Practical Endocrinology

RBC count

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Introduction

- Red blood cells make up almost 45 percent of the blood volume.
- Their primary function is to carry oxygen from the lungs to every cell in the body.
- Red blood cells are composed predominantly of a protein and iron compound, called hemoglobin, that captures oxygen molecules as the blood moves through the lungs, giving blood its red color.
- As blood passes through body tissues, hemoglobin then releases the oxygen to cells throughout the body.
- Red blood cells are so packed with hemoglobin that they lack many components, including a nucleus, found in other cells.

Principle:-

- The red blood cell count:- is the number of red blood cells per unit volume of whole blood.
- Normal red blood cells values at various ages are:
- Newborn: 4.8-7.2 million
- Adults (males): 4.9-5.5 million
- (Females): 4.4-5.0 million
- Pregnancy: slightly lower than normal adult values
- Children: 3.8-5.5 million

Aim of the experiment

RBC count is done to determine whether there is an adequate number of RBC in the circulation or not.

- 1- Manual method
- 2-Electronic cell counting

Manual red blood cell count material and instruments

- Anticoagulated whole (using EDTA or heparin as an anticoagulant) or capillary blood can be used.
- Hayem's solution (diluting fluid) composed of:
- Hgcl2 0.05 g
- Na2so4 2.5 g
- Nacl 0.5 g
- Distilled water 100 ml

- RBC pipette which is composed of a stem & a mixing chamber with a red bead, it is function is to mix blood with the substance and for differentiation from the WBC pipette.
- Haemocytometer “ Neubauer” chamber is counting chamber with a cover slip. The same counting chamber is used also for counting total white blood cells.
- Microscope
- Lancet
- Alcohol 70%
- Cotton

RBC Diluting Solution
(Hayem's Fluid)

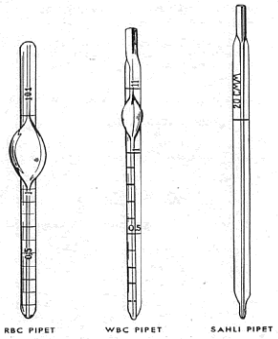
Mercuric chloride 0.5g (antiseptic)

Sodium Chloride 1g (provides isotonicity)

Sodium sulphate 5g (prevents Roleaux formation)

Dist.Water 200ml (Solvent)

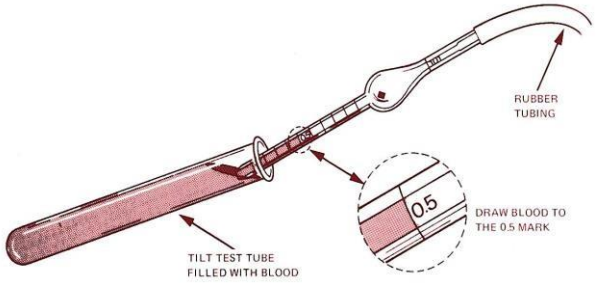
Procedure:

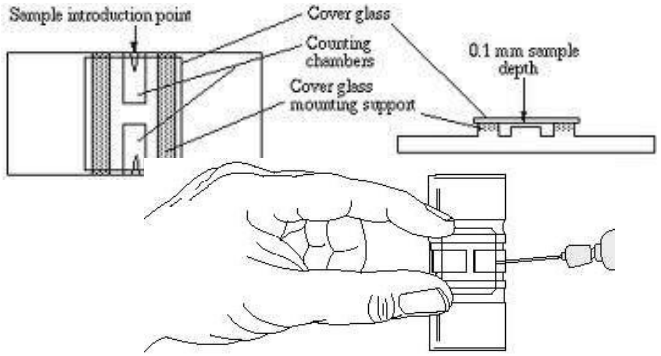


1. Dilution of the blood:
   - **Micropipette (20μ) 1:200 dilution.**

Pipette 4.0ml of diluting fluid into a tube

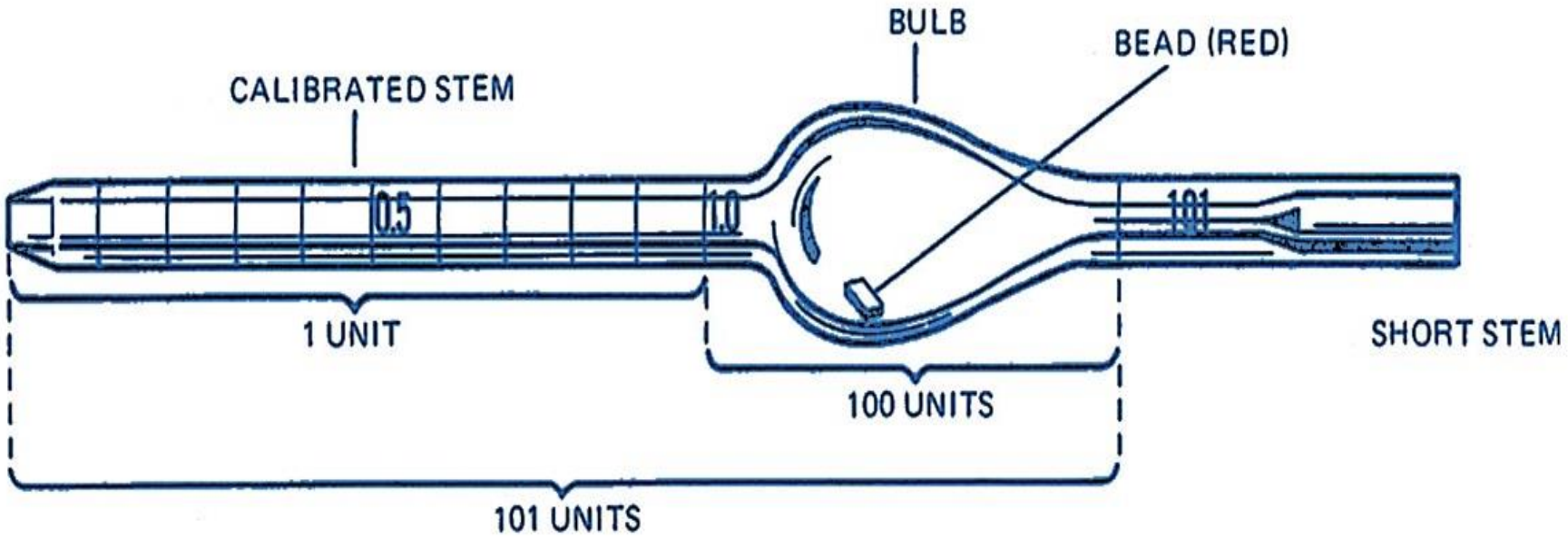
- Pipette 20μl of will mixed anticoagulated
- whole blood to the tube.
  - **Thoma red count pipette**.
- Draw the blood up to exactly the 0.5 mark and dilute to the 101 mark.
- Mix continuously for 2-3 minutes.

