Al-Rasheed University College
Pharmacy Department
2nd Stage / 2nd Course
(2019 – 2020)

Total RBCs Counting

Physiology Lab. / Lab. (5)

Assis. Lecturer
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Introduction:

- Red blood cells or erythrocytes are produced from the red bone marrow from precursors called (stem cell) or (hemocytoblasts)
- The major regulating factor for RBCs production is the oxygen.
- If the body is in a state of hypoxia (low oxygen level), the kidneys will produce a hormone called (erythropoietin) to stimulate the bone marrow to produce more RBCs
- RBCs maturation requires different nutrients; like proteins, iron and copper necessary for hemoglobin synthesis. Vitamins like, B12 and folic acid (B9) required for DNA synthesis in the stem cells of the bone marrow
- 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.

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

- **Functions of the RBCs:**
  - Transport O_2 from the lungs to the various tissues of the body and CO_2 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 CO_2 and water to produce carbonic acid (weak unstable acid) that breakdown to bicarbonate ions

Disorders of Erythrocytes:

- An imbalance between the rate of RBC production (erythropoiesis) and the rate of destruction.
- **There are mainly two types:**
  - **Polycythemia:** excess of RBCs production resulting in increased viscosity of the blood, reduced blood flow rate, and, if sever, plugging of the capillaries. It is of two types:
    - Primary Polycythemia (polycythemia vera)
    - Secondary Polycythemia
  - **Anemia:** deficiency in either RBCs or hemoglobin or both of them. Its types:
    - Nutritional anemia (most common)
    - Pernicious anemia
    - Aplastic anemia
    - Renal anemia
    - Hemolytic anemia
    - Hemorrhagic anemia

Polycythemia:

1- Primary Polycythemia (polycythemia Vera):

- cancer of the myeloid tissue.
- result in an RBC count as high as 11 million RBCs/ $1mm^3$ blood
- hematocrit as high as 80%.

2- Secondary Polycythemia:

- caused by smoking, air pollution, emphysema, high altitude, or other factors that create a state of hypoxemia.
- characterized by RBC count as high as 6 – 8 million RBCs/ $1mm^3$ blood

Anemia:

- Is defined as a decrease in the oxygen – carrying capacity of blood
- result from either a decrease in number of erythrocytes or a decrease in the size of the erythrocyte associated with low levels of hemoglobin or both of them.

1- Nutritional Anemia:

- The most common type of anemia
- caused by dietary deficiency, most commonly iron deficiency because iron is a component of hemoglobin.
- In case of hemoglobin deficiency, the size of RBCs is small and they are called microcytic
- also could be caused by folic acid deficiency which is required for normal formation of DNA and thus normal division of the hematopoietic stem cells
- deficiency in folic acid causing RBCs to be enlarged and have a shorter life span; they are called macrocytic

2- Pernicious Anemia:

- Normal development of RBCs requires vitamin B12
- Vitamin B12 is called (extrinsic factor), because it can not be synthesized by the bod and only achieved from food
- Parietal cells of the stomach secretes a chemical substance called (intrinsic factor), that prevent vitamin B12 destruction and promote its absorption

Causes:

- Due to either deficiency in vitamin B12 (the extrinsic factor) which is required for normal DNA formation;
- deficiency in the intrinsic factor

Risk Factors:

- Deficiency of vitamin B12 usually found in vegetarian people and can be treated with diet and oral supplements of B12 because human body cannot synthesize B12
- Deficiency in the intrinsic factor could be due to congenital abnormalities, gastric ulcer, and gastric bypass and also in old age people. Pernicious anemia due to intrinsic factor deficiency can only be treated by injectable vitamin B12 supplements.

Anemia: continue

3- Aplastic Anemia:

- It is caused by a defect in the bone marrow; the primary site of erythrocytes and leukocytes production
- the result is a deficiency in both (RBCs and WBCs).

4- Renal Anemia:

- It is due to chronic kidney disease that renders the kidneys unable to produce erythropoietin hormone which is essential for RBCs production.
- This type of anemia can be treated by regular administration of injectable erythropoietin hormone.

