Hematology Laboratory
Manual Red Blood cells Count

Red blood cell structure:-

Biconcave disk shape cells ; ideal for gas exchange, spectrin - elastic protein; allows shape change ,(7.5 micron)diameter,(2.0 micron) thick. mature cells are not nucleate (no nucleus),contains very few organelles; mainly a hemoglobin carrier(hemoglobin – 33% of cell mass); carries oxygen, no mitochondria; only anaerobic respiration.

Functions

(oxygen & carbon dioxide transport)

Red blood cell production (Erythropoiesis):-

RBC formed from yolk sac in the first embryonic stages at late stage it formed by liver,spleen and lymph node ,after birth it produce mainly by the red bone marrow and it dependent on a number of factors including iron (a component of hemoglobin), vitamin B_{12} and folic acid (which are necessary for normal mitosis), and erythropoietin, Which is a hormone produced by the kidney, regulates the rate of red cell production. Its levels depend on oxygen levels in the blood supplying the kidney. If oxygen levels fall below normal, more erythropoietin is released; this stimulates red cell production, thus increasing hemoglobin levels and the oxygen carrying capacity of the blood.

Fate of red blood cells :-

RBC mainly consist from heme and protein called goblin ,aged RBC damaged by macrophages ,kupffer cells ,specific cell from the reticulo - endothelial system located in the liver , spleen ,lymph node and bone marrow. when RBC damaged will result heme and globin,Heme return to the liver while goblin back to body proteins and used to produce new hemoglobin . life Spain of RBC is 120

.Erythrocyte Disorders :-

1.**Anemia;** - Any reduction in the total amount of hemoglobin in the blood ,that results when blood has lower than normal ability to carry oxygen

Causes of anemia;-

a. Insufficient erythrocyte count

i. **hemorrhagic anemia** - loss of blood from bleeding (wound, ulcer,etc.)

ii. **hemolytic anemia** - erythrocytes rupture (hemoglobin/transfusion

problems, infection)
iii. **a plastic anemia** - red marrow problems (cancer treatment, marrow disease, etc.)

b.Decrease in Hemoglobin
i.**iron-deficiency anemia** - low Iron levels (diet; absorption, bleeding, etc.)

ii.**pernicious anemia** - low Vitamin B12 (diet, intrinsic factor for Vit B absorption)
c.Abnormal Hemoglobin (usually genetic)
i.**thalassemia** - easily ruptured RBCs .
ii.sickle-cell anemia - sickle-shaped RBCs .

2.Polycythemia - excess RBC count, causes thick blood , it is an abnormally high level of red blood cells. Its type:-
a.polycythemia vera - bone marrow problem.
b.secondary polycythemia - increased erythropoietin release stimulated by hypoxemia due to high altitude (where the oxygen content of the air is reduced).
Normal range of number of RBC(million/mm^3) for few types:-

N	Type	No.RBC (Million/Mm^3)
1	Sheep & Gout	10-13
2	Horus	7-8
3	Cat &Dog	6-8
4	Rabbits	5.5-6.5
5	Poultry	2.5-3.5
6	Women	4.0-5.5
7	Men	4.5-6.0

. Red blood cell count (RBC count)
Principal;-The method consists of accurate dilution of a measured quantity of blood with an isotonic solution ,which will also prevent coagulation. Dilution is necessary because the cells of normal blood are so numerous that under the microscope individual cells are hardly seen.

Material and method ;-

1- The special pipette, consisting of a capillary tube marked with figures 0.5,1,and 101 with a bulb between the marks 1 and 101.The bulb contain small red glass bead.
2- Blood cell counting chambers are called (**haemocytometers);** the most commonly used type is the **Improved Neubauer chamber**. The Improved Neubauer chamber is a thick glass slide with two recessed central areas each having a finely ruled grid. The grid consists of 9 squares, each with an area of $1mm^2$. The central square is divided into 25 smaller squares, and each of these is further divided into 16 squares, giving a total of 400 squares. This central portion is used for red cell counts. The four corner squares are divided into 16 squares only are used for white cell counts. When the special (heavy) cover glass is placed over the recessed central area, the depth of the counting chamber is 0.1 mm.
3- Special thick cover slide of standard weight and thickness
4- Fluid to dilution the blood sample, which is called Hayme's solution, consist of
   -Mercuric chloride 0.5g : break down WBC, platelets and germs .
   -Sodium chloride 1.0g : keep the osmotic pressure
   -Sodium sulfate 5.0g : prevent blood clotting
   -Distilled water 200ml

Procedure

1. The counting chamber and the cover slip are cleaned and the cover slip placed on the lateral bars across the middle of the counting chamber.
2. Clean the figure and puncturing it gently by lancet.
3. Using the special pipette and quickly draw the blood up to the mark 0.5 and then immediately draw Hayme's solution up to the mark 101
4. Mix well for 1 – 2 min.
5. Discard the first three drops of mixture from the capillary prior to loading the hemacytometer
6. Introduce a little of the diluted blood into the chamber with the cover slid from the pipette. No pressure is required to fill the chamber, capillary action is quite sufficient.
7. Leave the cells to settle for 2 min.
8. Focus on the ruled area of the chamber using the x10 microscope objective. Change to the x40 objective and adjust focus.

9. Using the central large square only, count 5 groups of 16 small squares. Avoid counting the same cell twice.

Calculation

Blood was diluted 1 in 200 ,dilution blood factor =200

No. RBC in $1cc^3$ =N of RBC in5 small square x $\frac{\text{dilution factor}}{\text{volume of counting area}}$

Area of **one** small square = 0.04 mm^2

Depth of chamber = 0.1 mm

So volume of **one** small square = 0.1mm x 0.04 mm^2

= 0.00 4mm^3

Therefore, volume of 5 small sequare = 5 x 0.004mm^3

= 0,02mm^3

Therefore, No. of cells in 1 mm^3 blood = $N \times \frac{200}{0.02}$

So total No. of RBC in 1 mm^3 blood = **N x 10,000**

Express your result in terms of **10^6 cells/ mm^3**

Errors caused in RBC count technique:

1. not thoroughly mixing blood
2. inadequate shaking
3. failure to discard first 3 drops
4. not loading chamber properly (overfilling, trapped air bubbles)
5. counting cells inaccurately (skipping cells, counting cells twice, counting on wrong borders)
6. calculation error
7. clerical error