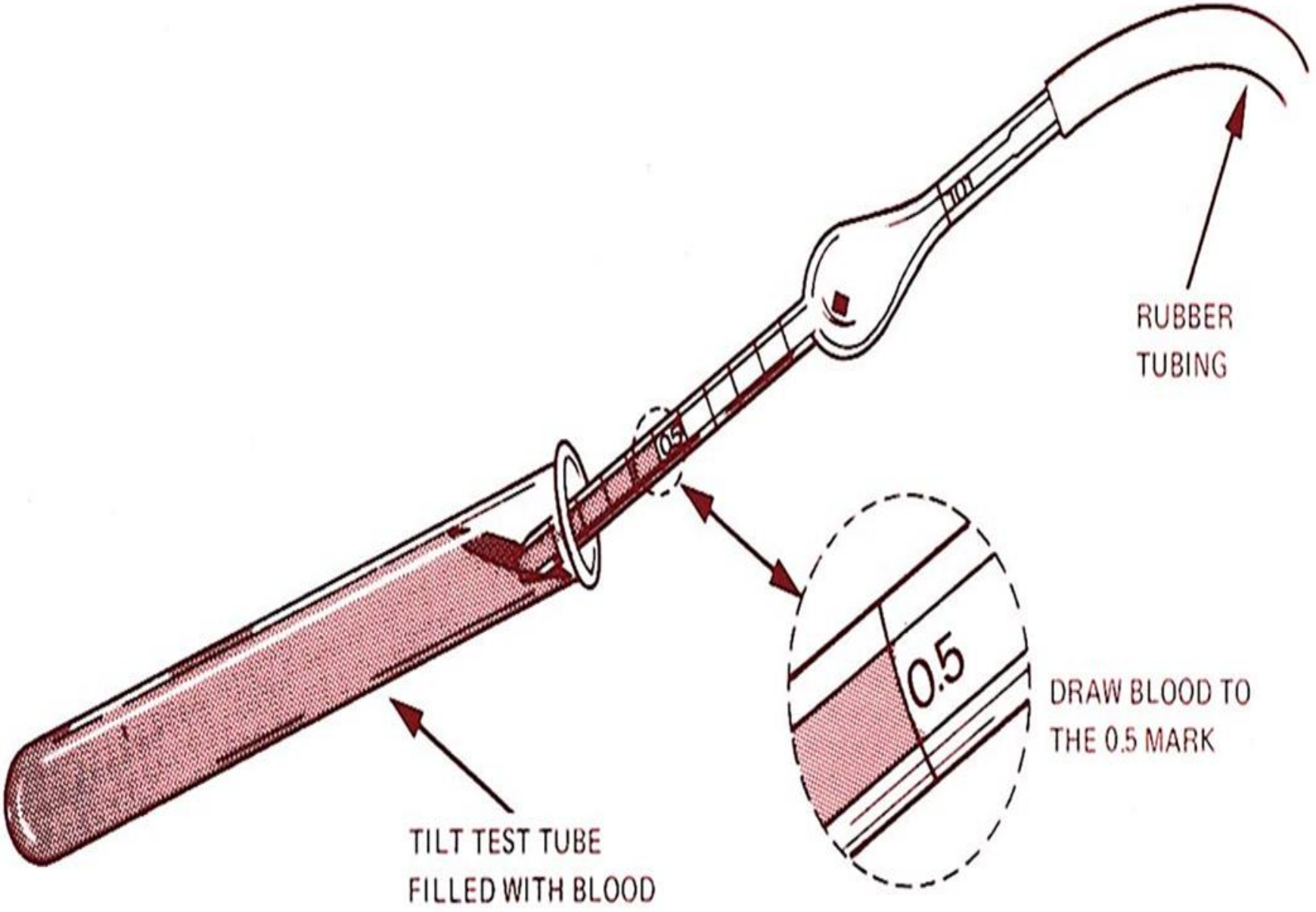




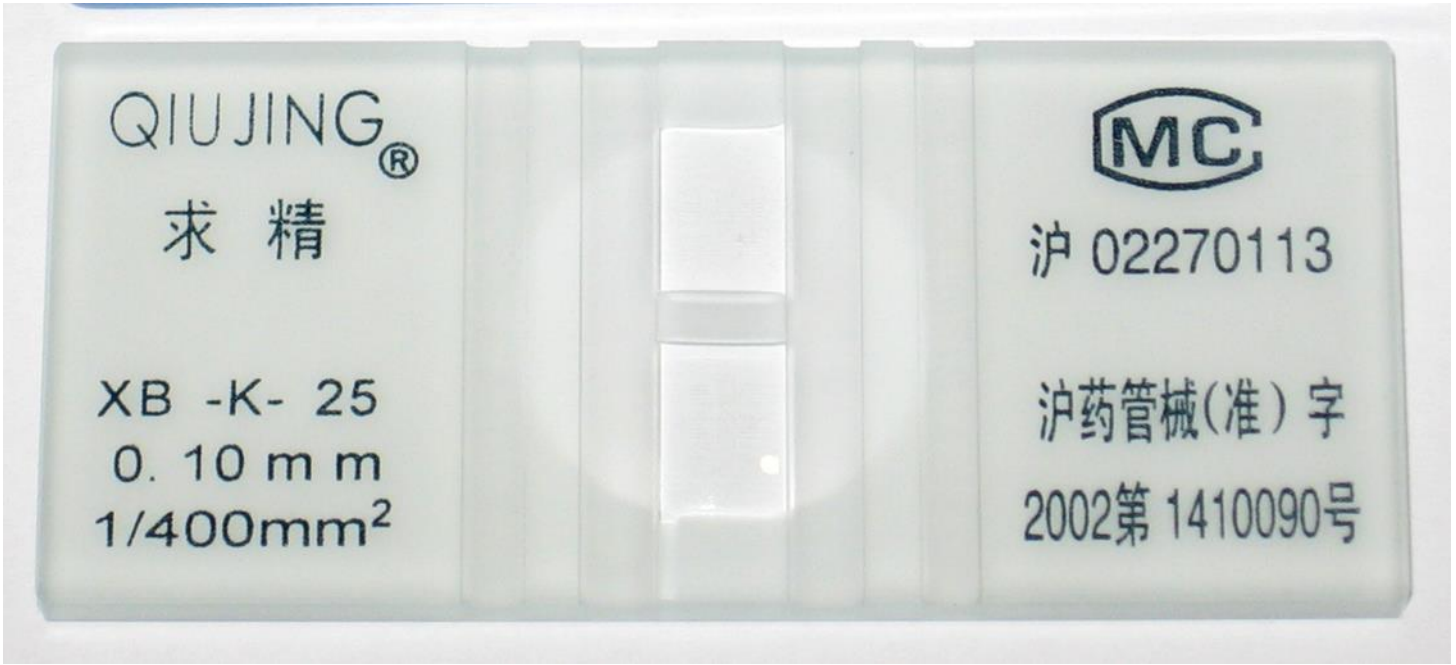
2. Load the cleaned hematocytometer.
3. Place the hematocytometer on the microscope stage, lower the condenser. Leave on bench for 2-5 minutes for the cells to settle.

- Wipe off any blood adhering to its outer side. 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 finger and thumb.