Anemia: continue

5- Hemolytic Anemia:

- Is a disorder of blood in which erythrocytes rupture or are destroyed at an excessive rate.
- caused by:
  - inherited defects within erythrocytes like (G6PD),
  - certain type of medications (anti-malarial drugs and sulfa drugs),
  - incompatible blood transfusion,
  - erythroblastosis fetalis (HDN)
  - some autoimmune disorders.

6- Hemorrhagic Anemia:

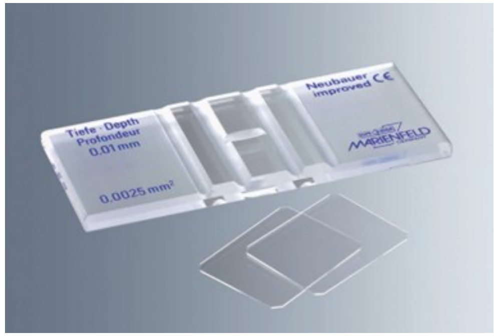
- It is caused by rapid loss of blood, it can result from trauma, ulcers or excessive menstrual bleeding.

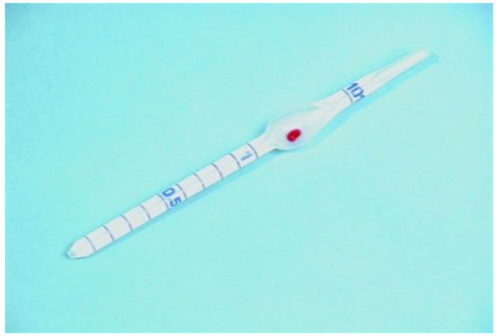
Objective (Aim) of The Experiment:

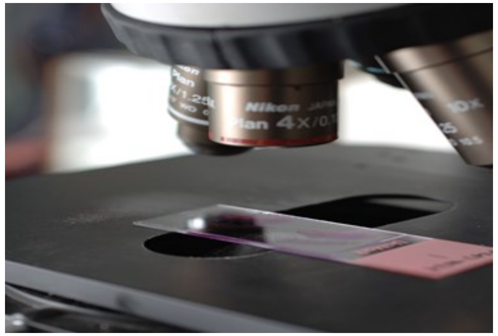
- To determine the total number of RBCs
- To evaluate the general shape of the RBCs for possible presence of certain disorders
- To determine the presence of RBC disorders (polycythemia or anemia)

Basic Requirements:

- 70% alcohol and cotton
- Sterile blood lancet
- Special RBC pipette
- Special slide (hemocytometer)
- Cover slip
- Special dilution fluid (Hayem's Solution) or (Gower's Solution)
- Microscope

