# Pathological Analysis Department

**Title of the lab : RBC Counting**

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 **Red Blood Cells Counting**

**The RBC counting**, performed manually or by ,the blood cells analyzer

**Introduction :**

The red blood cells count, is the number of red blood cells in mmᶟ of whole blood .the normal average in male is 5.500.000 cell\mmᶟ

(range 5.000.000 -6.000.000 cell\mmᶟ)in female 4.800.000cell\mmᶟ

Range (4.000.000-5.500.000 ) cell\mmᶟ and child from (10-12 years ) 5.400.000 cell\mmᶟ.

**Objective :**

To do red blood cells count of provided sample of blood with EDTA

**Principle** :

The blood is diluted 200 time with RBCs diluting fluid (1\200) in practice (20ul blood and 4 ml dilution )

Materials and instruments :

1-venous blood mixing with EDTA or capillary blood with heparin

2-neubauers chamber with cover slid

3-micropipette or red blood cell pipette

4-microscope

5-diluting fluid :**a-**disodium citrate solution its composition disodium citrate 3.8mg ,formalin 1ml and distilled water 99ml.

**b-**hayems fluid its composed of –mercuric chloride 0.5mg(act as antiseptic ),sodium chloride 1mg,sodium sulphate 5mg(have effect to prevent blood cloting ) and distilled water 200ml.

the purpose of this fluid its isotonic solution diluted blood , prevent lysis and prevent blood sedimentation.

**The causes of blood count decreasing :**

1-leukemia 2-bone marrow failure 3- hemorrhage 4-Anaemia

 5-RBCs lysis 6- Fluid overload (hemodilution)

**The causes of blood count increasing :**

1-defect in erythropoietin 2-polycethemia 3-cardaic failure

4-dehydrtion 5-smoking 6-vomiting 7-lungs fibrosis 8-type of abnormal Hb

**Physiological effect on Rbc count:-**

1-age 2-sex 3-Activity 4-nutrition 5-pregnancy 6-brest feeding

7-psychological Emotions 8-high altitude .

# Red Blood Cells Counting Principle:

A suitable dilution of blood (1/200) (20ul blood and 4 ml dilution )

 made in formal citrate solution and the number of RBC in the 1/5th of the RBC square are counted, using an improved Neubauer counting chamber. The result is expressed in RBC/l of blood.

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# Equipments:

* Microscopes.
* Automatic pipettes.
* Improved **Neubauer chamber**. **Disposable materials:**
* Formal citrate solution.
* Glass or plastic tubes.
* Cover slides.
* Yellow tips. - Blue tips.

# Method*:*

1. Pipette 4.00 ml of the diluting fluid in a glass or plastic tube.
2. Add 0.02 ml of well mixed blood to make dilution of 1/200.
3. Tightly seal the tube and mix the suspension for one minute.
4. Fill the counting chamber by means of capillary tube, and leave on the bench for 2-5 min for cells to settle.
5. Count the Red cell in the 1/5 of the RBC square i.e. 0.2 mm2.

*Calaulation:*

# No. of the counted cells.

Count (/l ) = \_ dilution X 106.

Volume of the blood ( μl)

# N

Count (/l ) = X 200 X 106 / l.

0.02

Number of red cells count = N x 10,000



