Total Red Blood Cells (RBC) Counting

Introduction:

- Normal erythrocytes are biconcave disks; the advantages are:
    - increases the surface area of the erythrocytes, the greater surface area makes it easier for gas to move into and out of the erythrocytes
    - They can bend or fold around its thin center, thus decreasing the size of the erythrocyte and enabling it to pass more easily through small blood vessels
- Erythrocytes are highly specialized cells that lose their nuclei and nearly all their organelles during maturation.
- Some of the major contents of RBCs include: lipids, ATP, and the enzyme carbonic anhydrase; while the main component is the pigmented protein (Hemoglobin) that occupies 1/3 of the RBC volume and gives the cell its color.

Functions of RBCs:

- Transport O2 from the lungs to the various tissues of the body and CO2 from the tissues to the lungs and this function is related to the Hb in the RBC
- Regulation of blood pH which is due to the presence of carbonic anhydrase in the RBC that catalyzes the reaction between CO2 and water

Erythrocytes (RBCs) Disorders:

An imbalance between the rate of RBC production (erythropoiesis) and the rate of destruction.Excess of RBC production called ***Polycythemia*** and deficiency either in RBCs or hemoglobin or both is called ***Anemia.***

A) Polycythemia:

Is characterized by an over production of erythrocytes, resulting in increased viscosity of the blood, reduced blood flow rate, and, if sever, plugging of the capillaries. It is of two types:

a. Polycythemia Vera (Primary polycythemia):
   Is due to cancer of the myeloid tissue.It can result in an RBC count as high as 11 million RBCs/ 1mm3 blood and a hematocrit as high as 80%.

b. Secondary Polycythemia:
   Is characterized by RBC count as high as 6 – 8 million RBCs/ 1mm3 blood, it is caused by smoking, air pollution, emphysema, high altitude, or other factors that create a state of hypoxemia.

B) Anemia:

Is defined as a decrease in the oxygen – carrying capacity of blood, it is generally associated with a low hematocrit, which can result from either a decrease in number of erythrocytes or a decrease in the size of the erythrocyte. There are several types of anemia and they are:

a. Nutritional Anemia:
   The most common type of anemia which is caused by dietary deficiency, most commonly iron deficiency because iron is a component of hemoglobin. It also could be caused by folic acid deficiency causing RBCs to be enlarged and more fragile.

b. Pernicious Anemia:
   It could be due to either deficiency in vitamin B12 (the extrinsic factor) or deficiency in the intrinsic factor which is produced by the parietal cells of the stomach.

c. Aplastic Anemia:
   It is caused by a defect in the bone marrow.

d. Renal Anemia:
   It is due to chronic kidney disease that renders the kidneys unable to produce erythropoietin hormone which is essential for RBCs production.

e. Hemolytic Anemia:
   Is a disorder of blood in which erythrocytes rupture or are destroyed at an excessive rate.

Principle of the Experiment:

This method depends on accurate dilution of a measured quantity of blood with an isotonic solution {Gower's solution}, which also prevents coagulation. Dilution is necessary because the red cells of normal blood are so numerous that under the microscope; cells are barely discernible.

Normal Value:

Male: 4.6 – 6.2 million cells/1mm3 of blood
Female: 4.2 – 5.2 million cells/1mm3 of blood

Objective of the Experiment:
To examine the blood sample and then determine whether the individual has anemia or polycythemia or normal value of red blood cells.

Apparatus and Reagents required:

1. **Special RBC pipette** (has red porcelain and large mixing chamber)
2. **Counting chamber (also called hemocytometer).** The improved Neubauer chamber has 2 types of counting squares, one for leukocytes counting which is located at the corners of the chamber and the other one for RBCs counting, which is located in the center of the chamber.
3. Cover slip
4. **Dilution fluid (Gower´s solution):**the solution is prepared using 33.3ml of glacial acetic acid, 12.5 gm of sodium sulphate and complete the volume to 200 ml with distilled water. Sodium sulphate preserves the RBCs while the glacial acetic acid in this high volume will cause the destruction of the WBCs and platelets

Note: the procedure is the same as the one of counting total WBCs except for the counting square used, which is located in the middle of the counting chamber and it is divided into 25 small squares and each small square is divided into 16 even smaller squares.

Calculations:

1. From the RBC pipette,

0.5 unit of blood is diluted to 101 units with Gower's solution

0.5 unit (B) → 101 unit (B+G)

Mix by rotating the pipette for 2 – 5 min then discards 3 drops which are equivalent to 1 unit.