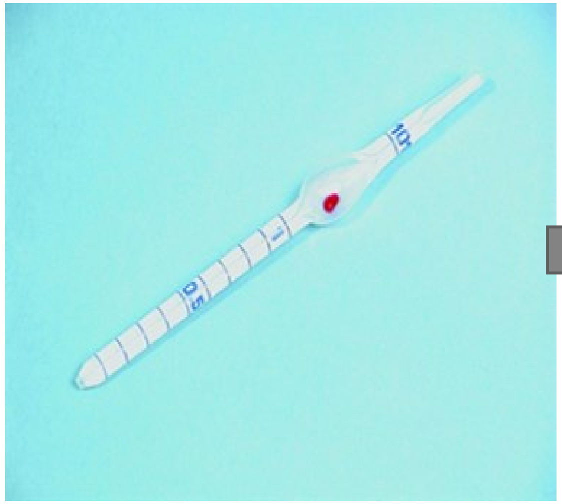


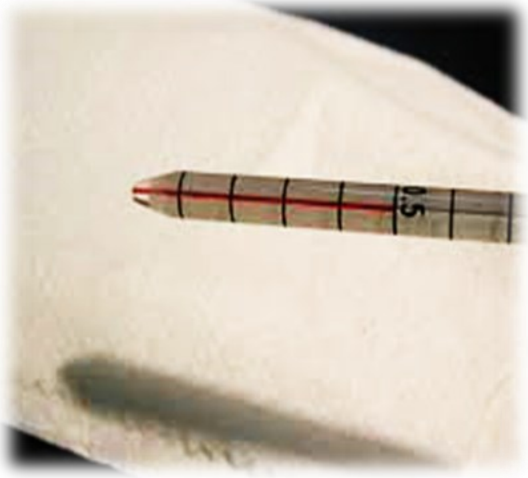


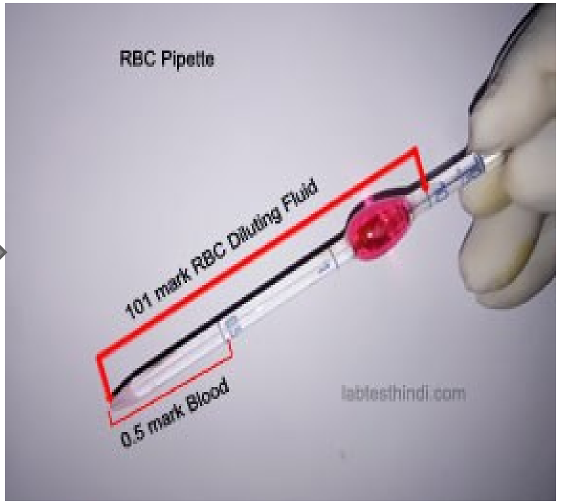


Procedure:

1- RBC Pipette







- Graduated (0.5 IU – 101 IU)
- Large mixing chamber
- Used to withdraw blood sample, and then dilute it with (Hayem's or Gower's solution)

Procedure:

2- Dilution Fluid:

Hayem's Solution:

- **Constituents:**
  - Sodium Chloride (preserve tonicity and prevent hemolysis)
  - Sodium Sulfate (as anticoagulant)
  - Mercuric Chloride (prevent hemolysis)
  - Distilled Water (for dilution)
- **This type of solution can not destroy WBCs and Platelets**

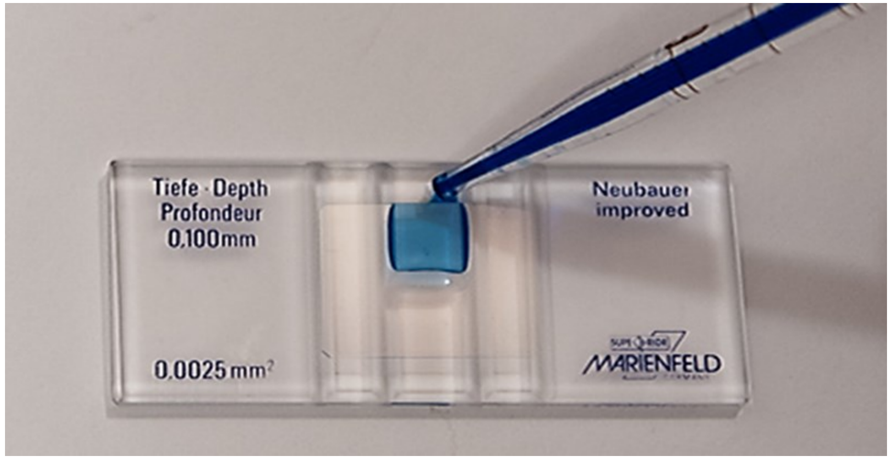
Gower's Solution:

