Al Muthanna University College of Pharmacy

Red blood cell counting

By

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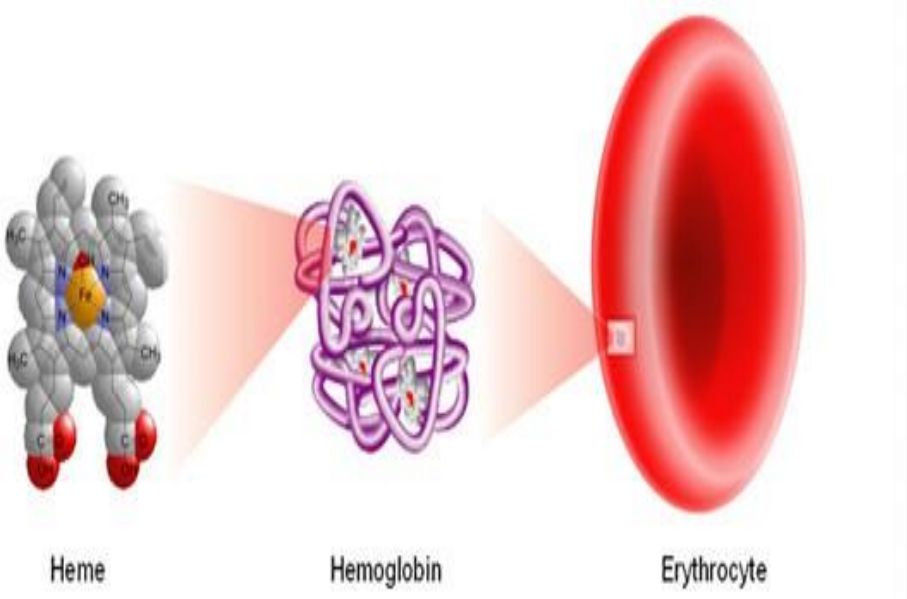
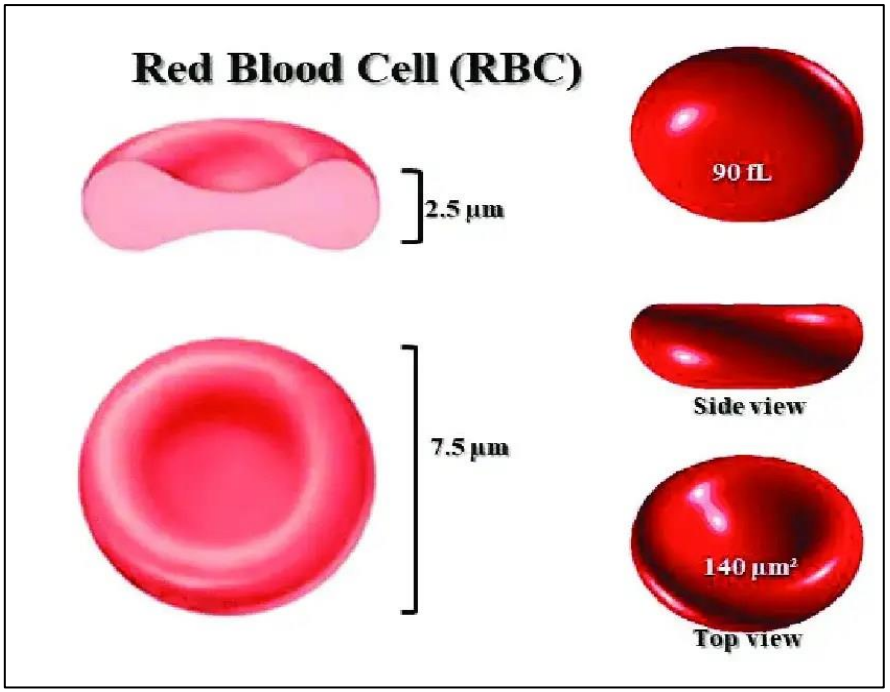
Second Stage



