University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Red Blood Cell (RBC) Count

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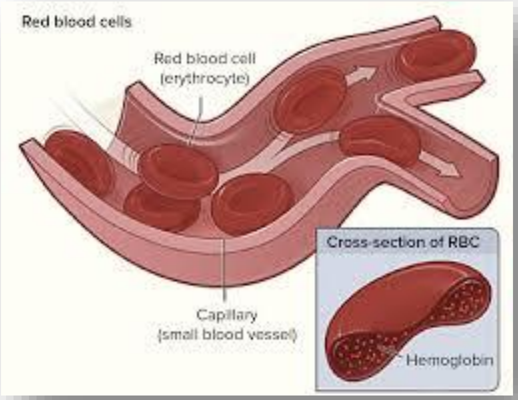

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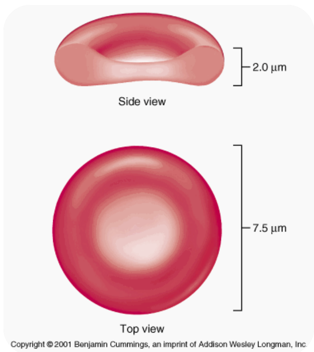
Introduction

- ***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.

- ***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 µm and thickness of 2.5 µm at the thickest point and 1 µm 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 CO_2.

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

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.

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 soluton (diluting fluid) composed of:

- $HgCl_2$ 0.05g
- Na_2SO_4 2.5g
- NaCl 0.5g
- Distilled water 100 ml

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).

1. Haemocytometer "Neubauer" chamber is the counting chamber with a cover slip. The same counting chamber is also used for counting total white blood cells.
2. Microscope
3. Lancet
4. Alcohol 70%
5. Cotton

Manual RBC Count Materials and Instruments

RBC Diluting Fluid

Microscope

RBC Pipette

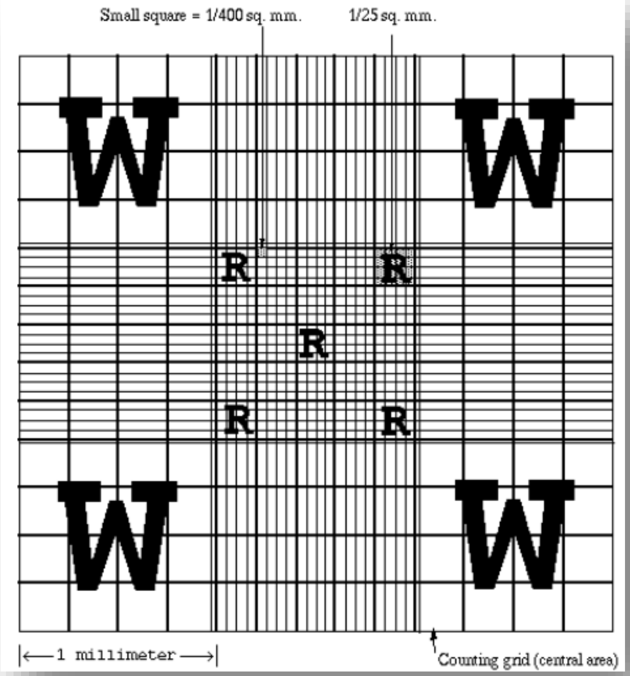
Neubauer Chamber

Alcohol Pad

Neubauer 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 3mm^2 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 mm^2 .