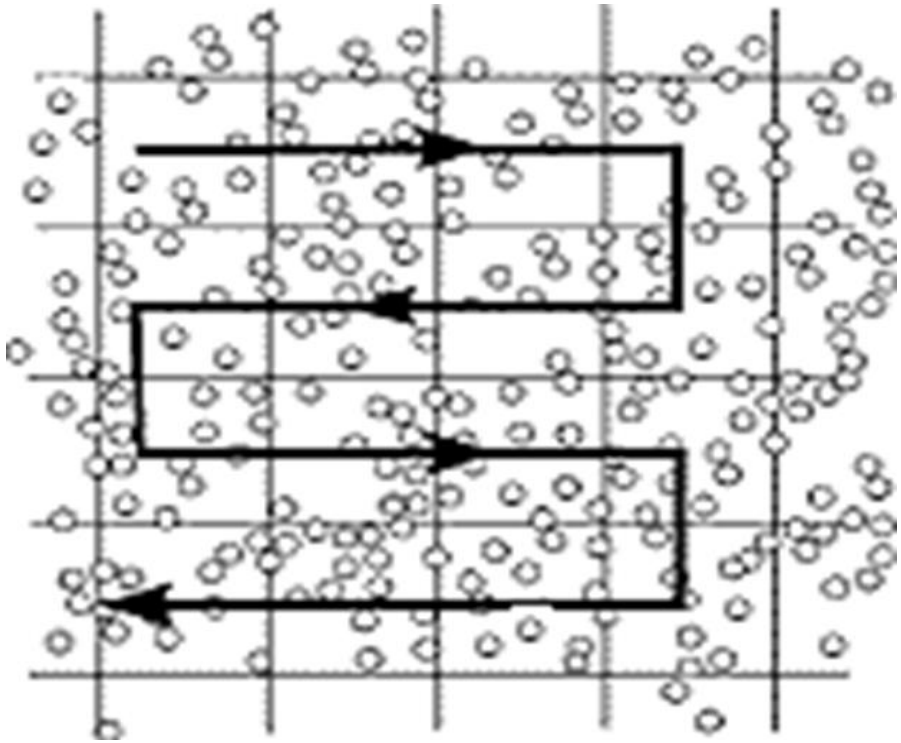

BULB
BEAD (RED)
CALIBRATED STEM
0.5
1.0
101
1 UNIT
SHORT STEM
100 UNITS
101 UNITS


RUBBER
TUBING
0.5
DRAW BLOOD TO
THE 0.5 MARK
TILT TEST TUBE
FILLED WITH BLOOD

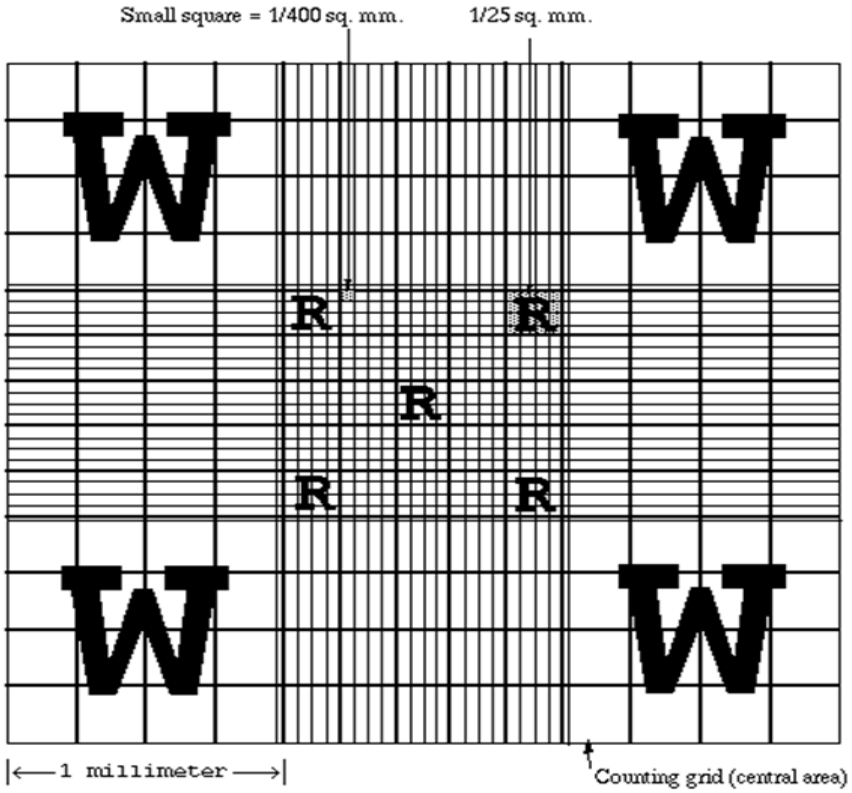
Neubauer Hemocytometer

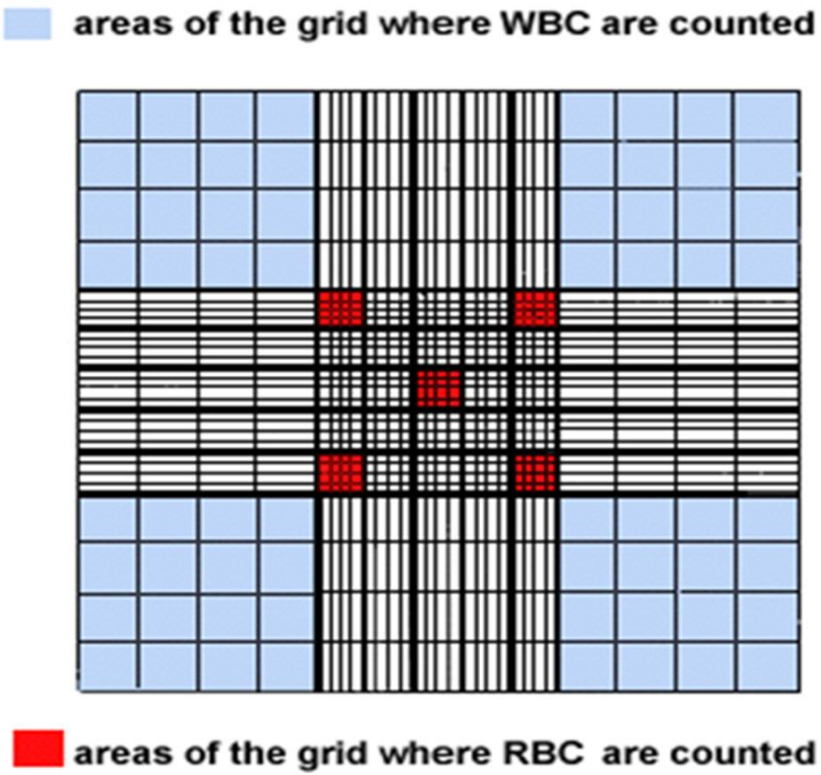


- Scan the counting area with 10x objective lens.
- Use the 40x objective, include all cells lying on the lower and left lines of any square; omit the cells on the upper & right hand lines.



Count the cells in 5 medium squares of 16 small squares i.e. 80 squares, one at each corner and one in the center.





Hemocytometer Chamber

calculation

- Count the number (N) of cells in 80 small squares located in 5 middle-sizes squares (four located at the four corner and one in the middle). The size of 80 small squares in which “N” number of cells are found is:

1/20 x 1/20 x 1/10 x 80 = 1/50 mm3

Where 1/20 mm:- is the slid line of the square.

1/10 mm:- is the depth of the counting chamber between cover slip and the ruling.

80:- is the number of small squares used to count.

- The total number of cells in 1mm3 are =N x 50 (**before** diluting the sample)

- The actual total number of cells **after** dilution should be = N x 50 x **200**= N x 10000

Pathological conditions:-

- Polycythemia is a disease of unknown origin that results in an abnormal increase in red blood cells due to over production of red blood cells in the bone marrow not caused by physiologic need (primary polycythemia vera), while secondary polycythemia vera occur in response to hypoxia.

- Anemia: is a general term that refers to a decrease in red blood cells.