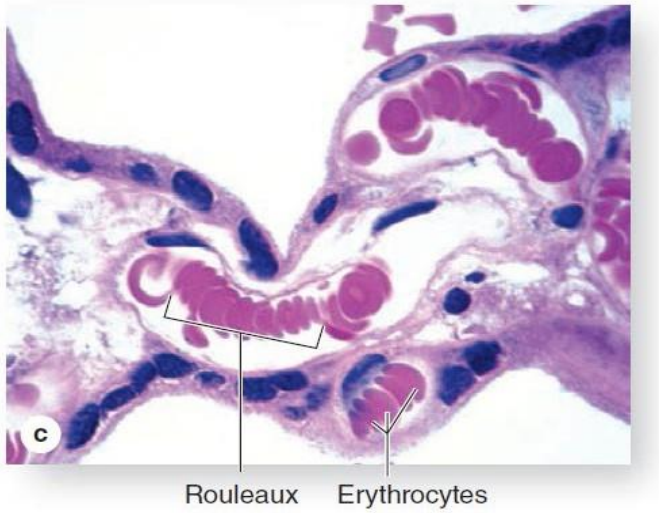
Introduction

Red blood cells (erythrocytes) are no nucleated, biconcave discs. The red cell membranes flexible. Each RBC has a mean diameter of about 7.5µm and thickness of 2.5µm at the thickest point and 1µm or less at the center.

Erythrocyte cytoplasm lacks all organelles but is densely filled with **hemoglobin** which enables them to transport oxygen around the circulation. RBCs also contain the **carbonic anhydrase enzyme** which enables them to carry CO2.



- The biconcave shape provides a large surface-to-volume ratio and facilitates gas exchange.
- Erythrocytes are normally quite flexible, which permits them to bend and adapt to the small diameters and irregular turns of capillaries.
- In larger blood vessels RBCs may adhere to one another loosely in stacks called rouleaux.



- Erythrocytes are Lacking mitochondria, and they depend on anaerobic glycolysis for their minimal energy needs. Lacking nuclei, they cannot replace defective proteins.

Human erythrocytes normally survive in the circulation for about **120 days**. By this time defects in the membrane's cytoskeletal meshwork or ion transport systems begin to produce swelling or other shape abnormalities. Old or worn-out RBCs displaying such changes are recognized and removed from circulation, mainly by macrophages of the spleen, liver, and bone marrow.

The process of RBC development from stem cells takes about **7 days** and is called **erythropoiesis**.

It takes about **20 seconds** for a red blood cell to circle the whole body.

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

Normal range of RBCs:

Adults: 4.8-7.2 million (**male**) and 4.9-5.5 million (**female**).

Pregnancy: slightly lower than normal adult values.

Children: 3.8-5.5 million.