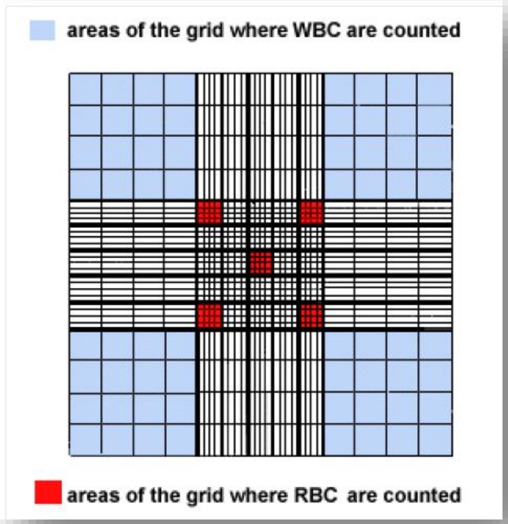


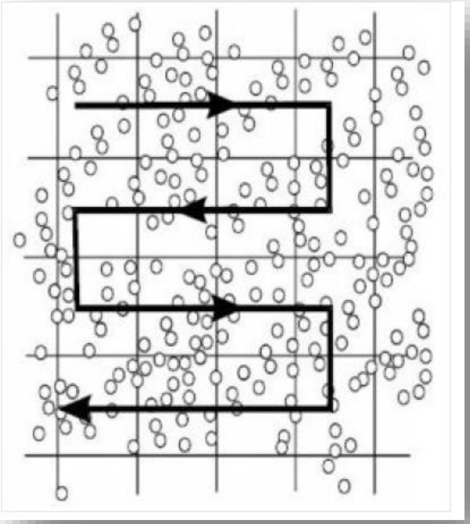
Procedure

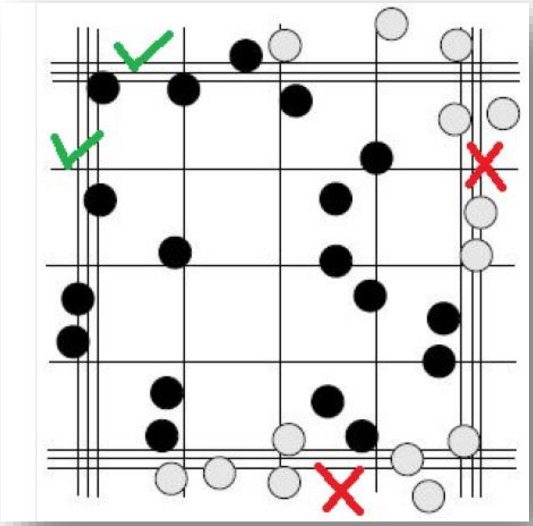
- Wipe your partner's finger with cotton soaked with alcohol and allow it to dry. With a sterilized disposable lancet , make a small prick on the finger tip. When a drop of reasonable size gets collected , hold the RBC pipette slightly tilted from the vertical position , apply its tip to the drop, and aspirate blood to the 0.5 mark.
- 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.
- Aspirate the diluting fluid to 101 mark, thus making 1: 200 dilution of blood.
- Hold the pipette horizontally and shake it with both hands between index finger and thumb for 2-3 min.
- Blow out quarter of the contents to remove the pure diluting fluid in the stem.
- Prepare the counting chamber and cover it with a cover slip.
- Hold the pipette 45° and touch its tip gently on the surface of the counting platform where it projects beyond the cover slip and a small amount will be drawn under the cover slip.

Procedure

- Place the Neubauer chamber on the stage of the microscope and leave it for 2 min to allow the blood cells to get settled.
- Scan the counting area with a 10x objective lens.
- 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.
- 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 :

$1/20 \times 1/20 \times 1/10 \times 80 = 1/50\ mm^3$

Where 1/20 mm is sideline 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 1 mm^3 is $= N \times 50$ (diluted sample)
- ✓ The actual total number of cells before the dilution should be :
- ✓ $N \times 50 \times 200 = N \times 10000$

Sources of Errors

- Errors in dilution, counting, or calculation.
- Inadequate or improper shaking of the pipette after dilution.
- Failure to wipe excess blood from the end of the pipette.
- Drying of sample during or prior to counting.
- Overflow of fluid into moat.

Sources of Errors

- Improper adjustment of light source.
- Failure to allow cells to settle into a single plane prior to counting.
- Inadequate cleaning of glass wares.
- Failure to discharge diluting fluid from the capillary prior to charging the chamber.
- Failure to focus microscope up and down during counting.

Some Medical Consideration

- The medical condition in which RBC count decreases (lower than the normal value) is called ***anemia***. The condition in which RBC count increases (more than normal) is called ***polycythemia***.
- 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.
- There is a normal physiological increase in RBC count at high altitudes and also after strenuous physical training . At high altitudes, less atmospheric weight pushes air into the lungs causing a decrease in the partial pressure of oxygen and hypoxia. With the strenuous physical training, increased muscle demands more oxygen.
- Medications such as gentamicin and methyldopa have been associated with an increase in the number of RBCs.
- Smokers have a higher number of RBCs than non-smokers.

Some Medical Consideration

- There are also some pathological reasons that lead to an increase in RBCs. Polycythemia Vera is a disease of unknown origin that results in an abnormal increases in RBCs. ***Polycythemia Vera*** also called a "Primary Polycythemia" because the overproduction of RBCs does not come from hypoxia. The term "Vera" means true, thus polycythemia Vera refers specifically to the overproduction of RBCs in the bone marrow not caused by a physiological need. Polycythemia Vera is treated by a radioactive phosphorus to slow down the bone marrow overproduction 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.
- Examples of "***secondary polycythemia***" that occurs in response to hypoxia are : chronic lung disease in adults and children with congenital heart defects characterized by cyanosis.

Some Medical Consideration

- ***Anemia*** is a general term that refers to a decrease in the number of RBCs . Actually it can occur from a decrease in the number of RBC, or Hemoglobin, or both.

- ***A lower than normal RBCs can result from a number of causes including:***

- Massive RBCs loss as in acute hemorrhage .
- Bone marrow failure.
- Erythropoietin deficiency, which is the primary cause of anemia in patients with chronic kidney disease.
- Hemolysis, or RBC destruction caused by transfusions and blood vessel injury.
- Malnutrition.
- Nutritional deficiencies, including deficiencies in iron, folate, and vitamins B-6 and B-12.
- Pregnancy.
- Thyroid disorders.
- Chemotherapy or radiation side effects from treatment of bone marrow malignancies such as leukemia that can result in bone marrow suppression.

Fascinating Fact