Al-Rasheed University College Pharmacy Department
2nd Stage / 2nd Semester
2021-2022

Total RBCs Counting

Physiology lab #4

Done by:

MSc Mohammed Akram Al-Mahdawi

Introduction

- Red blood cells or erythrocytes are produced from the red bone marrow from precursors called (stem cell)
- The major regulating factor for RBCs production is the oxygen.
- 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
- 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.
- Advantages of biconcave shape;

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

The reaction between CO2 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

Disorders of Erythrocytes

- Anemia: deficiency in either RBCs or hemoglobin or both of them.
- Anemia types:

Nutritional anemia (most common)

Pernicious anemia

Aplastic anemia

Renal anemia

Hemolytic anemia

Hemorrhagic anemia

Polycythemia

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%.

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.

- **1- Nutritional 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

Caused by folic acid deficiency which is required for normal formation of DNA

Deficiency in folic acid causing RBCs to be enlarged and have a shorter life span; they are called macrocytic

Anemia

- **2- Pernicious Anemia:**

Normal development of RBCs requires vitamin B12

Vitamin B12 is called (extrinsic factor).

Parietal cells of the stomach secretes a chemical substance called (intrinsic factor), that prevent vitamin B12 destruction and promote its absorption.

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

- **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

- **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:**

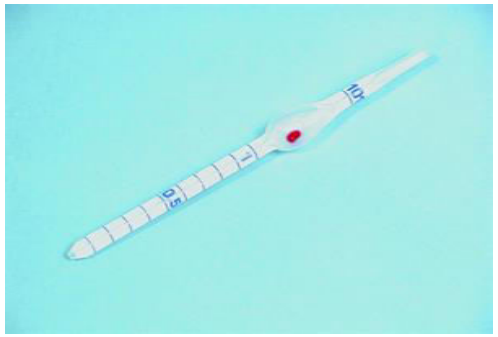
It is caused by rapid loss of blood, it can result from trauma, ulcers or excessive menstrual bleeding.

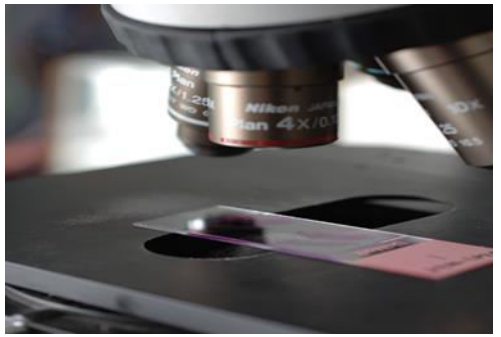
Objective (Aim) of The Experiment:

- 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**

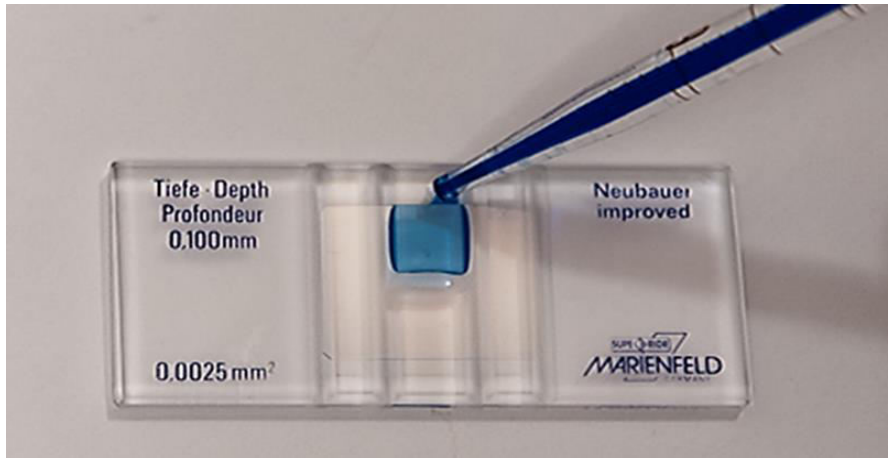
Gower's Solution:

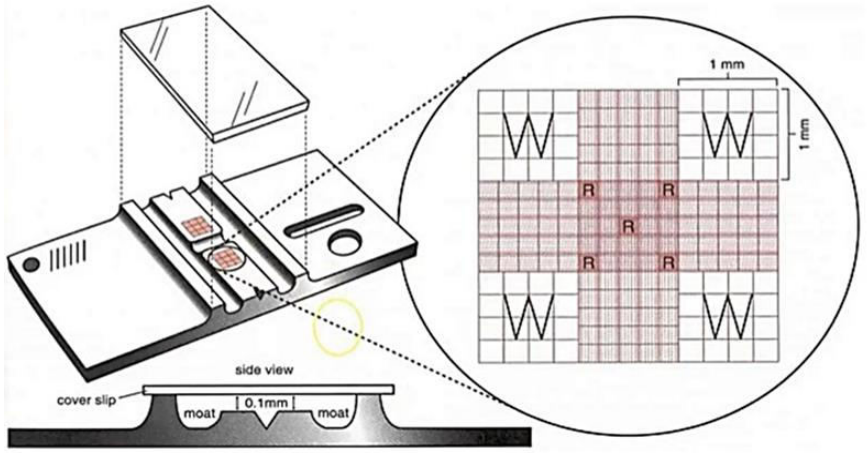
- **Constituents:**
  - Glacial acetic acid 33% (for destruction of WBCs and platelets)
  - Sodium sulfate (as anticoagulant and prevent RBCs hemolysis)
  - Distilled water (for dilution)
- **This type od solution can destroy WBCs and Platelets**