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

Purposes of RBC 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

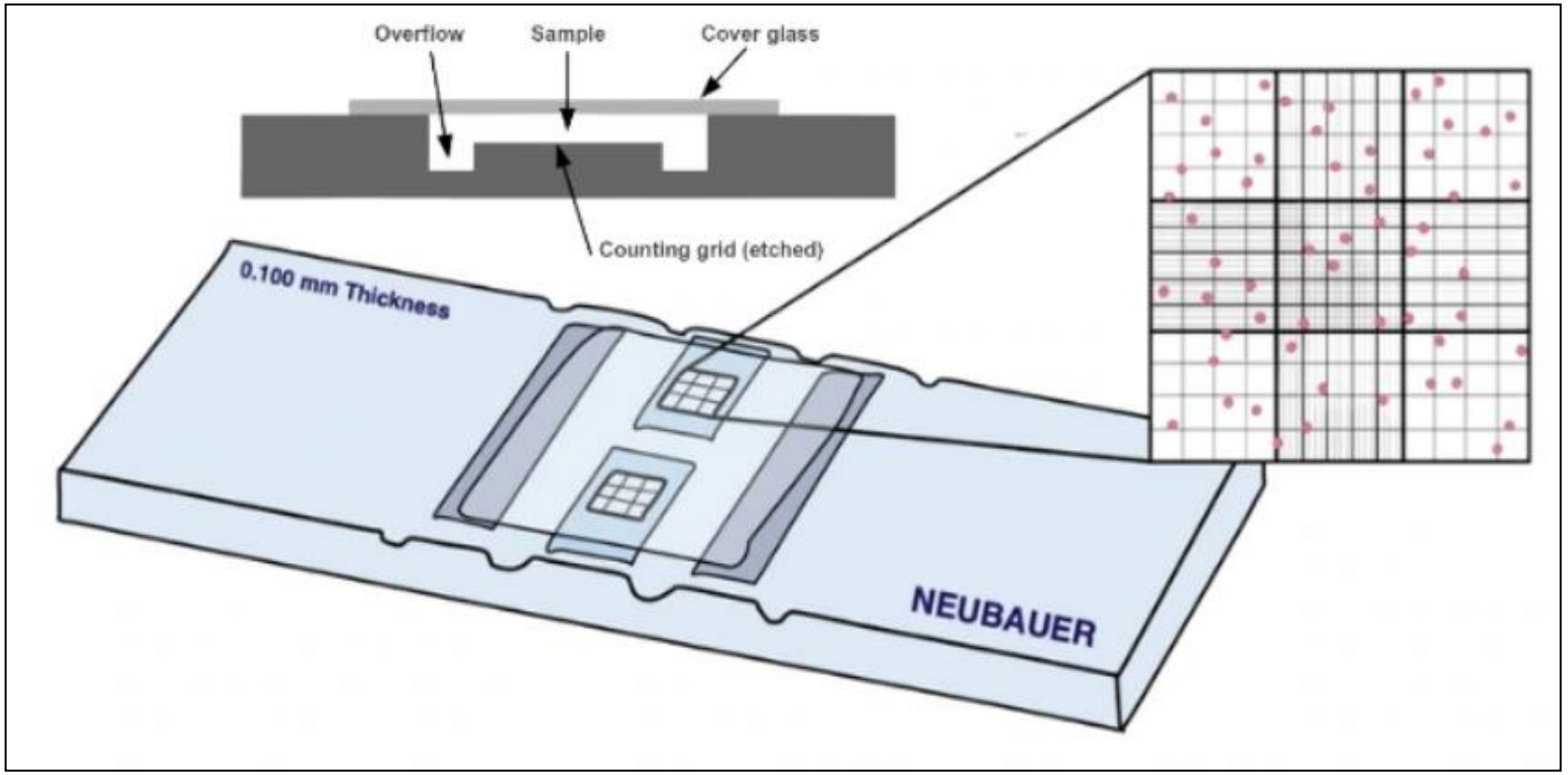
To evaluate the number of red blood cells (RBCs); to screen for, help diagnose, or monitor conditions affecting red blood cells.