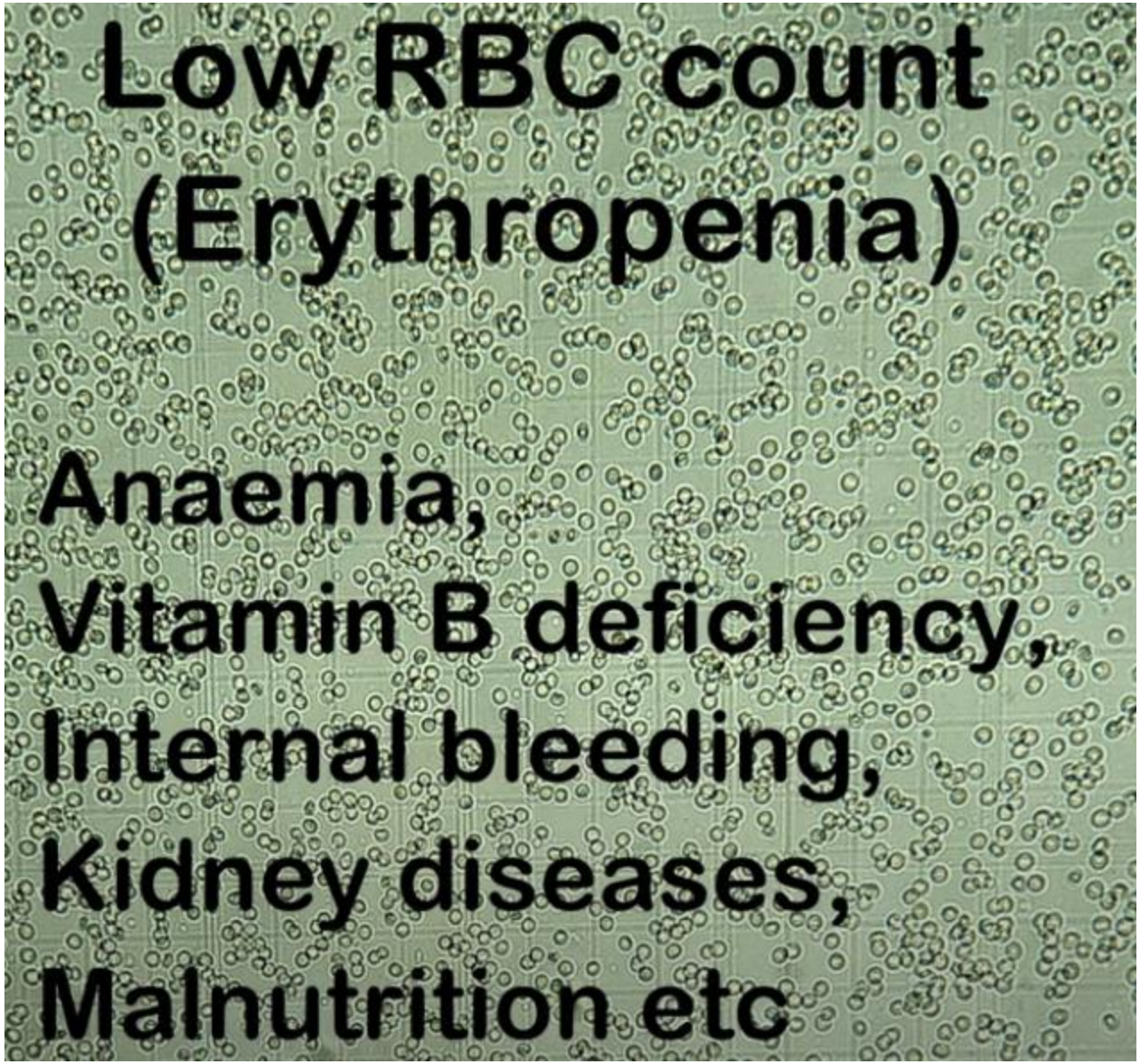
Anemia can occur from either a decrease in the number of red blood cells, a decrease in the hemoglobin content, or both.

