- anti-Rouleaux.
- preserve RBC shape.

(**Special pipette)**

Procedure :

1. using of diluting fluid called Hayem's solution .
2. counting chamber (Neubauer chamber).
3. red cell pipette .
4. cover slip .
5. Microscope .

Method:

1. Use a clean and drayed counting chamber , cover slip and pipette.
2. SuΓk up the blood to the mark 0.5 .
3. Clean the out side of pipette from blood with cotton .
4. Suck up the diluting fluid (Hayem's solution) to the mark 101 .
5. Rotate the pipette to mix blood with Hayem's solution .
6. Discard a drop or 2 drops of the fluid and place the tip of the pipette against the edge of cover slip , diluting blood will enter the counting chamber by capillary attraction until the platform completely covered .
7. Place the counting chamber on microscope and leave it for 2 minutes to settle cells .
8. Examine the slide under the low-power first , focusing on central square then focusing under higher power .
9. Count the chamber of red cells in 5 medium square of the large central square. (i.e. 80 small square it means 5 × 16 = 80) .
10. Count the cells using Γ shape method .

Calculation :

1. The 0.5 units of blood have been diluted by 99.5 units of diluting fluid (0.5 → 101) units , it mean the dilution is 1 in 200 . 2. X mean the number of RBC in 80 small square .
3. The size area of 80 small square is 80 × 1/4000 cu.mm = 1/50 cu.mm .
4. X cells in 1/50 cu.mm of diluted blood is 50X in 1 cu.mm.
5. The number of cells in undiluted blood is 50X × 200 = 10000X .

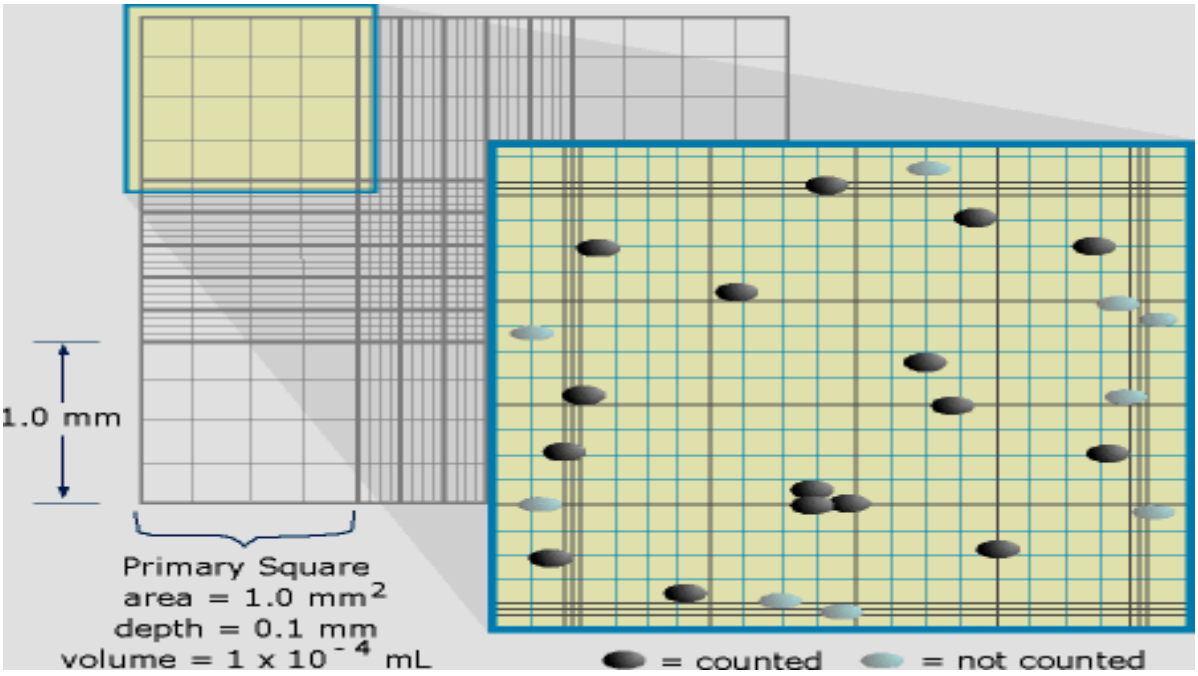
White blood cell count

Introduction white blood cells or Leucocytes are much less in number than red blood cells or erythrocytes in the circulating blood leucocytes in various domestic animals species defers from animal species to another as follow

animal	***Horse***	***Cow***	***Sheep***	***Goat***	***Dog***	***Cat***	***Chicken***
Total leukocyte count per cu.mm.	***8000 – 11,000***	***7000 – 10,000***	***7000 – 10,000***	***8000 – 12,000***	***9000 – 13,000***	***10,000 – 15,000***	***20,000 – 30,000***

The main function of WBC is defense mechanism against foreign body that enter the body . So in bacterial infections , the WBC especially neutrophils may be increased greatly (leucocytosis in general) or (neutrophilia) , but in viral infection WBC especially neutrophils decreased in number (leucopenia or neutropenia) .

Turk s solution consist of : 1. glacial acetic acid ……. 1 ml (for destroy RBC) . 2. 1% gentian violet solution ……. 1 ml (for staining nucleus of WBC) . 3. distilled water ……100 ml .



Procedure :

1. Use especial pipette which has white glass bead , the mark above the bulb is 11.
2. Blood diluted fluid is Turk's solution which consist of glacial acetic acid , gentian violet , D.W .
3. Counting chamber ((Neubauer ruling chamber).
4. Suck the blood until reach mark 0.5 and then suck the Turk's solution until mark 11.
5. Leave the pipette for 5 min. to ensure destroyed reb blood cells and staining WBC.

6. Discard 2 drop at first before putting one drop on counting chamber between cover slip and the slide.
7. Count the WBC in each 4 large squares , using low power for these counts .

Calculation

1. Since the blood was diluted to mark 11 it means the dilution is 1 in 20 (0.5→11) .

2. X is the average number of WBC in one large square .

3. Since the area of large square in 1 mm. , and the depth is 0.1 mm. the X mean number of WBC in 0.1 mm. of diluted blood.

4. X × 10 mean number of WBC in 1 c mm. , of diluted blood.

5. Since the blood was diluted for 20 times , the number of WBC in c. mm. of normal blood is X × 10 × 20 (X×200).