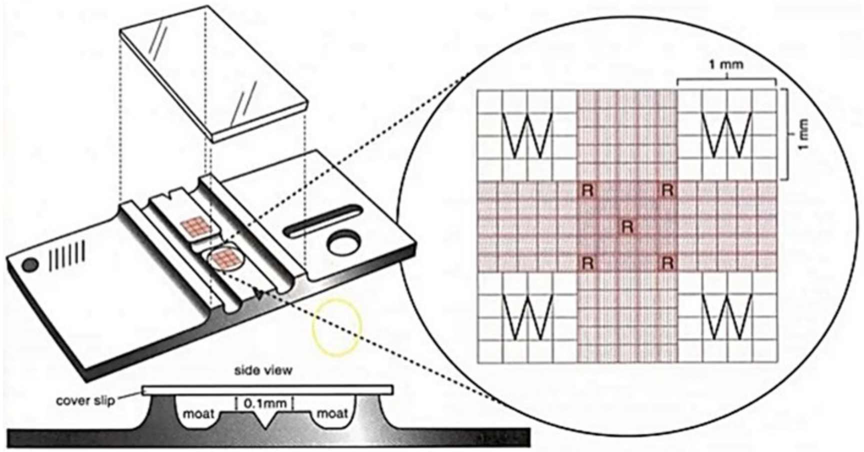
- **Constituents:**
  - Glacial acetic acid 33% (for destruction of WBCs and platelets)
  - Sodium sulfate (as anticoagulant and prevent RBCs hemolysis)
  - Distilled water (for dilution)
- **This type od solution can destroy WBCs and Platelets**

Procedure:

3- Hemocytometer (Counting Chamber)

- Hemocytometer is a special slide containing a counting chamber
- It is used for manual measurement of different types of cells in blood and other body fluids
- Used to determine the total number of:
  - WBCs in a blood sample, cerebrospinal fluid sample, and synovial sample
  - RBCs in a blood sample
  - Platelets in a blood sample
  - Sperms in a seminal fluid sample





How to Calculate Total Number of RBCs?

- **Part One: derived 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)

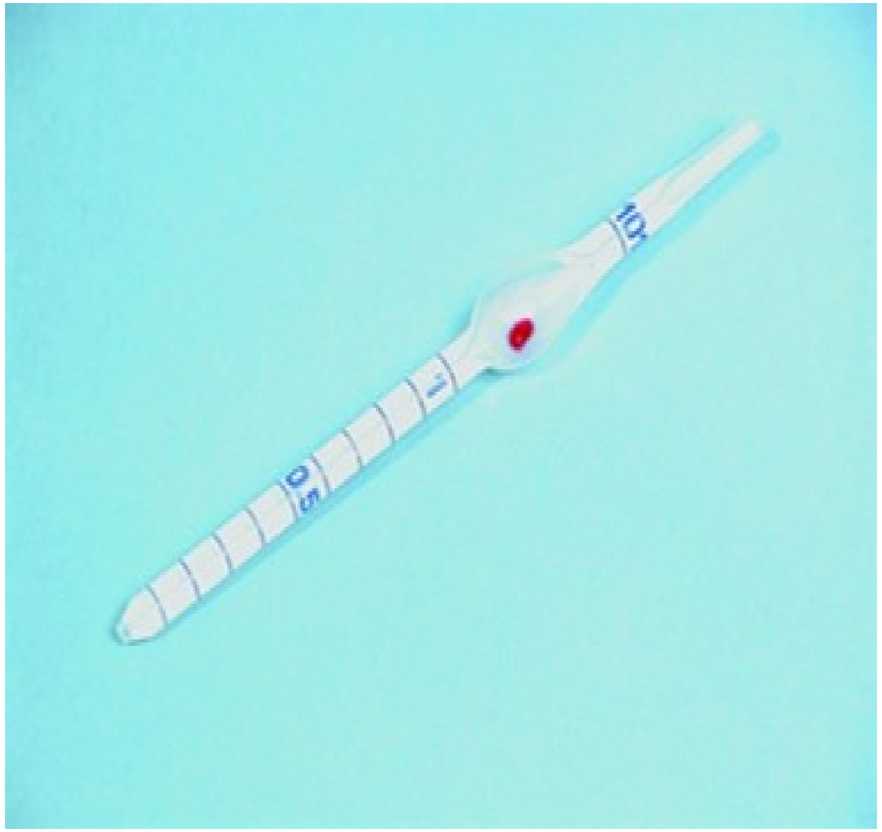
Mix by rotating the pipette for 2 – 5 min then **discards 3 drops which are equivalent to 1 (IU) unit. (3 drops = 1 IU (B+G))**

  - 0.5 unit (B) → 101 unit (B+G) – 1 unit (B+G)
  - 0.5 unit (B) → 100 unit (B+G)
  - [0.5 unit (B) ≡ 10 unit (B+G)] x 2
  - 1 unit (B) ≡ 200 unit (B+G)
  - 1 unit = $1mm^3$

<ins>**Since**</ins> [1 mm^3 (B) ≡ 200 mm^3 (B+G)] / 200

<ins>**So**</ins> 1/200 mm^3 (B) ≡ 1 mm^3 (B+G)

1 mm^3 (B+G) ≡ 1/200 mm^3 (B)



How to Calculate Total Number of RBCs?