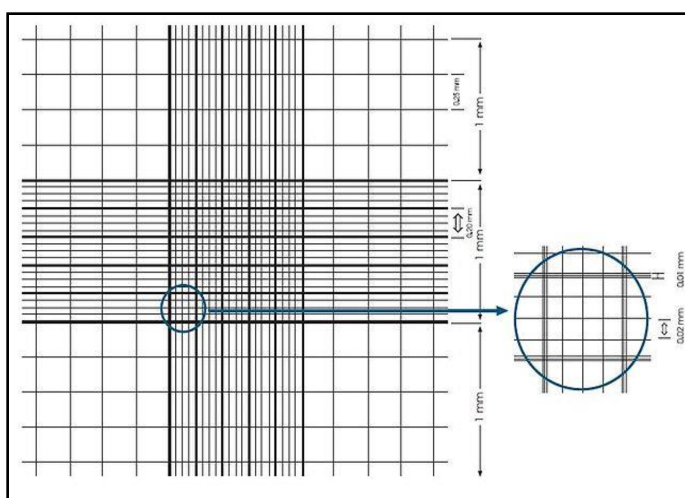
0.5 unit (B) → 101 unit (B+G) – 1 unit (B+G)
0.5 unit (B) → 100 unit (B+G)
0.5 unit (B) ≡ 100 unit (B+G)
1 unit (B) ≡ 200 unit (B+G)
1 mm^3 (B) ≡ 200 mm^3 (B+G)
1/200 mm^3 (B) ≡ 1 mm^3 (B+G)

1 mm^3 (B+G) ≡ 1/200 mm^3 (B)

2. Form the hemocytometer slide:

Since we are counting RBCs, then the middle square of the counting chamber should be used.
The volume that covers one intermediate square is:
V= L * W * D
= 1/5mm * 1/5mm * 0.1mm
= 1/250mm^3 (B+G)
Since we are counting RBCs, then we need to calculate the volume of 5 intermediate squares:
V= 1/250mm^3 (B+G) * 5

Then V= 1/50 mm^3 (B+G)



Volume	**Cells**
1/50mm^3 (B+G)	R
1mm^3 (B+G)	?

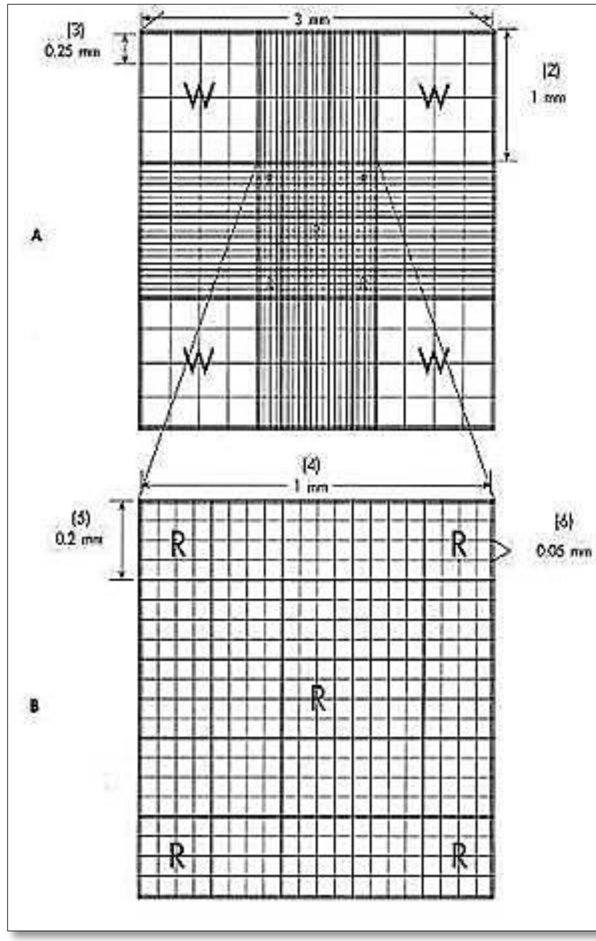
Then 1mm^3 (B+G) = 50R

Since $1mm^3$ (B+G) ≡ $1/200mm^3$ (B) ------------- (from the pipette equation)
Then:

Volume	**Cells**
$1/200mm^3$ (B)	50R
$1mm^3$ (B)	?

Then $1mm^3$ (B) = 10000R

R = R1 + R2 + R3 + R4 + R5
(From the counting chamber)



Home work:

1- Why the value of RBC counting in females less than males?
2- Why there is an increase in the RBC count in individuals with hypoxia or those who are living in high altitudes?
3- What are the major bad consequences of anemia?
4- How to treat patients with nutritional anemia?
5- How to treat patients with pernicious anemia?
6- How to treat anemia of chronic kidney disease?
7- What is the function of the intrinsic factor and its location?
8- What is the difference between total RBC count and WBC count according to procedure requirements and calculations?