

Blood and its Components

Blood is an essential fluid carries out the critical functions of transporting oxygen and nutrients to our cells and getting rid of carbon dioxide, ammonia, and other waste products. In addition, it plays a vital role in our immune system and in maintaining a relatively constant body temperature. Four of the most important ones are red blood cells, white blood cells, platelets, and plasma.



Red Blood Cells Shape

•Red blood cells (RBCs) or erythrocytes, are the round shape, biconcave discs, present in the blood that helps in the transport of gases throughout the body.

•The biconcave shape helps the RBCs in rendering the red cells quite flexible so that they can easily pass through the capillaries. On an average, the size of the Red Blood Cells (RBCs) is 7.2 - 7.4 mm (microns).





•The mature RBCs are non-nucleated cells with an Iron-containing pigment known as Hemoglobin which helps in the transport of oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs for excretion.

• The Average lifespan of Red Blood Cells (RBCs) is 100 – 120 days.

• The red blood cell (RBC) count is the number of red blood cells per unit volume of whole blood.

- Normal range of RBCs:
- Adults male: 4.8-7.2 million cells / cu.mm
- adult female :4.9-5.5 million cells / cu.mm
- Pregnancy: slightly lower than normal adult values.
- Children: 3.8-5.5 million cells / cu.mm.

• RBCs are non-nucleated, biconcave discs. The red cell membrane is flexible and exhibits a remarkable deformability.

• The number of RBCs varies with age, sex, and altitude.

• Each RBC has a mean diameter of about 7.2 μ m and thickness of 2.5 μ m at the thickest point and 1 μ m or less at the center.

The main constituent of the RBCs is hemoglobin which enables them to transport oxygen around the circulation. RBCs also contain the carbonic anhydrase enzyme which enables them to carry CO2.



Normal structure of RBC are non-nucleated and biconcave discs





Red blood cells count

- A RBC count is a blood test that tells how many red blood cells (RBCs) you have
- The RBC measurement is used to help diagnose red blood cell disorders, such as anemia.

Purposes of RBCs Count Experiment:

1- As part of a complete blood count (CBC), during a health checkup, or when a healthcare practitioner suspects that you have a condition such as anemia or polycythemia.

2- To evaluate the number of red blood cells (RBCs); to screen for, help diagnose, or monitor conditions affecting red blood cells.

3- To learn how to use the manual method in the lab to get the number of red blood cells.

Method of RBC Count:

Visual haemocytometer (manual method)

Despite the fact of the recent technical development of scientific laboratories, the neubauers chamber remains the most common method used for cell counting around the world.



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Manual RBC Count Materials and Instruments

1. Anticoagulated whole blood (using tube EDTA or heparin as an anticoagulant) or direct capillary blood can be used.

2- Haemocytometer (Neubauers) chamber is counting chamber with cover slide.

3. Hayem's soluton (diluting fluid) composed of:

Mercuric chloride 0.5 g, sodium chloride 0.5g, distal water 200ml

4- RBC pipette which is composed of stem and mixing chamber with a red bead that facilitates the mixing of blood with the diluting fluid (also used to differentiate RBC pipette from WBC pipette).

- 5- light microscope
- 6- lancet
- 7- Alcohol 70%
- 8- Cotton



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Neubauers Chamber

- Neubauer's chamber is a thick glass plate with the size of a glass slide (30mmx70mmx4mm). The counting region consists of two ruled areas. There are depressions or the moats on either side or in between the areas on which the squares are marked thus giving an "H" shape.
- The ruled area is 3mm² divided into 9 large squares each with a 1 mm area. The large central square (which can be seen in its entirely with the 10x objective), is divided into 25 medium squares with double or triple lines. Each of these 25 squares are is again divided into 16 small squares with single lines, so that each of the smallest squares has an area of 1/400 mm²



hemocytometer chamber

Procedure

1- Fill the RBC pipette up to the 0.5 mark with the blood specimen and wipe out the pipette externally to avoid false high results.

2- Fill the same pipette with the RBC diluting fluid (preferably Hayem's Fluid) up to the mark 101.

3- Be cautious that there should be no air bubble in the pipette bulb. pipette (horizontally) between your palms.



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4- Mix the Blood and Diluting fluid in the pipette gently for about 3 minutes, the 3-4 drops discarded and adrop of diluted blood placed into the haemocytometer chamber after cleaning and fixation of special cover slid and then allowed to settle for 1 minuting.

5- Examine the chamber under low power the counting the total number of RBC in five medium squares including the four corners and center one.



Calculation of RBCs:

Total RBC Count = N × Dilution / Area × Depth N × 200 (or 100 as the dilution is made) / $(1/5 \times 0.1)$ Total RBC count = N × 10,000 / mm³

MEDICAL CONSIDERATIONS

Pathological conditions:

A- High RBC count (Polycythemia)

Polycythemia is a disease of unknown origin that results in an abnormal increase in red blood cells due to over production of red blood cells in the bone marrow not caused by physiologic need (primary polycythemia vera), while secondary polycythemia occurs in response to hypoxia. smokers also have a higher number of red blood cells than non-smokers.

B- Low RBC count (Erthropenia)

Anemia: is a general term that refers to a decrease in red blood cells.





Anemia can occur from:

- 1. Red blood cell (RBC) count
- 2. Hemoglobin content
- 3. Packed cell volume (PVC).

Generally, reduction in RBC count, hemoglobin content and PCV occurs because of:

- 1. Decreased production of RBC
- 2. Increased destruction of RBC
- 3. Excess loss of blood from the body.

CLASSIFICATION OF ANEMIA

Anemia is classified by two methods:

- 1. Morphological classification
- 2. Etiological classification.

MORPHOLOGICAL CLASSIFICATION

Morphological classification depends upon the size and color of RBC. Size of RBC is determined by mean corpuscular volume (MCV). Color is determined by mean corpuscular hemoglobin concentration (MCHC). By this method, the anemia is classified into four types

1. Normocytic Normochromic Anemia

Size (MCV) and color (MCHC) of RBCs are normal. But the number of RBC is less.

2. Macrocytic Normochromic Anemia

RBCs are larger in size with normal color. RBC count is less.

3. Macrocytic Hypochromic Anemia

RBCs are larger in size. MCHC is less, so the cells are pale (less colored).

4. Microcytic Hypochromic Anemia

RBCs are smaller in size with less color.





table show the morphological anemia

Type of anemia	Size of RBC (MCV)	Color of RBC (MCHC)
Normocytic normochromic	Normal	Normal
Normocytic hypochromic	Normal	Less
Macrocytic hypochromic	Large	Less
Microcytic hypochromic	Small	Less

ETIOLOGICAL CLASSIFICATION OF ANEMIA

On the basis of etiology (study of cause or origin), anemia is divided into five types:

- 1. Hemorrhagic anemia
- 2. Hemolytic anemia
- 3. Nutrition deficiency anemia
- 4. Aplastic anemia
- 5. Anemia of chronic diseases.

C- Normal Physiological conditions of RBC count

- A normal physiological increase in the RBC count occurs at altitudes or after strenuous physical training high.
- The drug gentamicin have been associated with increasing the number of red blood cells.