- **Part Two: Derived from (hemocytometer)**

Calculate the volume of (1) small square

$V = L(\text{length}) * W(\text{width}) * D(\text{depth})$

$= 1/5mm * 1/5mm * 0/10mm$

$= 1/250\ mm^3\ (B+G)$

we have to calculate the volume of **<u>(5)</u>** small squares

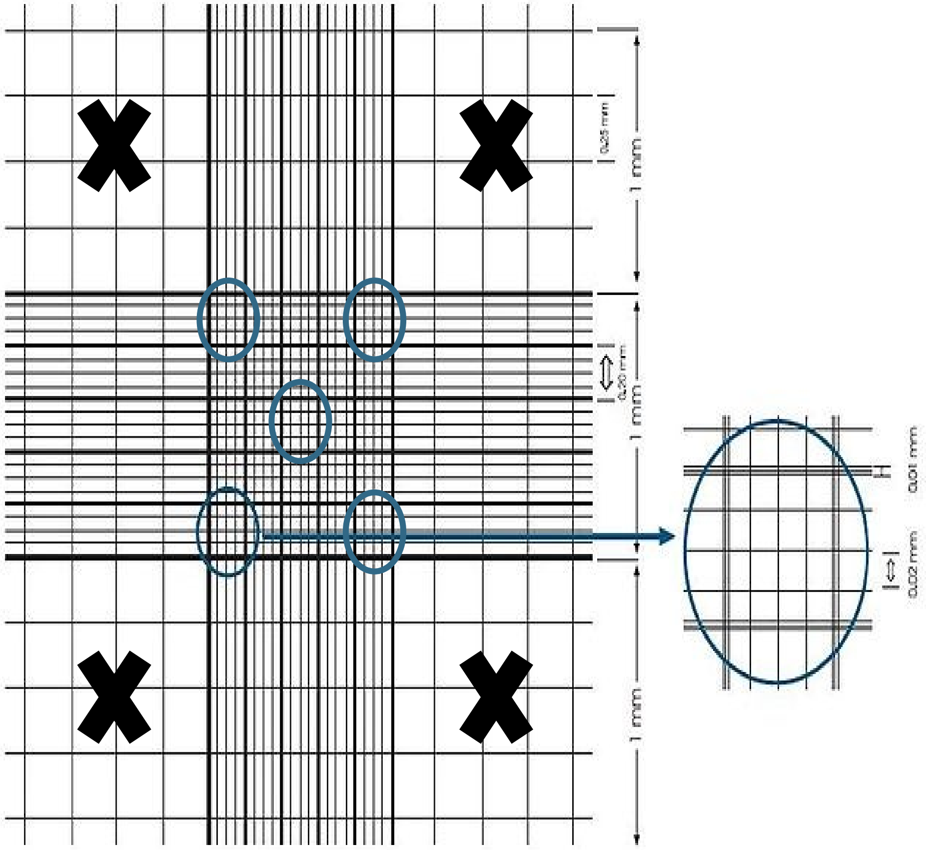
$V = 1/250\ mm^3\ (B+G) * 5$

$= 1/50\ mm^3\ (B+G)$

<u>Volume</u>	**<u>Cells</u>**
$1/50\ mm^3$ (B+G)	R
$1\ mm^3$ (B+G)	?