3

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

Methods of RBC count

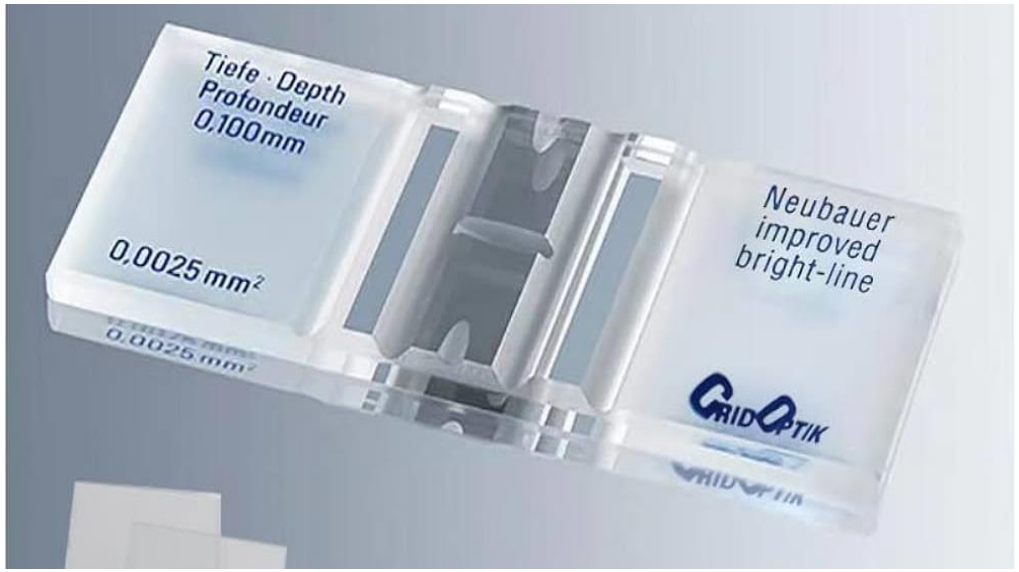
1. Manual Method: Despite the fact of the recent technical development of scientific laboratories, the **Neubauer chamber** remains the most common method used for cell counting around the world.



2. Automated (Electronic Cell Counting) Method: This technique uses changes in electrical resistance to count cells and provide an assessment of cell volume (depending on the counter). It is used to measure RBC in blood samples as well as samples of body cavity fluids (peritoneal, pleural).

Manual RBC count materials and instruments

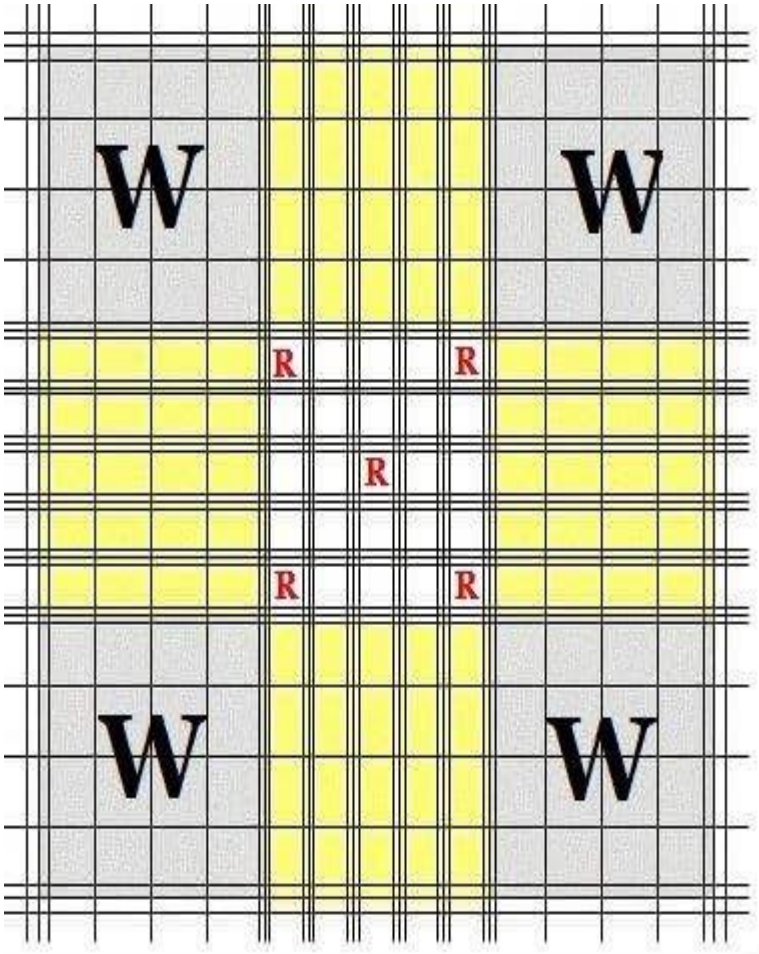
1. Anticoagulated whole blood (using EDTA or heparin as an anticoagulant) or capillary blood can be used.
2. Hayem's solution (diluting fluid)
3. RBC pipette which is composed of stem and mixing chamber with a red bead that facilitates the mixing of blood with the diluting fluid (also used to differentiate RBC pipette from WBC pipette).
4. Haemocytometer **"Neubauer" chamber** is the counting chamber with a cover slide.
5. Microscope
6. Lancet, Alcohol 70%, Cotton



