Red blood cell (RBC) count

Introduction and principle: The red blood cell count is the number of red blood cells per unit volume of whole blood.

Normal red blood cells values are:

- **Adults: (males): 4.9 - 5.5 millions / mm^3** .
- **(Females): 4.4 - 5.0 millions / mm3 .**
- **Newborns: 4.8 - 7.2 million**
- **Pregnancy: slightly lower than normal adult values**
- **Children: 3.8 – 5.5 million**

Each RBC is a biconcave disc having a diameter of 7.2 microns and a thickness of 2.2 microns. These cells contain the pigment hemoglobin which enables them to transport oxygen around the circulation. They also contain the enzyme **carbonic anhydrase** which enables them to carry CO_2. RBC count is done to determine whether there is an adequate number of RBC in the circulation or not.

Methods

1. Manual method
2. Electronic cell counting

Manual Red blood cell count material and instruments

1. Anticoagulated whole blood (using EDTA or heparin as an anticoagulant) or direct capillary blood can be used.
2. Hayem's solution (diluting fluid) composed of:
   - **$HgCl_2$ 0.05 g → to fix the cells and act as a preservative**
   - **$Na2SO_4$ 2.5 g → to prevent Rouleaux formation**
   - **NaCl 0.5 g → to maintain isotonicity**
   - **Distilled water 100 ml**
3. RBC pipette: which is composed of a stem & a mixing chamber with a red bead, its function is to mix blood with the substance and for differentiation from the WBC pipette.
4. Haemocytometer "Neubauer" chamber is counting chamber with a cover slip. The same counting chamber is used also for counting total White blood cells.
5. Microscope
6. Lancet
7. Alcohol 70%
8. Cotton
9.

Procedure:

- Wipe your partner's finger with cotton soaked with alcohol and allow it to dry. With a sterile disposable lancet do small prick on the finger tip, when a drop of reasonable size

has collected, hold the red cell pipette slightly tilted from the vertical position, apply its tip to the drop and aspirate blood to the mark 0.5.

- Wipe off any blood adhering to its outer side (be careful not to touch the tip itself).

If the blood gets beyond 0.5 marks tap the tip gently till the blood is exactly at the mark. Never allow the blood to clot inside the pipette. If the blood clots in the pipette blow the sample out, clean the pipette and begin all over again.

- Aspirate diluting Hayem's solution to the 101 mark, thus making 1:200 dilution of blood.
- Hold the pipette horizontally and role it with both hands between index finger and thumb.
- Blow out a 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^{o} & Touch its tip gently on the surface of the counting platform where it projects beyond the cover slip and a small amount of solution will be drawn under the cover slip.
- Place the Neubauer chamber on the stage of the microscope and allow the cells to settle for 2 minutes.
- Scan the counting area with 10X objective lens.
- Use the 40X objective, include all cells lying on the lower and left lines of any square; omit the cells on the upper & right hand lines.
- Count the cells in 5 medium squares of 16 small square i.e. 80 small squares, one at each corner and one in the center

PRECAUTIONS

1. The pipette must be dry and free from clotted blood and the bead must roll freely.
2. The pipette must be almost horizontal while filling it with blood. The tip must not press against the subject's skin or be lifted out of the blood drop (otherwise air will enter it).
3. The dilution of blood must begin immediately after the blood is drawn or else the blood may clot.
4. Leakage of fluid from the pipette must be avoided while mixing the bulb contents.
5. After charging the hemocytometer, the pipette must be washed in running water following which alcohol is used to remove water. Finally, the pipette is washed with acetone to remove the alcohol and left to dry.
7. Finger should not be squeezed once pricked, because the interstitial fluid will mix with blood and give wrong value.
8. As soon as the blood is drawn into the pipette, it should be immediately diluted with the RBC diluting fluid to prevent clotting.
9. There should not be any air bubbles in the pipette when blood or diluting fluid is sucked.
10. Do not tilt the microscope.
11. Cover slip should be placed uniformly over the ridges.
12. During charging care should be taken to prevent fluid from entering into the trenches

Calculation

Count the number (N) of cells in 80 small squares located in 5 medium-sized squares (four located at the four corners and one in the middle). The size of 80 small squares in which "N" numbers of cells are found is:

1/20 x 1/20 x 1/10 x 80 = 1/50 $mm^{3.}$

Where 1/20 mm is the sideline of the square, 1/10 mm is the depth of the counting chamber between coverslip and the ruling, 80 is the number of small squares used to count.