<u>Then</u> $1\ mm^3$ (B+G) = 50R

<u>Since</u> $1mm^3$ (B+G) ≡ 1/200 mm3 (B) ----------- (from the pipette equation)

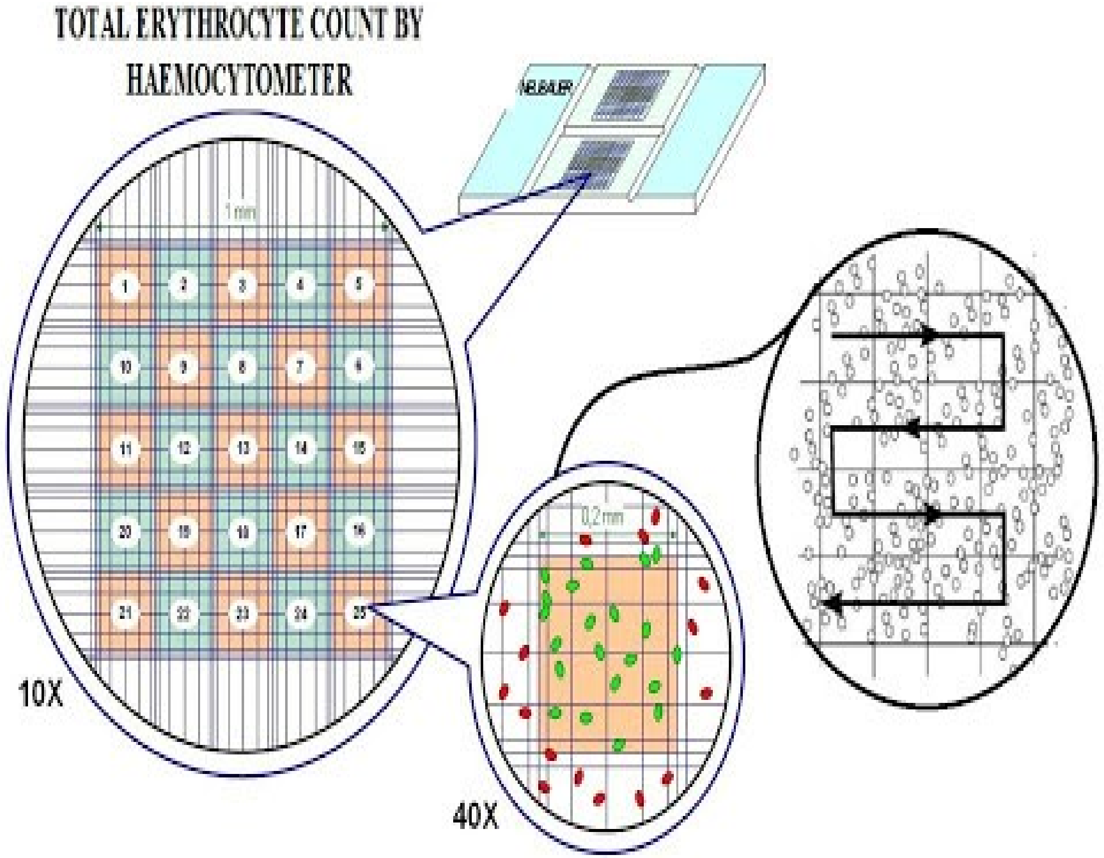


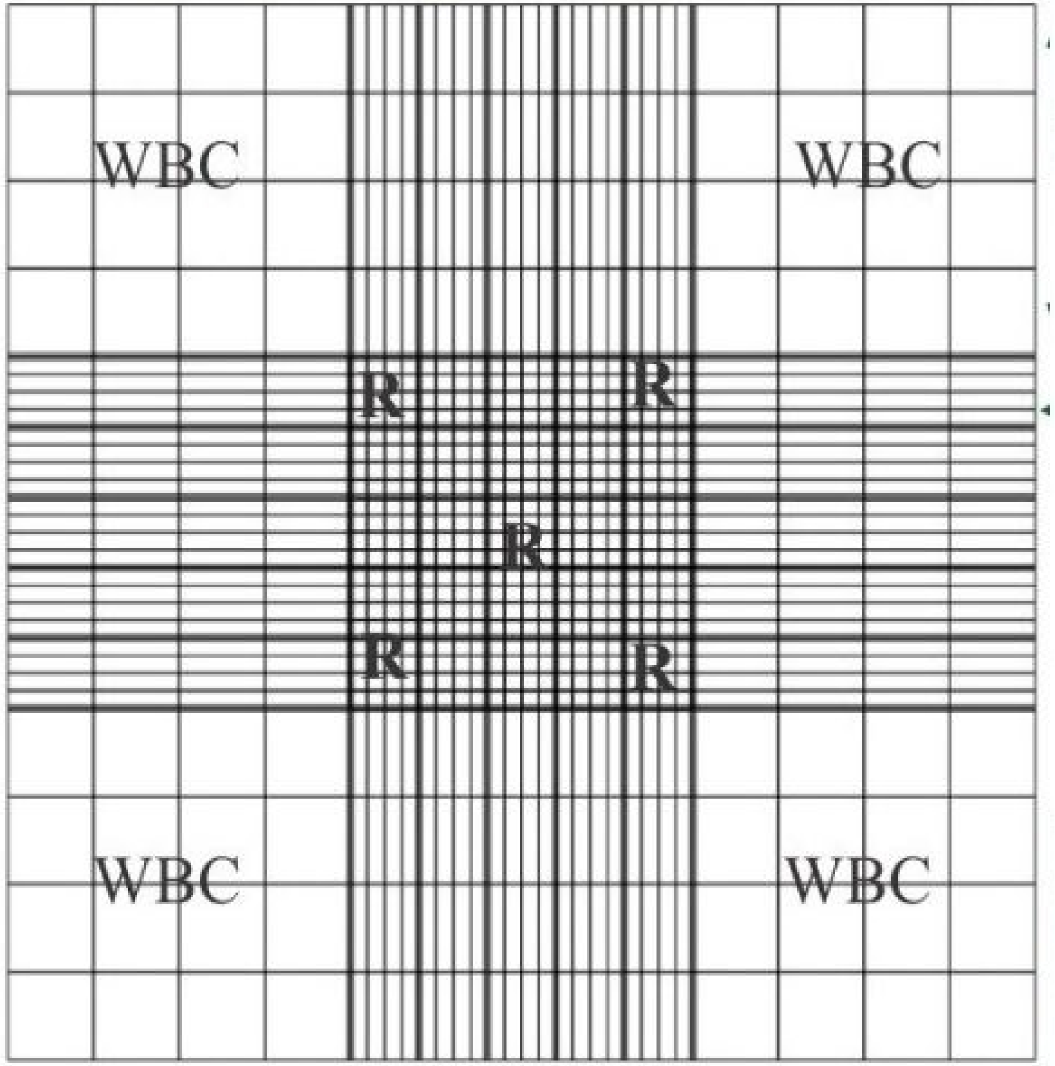
<u>Then:</u>

$1/200\ mm^3$ (B)	50R
$1\ mm^3$ (B)	?

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

R = R1+R2+R3+R4+R5


TOTAL ERYTHROCYTE COUNT BY
HAEMOCYTOMETER
NEUBAUER
1 mm
1
2
3
4
5
10
9
8
7
6
11
12
13
14
15
20
19
18
17
16
21
22
23
24
25
10X
0,2 mm
40X