Neubauer chamber

Neubauer's chamber is a thick glass plate with the size of a glass slide. The counting region consists of two ruled areas. There are depressions or the moats on either side or in between the are as on which the squares are marked thus giving an "H" shape.

The ruled area consist of 9 large squares. The large central square (which can be seen in its entirely with the10x objective), is divided in to 25 medium squares with double or triple lines. Each of these 25 squares are is again divided into 16 small squares with single lines.



Procedure

1. Wipe your partner’s finger with cotton soaked with alcohol and allow it to dry. With a sterilized disposable lancet, make a small prick on the finger tip. When a drop of reasonable size gets collected, hold the RBC pipette slightly tilted from the vertical position, apply its tip to the drop, and aspirate blood to the 0.5 mark.
2. Wipe off any blood adhering to its outer side. If the blood gets beyond 0.5 mark, tap the tip gently till the blood is exactly at the 0.5 mark. **Never allow the blood to clot inside the pipette**, if this happened then you have to blow the sample out, clean the pipette, and start over again.
3. Aspirate the diluting fluid to101 mark, thus making 1:200 dilution of blood.
4. Hold the pipette horizontally and shake it with both hands between index finger and thumb for 2-3min.
5. Blow out quarter of the contents to remove the pure diluting fluid in the stem.

6. Prepare the counting chamber and cover it with a cover slide.

7. Hold the pipette 45° and touch its tip gently on the surface of the counting platform where it projects beyond the coverslip and a small amount will be drawn under the coverslip.

8. Place the Neubauer chamber on the stage of the microscope and leave it for 2min to allow the blood cells to get settled.

9. Scan the counting area with 10x objective lens.

10. Use the 40x objective lens, include all the cells lying on the lower and left lines of any square, omit the cells on the upper and right lines.

11. Count the cells in the 16 small squares of the 5 large squares of RBC (80 small squares), one at each corner and one in the center.