Therefore the total numbers of cells in 1 mm^3 are = N x 50 (diluted sample)

The actual total number of cells before dilution should be:

N x 50 x 200 = **N x 10000**

Medical considerations

Medical condition in which a decrease in Hb/or RBC count is called **anemia** while the condition of increased number of RBC is called **polycythemia.**

- Males have a RBC count more than females due to many factors like the male hormone "androgen" and also due to the large muscle mass in males that need more O_2. Another factor is that the female loses an amount of blood during the menstrual cycle.

Polycythemia is of two types:

- **Primary polycythemia** " **Polycythemia Vera** " is a disease of unknown origin that results in an abnormal increase in red blood cells. In Polycythemia Vera, the overproduction of red blood cells does not result from hypoxia or a physiologic need. Hydration is an important consideration when caring for patients with abnormally high red blood cell counts. Very high RBC mass will slow blood velocity and increase the risk of intravascular clotting.
- **Secondary polycythemia**, occurs in response to hypoxia, are chronic lung disease in adults and children with congenital heart defects characterized by cyanosis. A normal physiological increase in the RBC count occurs at high altitudes. At high altitudes, less atmospheric oxygen is pushing into the lungs, causing a decrease in the partial pressure of oxygen and hypoxia. In addition, after strenuous physical training, RBC count is increased. It is unclear what causes the increase in red blood cells with exercise. Furthermore, smokers also have a higher number of red blood cells than non-smokers.
- a. Emphysema.
- b. Congenital heart disease.
- c. Poisoning by chemicals like arsenic and phosphorus.
- d. Repeated small hemorrhages.
- e. High altitudes.

A lower than normal RBC (**Anemia**) can result from a number of causes, including:

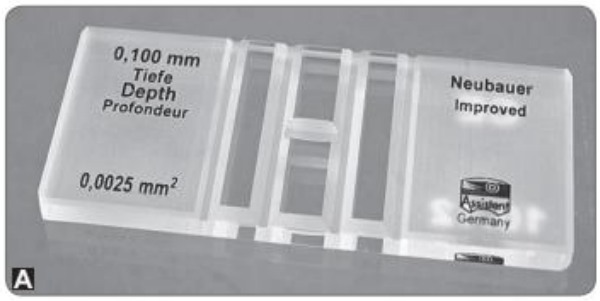
- Massive RBC loss, such as acute hemorrhage

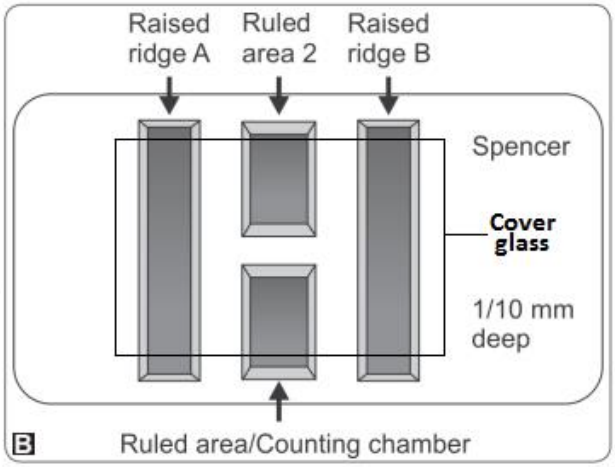
- Excessive destruction of abnormally rigid red blood cells
- Lack of substances needed for RBC production such as B12,Iron ,folic acid deficiencies & erythropoietin deficiency in chronic renal failure.
- Chemotherapy or radiation side effects from treatment of malignancies can result in bone marrow suppression.

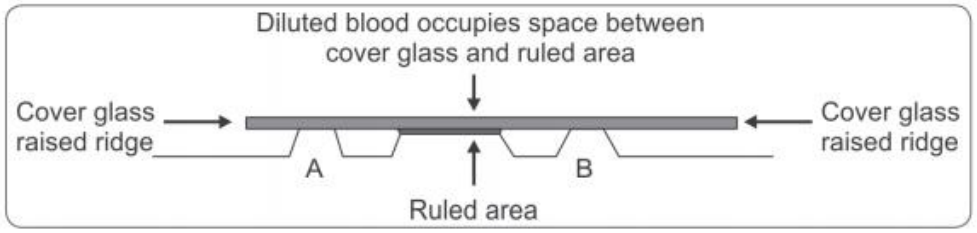
Anemia can occur from either a decrease in the number of red blood cells, a decrease in the hemoglobin content, or both.

Physiological Variations

1. Diurnal variation: Variation of about 5% may occur per period of 24 hours. The count is least during sleep and maximum during evening.
2. Muscular exercise: The count increases temporarily due to hypoxic stimulus.
3. Altitudes: RBC count increases with increase in altitude.
4. High external temperature: RBC count increases with increase of temperature.
5. RBC count also depends on the age and sex of a person.







Neubauer counting chamber and grids