Procedure:

3- Hemocytometer (Counting Chamber)

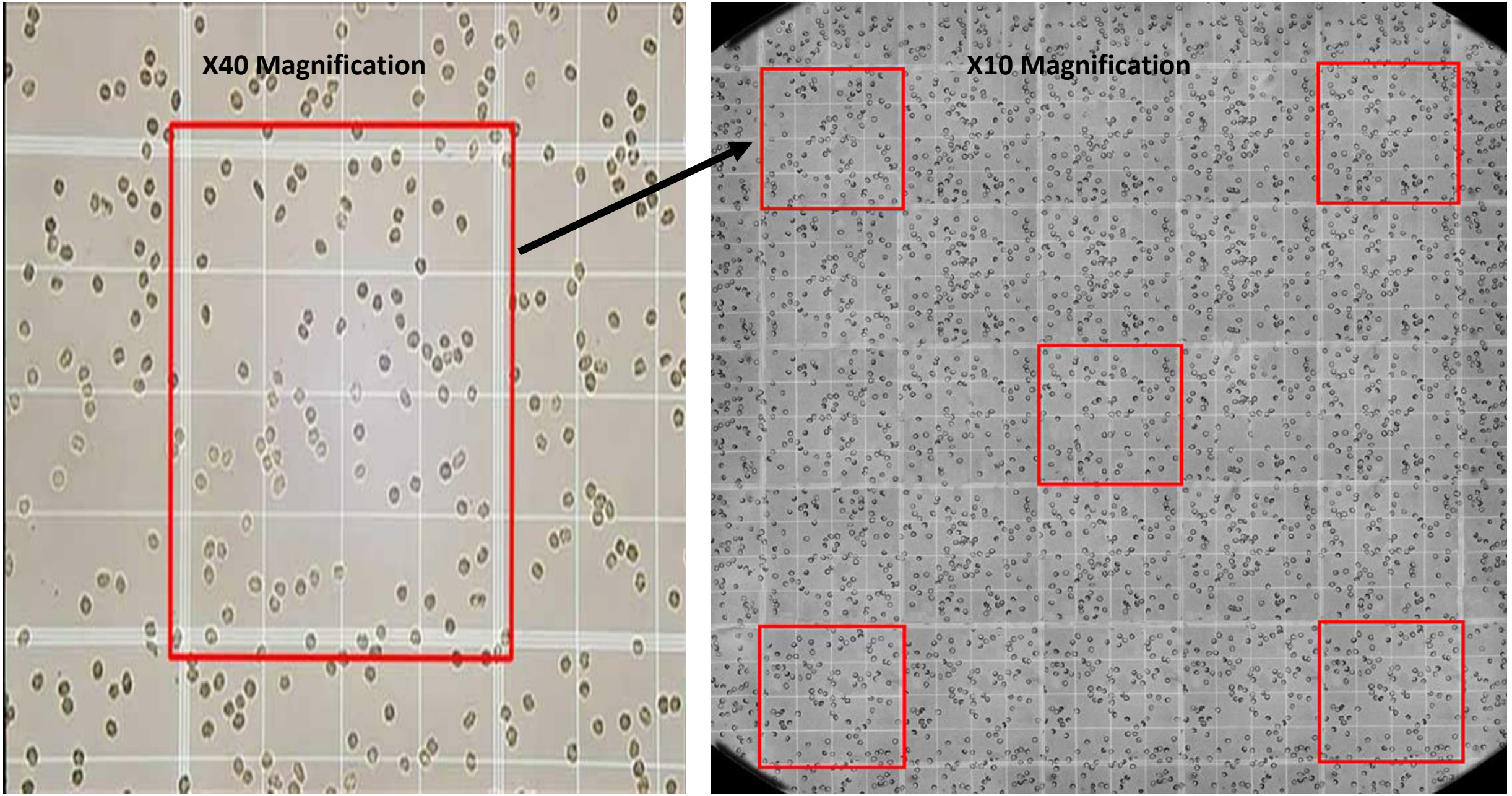
- Hemocytometer is a special slide containing a counting chamber
- It is used for manual measurement of different types of cells in blood and other body fluids
- Used to determine the total number of:
  - WBCs in a blood sample, cerebrospinal fluid sample, and synovial sample
  - RBCs in a blood sample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





Procedure

- 1- Sterilize your finger and then use a lancet to puncture the tip of the finger.
- 2- Draw the blood by using a mouth suction technique by the pipette
- 3- Draw the blood to the 0.5 mark of the pipette
- 4-Dilute with a hayem solution and continue to the 101 mark of the pipette.
- 5-Mix the pipete to dilute the blood properly
- 6- Discharge 1 to 2 drops from the pipette to ensure that mixing has been done properly
- 7- Put a cover glass on the top of the hemocytometer
- 7- Place a 1 drop on each of the tips of the hemocytometer
- 8- Put the hemocytometer on the microscope and first adjust at 10X magnification, then 40X magnification.
- 9- Count the total RBCs inside of the tertiary square.


X40 Magnification
X10 Magnification


Ramadan
Kareem