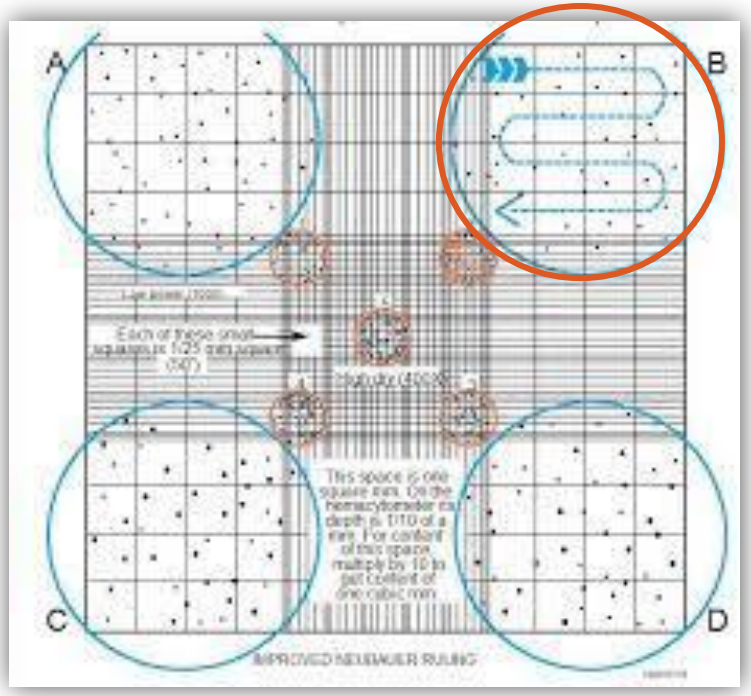
Calculation

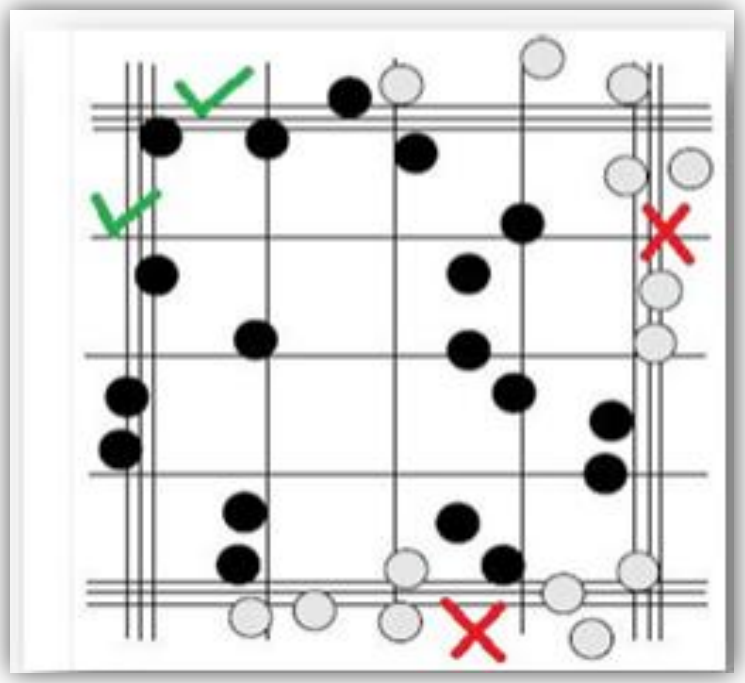
- Count the number of RBCs (N) in 80 small squares that are located in the 5 middle size squares (four at the corners and one in the middle).
- The volume of the 80 small squares in which ”N” of cells will be counted is:

1/20×1/20×1/10×80=1/50mm3

Where **1/20 mm** is side line of the square, **1/10** mm is the depth of the counting chamber between the coverslip and the ruling area, and 80 is the number of the small squares in the 5 large squares of RBC (16×5).

- Thus, the total number of cells in 1mm3 is =N×50 (diluted sample)
- The actual total number of cells before the dilution should be: N×50×200=N×10000


A
B
C
D
Each of these small squares is 1/25 mm square (1/25)
This space is one square mm. On the hemacytometer its depth is 1/10 of a mm. For content of this space multiply by 10 to get content of one cubic mm.
IMPROVED NEUBAUER RULING



Notes:

- Males have a RBC count more than females because of many factors such as: the male hormone “androgen”, the large muscle mass of males that need more oxygen, and females loss an amount of blood during the menstrual cycle.
- Medications such as **gentamicin** and **methyldopa** have been associated with an increase in the number of RBCs.
- Hydration is an important consideration when caring of patients with abnormally high RBCs. Too high RBCs mass slows down velocity of the blood and increases the risk of intravascular clotting.

Clinically Terms

Anemia is the condition of reduction in number of erythrocytes below the normal range. With fewer RBCs per milliliter of blood, tissues are unable to receive adequate O2. Symptoms of anemia include lethargy, shortness of breath, fatigue, skin pallor, and heart palpitations.

Sickle cell anemia is caused by a homozygous mutation causing an amino acid substitution in hemoglobin, which renders the mature RBCs deformed and slightly rigid and can lead to capillary blockage.

Polycythemia or Erythrocytosis is the condition of increased number of erythrocytes in blood. It could be normal condition as a physiologic adaptation found, for example, in individuals who live at high altitudes, where O2 tension is low.

Any Question