Erythrocytes are incomplete cells, lacking nucleus, unable to synthesize proteins which explains the limited lifetime (120 days). It has a biconcave disc shape that is the adaptation to O₂ transport function (great area for a low volume). Erythrocyte count is a current clinical exploration. It may be performed by two methods: microscopic (in hemocytometer) and electronic method.

Microscopic method

PRINCIPLE: the red cells are counted in a known volume of blood that has been diluted to a known proportion. The number of erythrocytes is calculated per mm³.

MATERIALS REQUIRED: needles, cotton, alcohol, Hayek solution, Thoma counting chamber, pipette Potain for collecting and dilution of the blood. Hayem solution is a hypertonic solution required to stabilize erythrocytes; it contains:

- Sodium chloride...... 1 g
- Sodium sulphate ....... 5 g
- Mercury dichloride...... 0.5 g
- Distilled water ...... 200 ml

You can also use saline solution.

Potain pipette consists of a graduated capillary tube with a bubble containing a red pearl. The capillary part under the dilution bubble is divided into 10 units and upon the bubble the division 101 is marked. The volume of the capillary part is 100 times smaller than the volume of the bubble. When the blood is drawn to the 0, 5 marks and the diluting fluid to the 101 mark, the dilution of the blood is 1/200. The dilution is 1/100 if the blood is drawn to the 1 mark. The solution left in the stem of the pipette is not mixed with the solution in the bulb and therefore must be discarded before the mixture is placed in the counting chamber.

The counting chamber is a thick glass slide on which a network with known size is marked. The slide is made thus, that by applying a cover glass, between the surface on which the network is marked and the cover glass surface, there is a space of 1/10 mm height.
Figure no. The counting chamber (a) front view, (b) top view.

The network of Thoma chamber is a square having an area of 1 mm² and is divided by triple lines into 16 squares with 1/5 mm side. Each such a square (1/5) is subdivided into another 16 squares with 1/20 mm side.

TECHNIQUE
1. The counting chamber and the cover glass must be very clean. This is why both of them are wiped with clean gauze. The cover glass is then applied on the chamber.
2. The chamber is put on the microscope scale. The surface of the network will be brought into the optical axis of the microscope. Moving the macrovisa, the objective of the microscope is lowered near the surface of the coverglass (the object lens 10X or 20X). Looking through the microscope, the objective is raised with the help of the macrovisa till the image of the network appears. The chamber is checked to be clean.
3. The pulp of the finger is disinfected with alcohol and is pricked with sterile needle. The first blood drop is wiped away, the blood is drawn up promptly and exactly to the 0.5 (or 1.0) mark and diluted immediately (the dilution fluid is drawn up to the 101 mark). If there has been a slight excess of blood drawn up, this may be removed by touching the tip of the pipette lightly with a piece of gauze.
4. The pipette tip is closed with the forefinger, the rubber tube (which has been used for aspiration) is bent and the pipette is fixed with the thumb. The pipette fixed this way is shaken immediately for about 2-3 minutes to facilitate the mixing. The first 2-3 drops from the pipette are discarded to eliminate the cell-free fluid from the capillary tube.
5. A drop of diluted blood is put at the orifice made by the coverslip and counting chamber. The chamber is filled by capillary action, the fluid being allowed to enter in a controlled manner so that it spreads slowly and evenly over the entire surface.
6. Moving the micro visa until the image becomes clear; it is checked if there are air bubbles. The procedure must be repeated in this case.
The chamber is allowed to stand for a few minutes to permit settling of the red cells.
7. The erythrocytes are counted in 5 squares with the side of 1/5 mm (since each of these squares contains 16 small squares, the red cells are counted in a total of 80 small squares - with the side of 1/20 mm). The four large squares in the corners and a central large square are counted. For any group of 16 small squares, the cells are counted in each small square, including in the count those cells that touch any one of the three lines or the single line on the left-hand and the top borders of the squares, but excluding those cells that any of the lines on the right-hand and bottom borders of the square. The cells are counted in each small square, first from left to the right beginning with the top row of four small squares, then from right to
the left for the next row, and so on. The number of cells for each of the 5 groups of 16 squares is recorded separately and the results are added.

![Network chamber included: the center type Thoma](image1)

**Calculation of the red-cell count**

Calculated: \[ \frac{N}{n \times d \times v} \]

N = number of erythrocytes counted
n = number of squares read (80)
d = dilution (1/200)
V = volume of a square = \( \frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} = \frac{1}{4000} \)
X = N \times 10000 / mm³

![Loading counting chamber](image2)
**INTERPRETATION**

Normal values are between:

- 4 to 5 million / mm³ for women
- 5 to 5.5 million / mm³ for men

Physiological changes in the number of erythrocytes are subject to:

**Physiological variations:**

1. **Sex.** The number is increased at male because the sexual male hormones—testosterone—stimulate the erythropoietin secretion and production of proteins.

2. **Age.** The new-born baby has a physiological polycythemia in the first 1-3 days after the birth. This is a sham polycythemia explained by the fact that the baby does not drink enough milk, but in the same time the water is lost through perspiration, urine and a hem concentration occurs. After 6-12 weeks an anemia is present because of the fetal Hemoglobin (HbF) and this persists until the fifth month.

3. **Altitude.** The people who live for instance at 5000 m height (mountains) have 7.0-7.5 mil erythrocytes/mm³. The explanation of this increased number of red blood cells is the hypoxia which stimulates the erythropoiesis.

4. **Exercise.** The increased RBC number in the period of exercise is due to two mechanisms:
   a) The permeability of the capillaries increases and the water is lost in the interstitium (hemoconcentration).
   b) The spleen contracts and throws erythrocytes in circulation (the spleen hematocrit is 70-80%)

5. **Digestion.** After eating, the water is absorbed in the blood and decreases the RBC number.

**Pathological changes**

The increase of the erythrocyte number over 6.0-6.5 mill. /mm³ is called polycythemia or polyglobulia. Polyglobulia is of two types.

a) polycythemia vera - primary polyglobulia. The cause is unknown. The RBC number is high increased up to 10-12 mills. /mm³

b) Secondary polycythemia occurs in the diseases in which there is hypoxia. For instance: pulmonary sclerosis, heart failure, intoxications with carbon monoxide.

The decrease of RBC number under 4.0 mills. /mm³ is called anemia. Anemias are functionally classified in two groups:

a) Aregenerative or hyporegenerative anemias in which the bone marrow diminishes the production of RBCs. For instance exposure to X and gamma radiations, deficiency of some plastic factors that are necessary for the Hb synthesis, toxic substances

b) Regenerative anemias in which the bone marrow functions normally. This type of anemia occurs after hyperhemolysis or hemorrhages.