WBC
WBC
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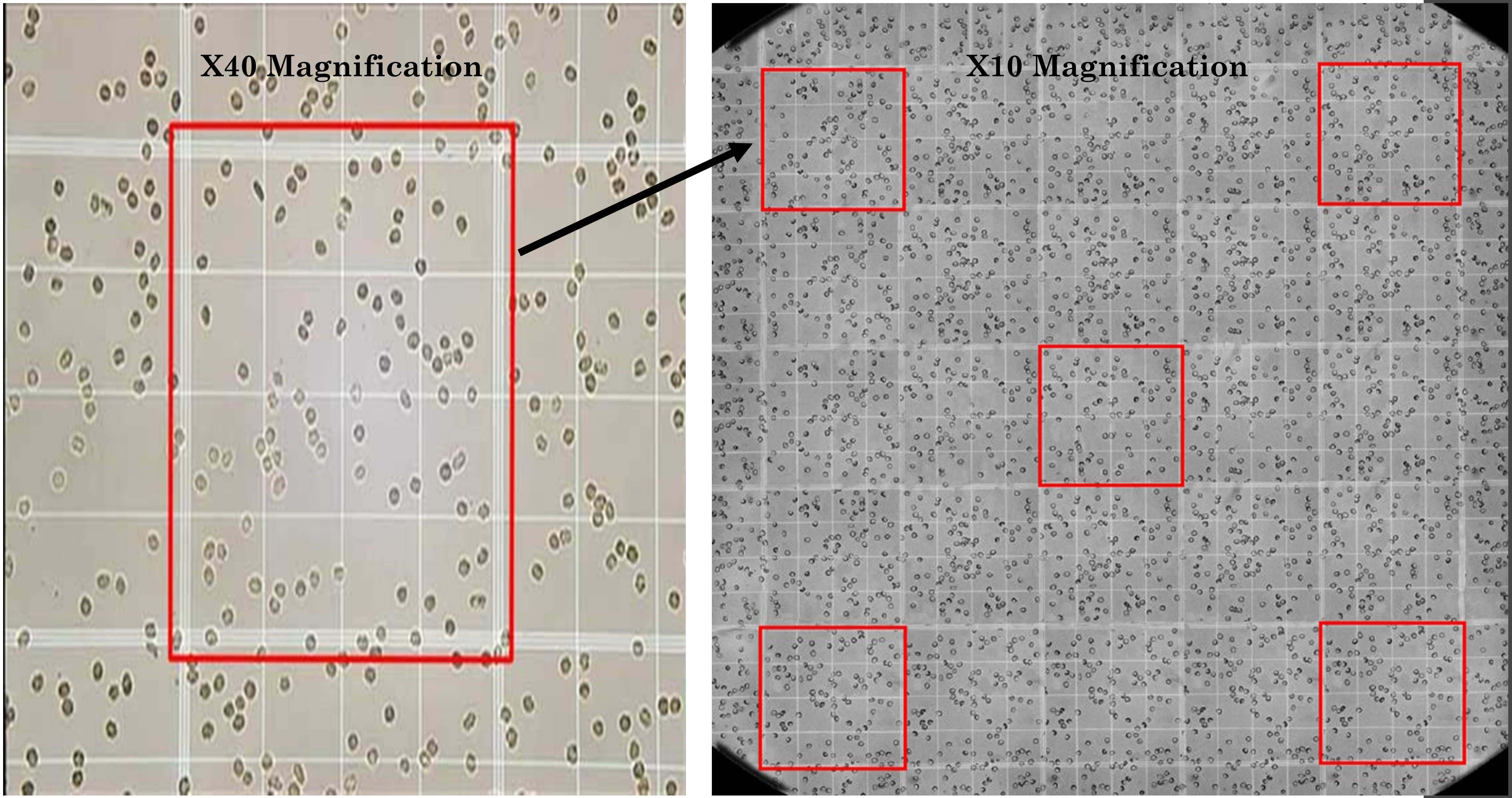
X40 Magnification
X10 Magnification

Example:

- **Determine the RBC counting for a <u>male</u> patient if you have the following readings: (R1= 110, R2= 135, R3= 98, R4= 120, R4=84). State if the result is normal, Anemia or Polycythemia?**

Answer:

R total = R1 + R2 + R3 + R4 + R5

= 110 + 135 + 98 + 120 + 84

= 547

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

Then 10000 * 547 = 5470000 RBCs / $1mm^3$ of blood

The result is **(Normal)** because the normal value for male is:

4.6 – 6.2 million cells / $1mm^3$ of blood

Example:

- **determine the RBC counting for a <u>female</u> patient if the RBCs count of five squares is 285. State if the result is normal, polycythemia or anemia?**

Answer:

R total = 285

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

Then 285 * 10000 = 2850000 RBCs / $1mm^3$ of blood

The result is **(Anemia)** because the normal value for female is:

4.2 – 5.2 million cell / $1mm^3$ of blood


STAY HOME
STAY SAFE