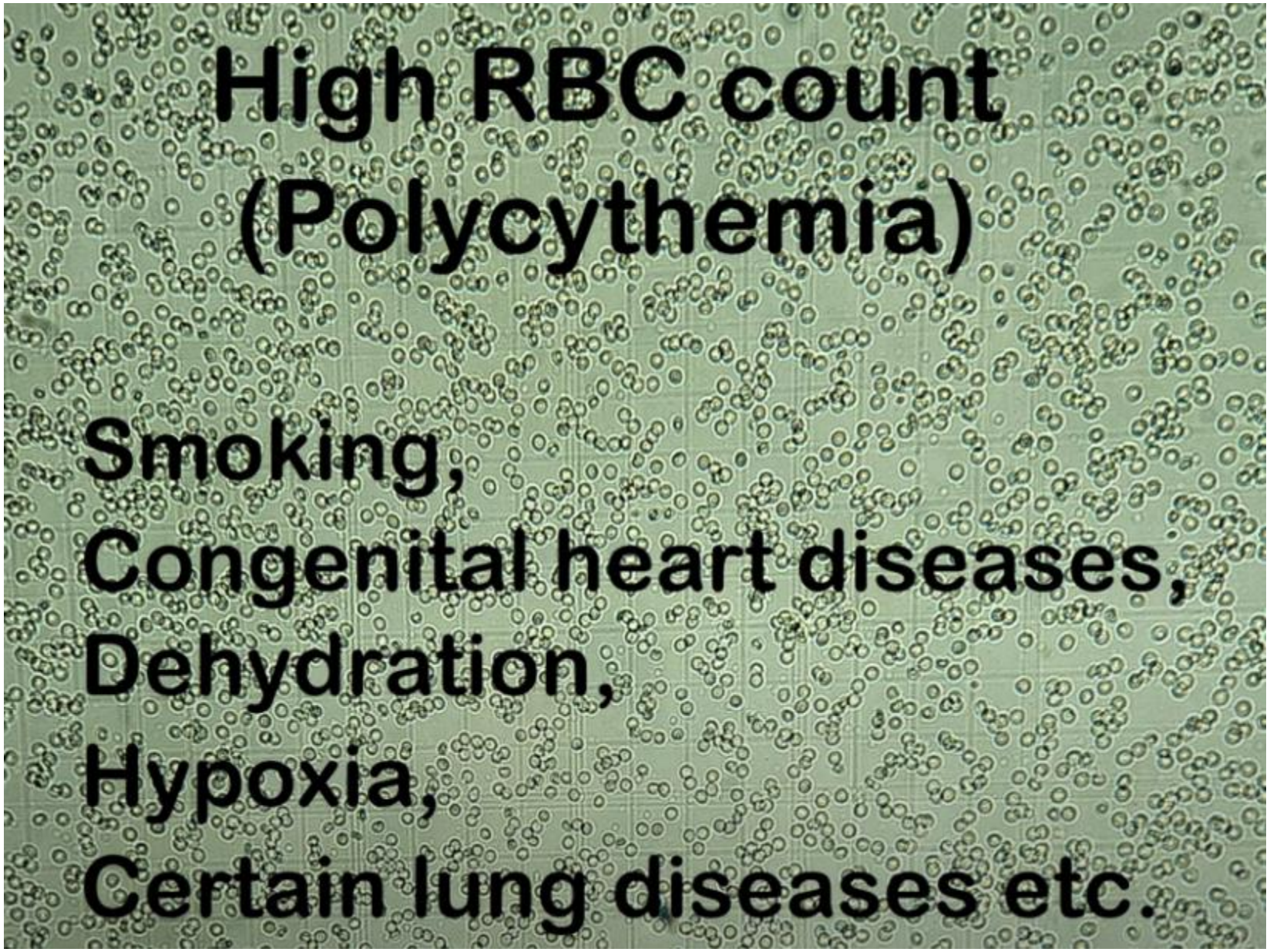
A lower than normal RBC can result from a number of causes, including:

- Massive RBC loss, such as acute hemorrhage
- Abnormal destruction of RBC
- Lack of substances needed for RBC production
- Chemotherapy or radiation side effect from treatment of bone marrow malignancies such as leukemia can result in bone marrow suppression.

normal physiological conditions

- A normal physiological increase in the RBC count occurs at high altitudes or after strenuous physical training.
- The drugs gentamicin and methyldopa have been associated with increasing the number of red blood cells.
- Smokers also have a higher number of red blood cells than non smokers


Low RBC count
(Erythropenia)
Anaemia,
Vitamin B deficiency,
Internal bleeding,
Kidney diseases,
Malnutrition etc


High RBC count
(Polycythemia)
Smoking,
Congenital heart diseases,
Dehydration,
Hypoxia,
Certain lung diseases etc.

ERRORS OF RBC COUNT

1. Diluent should be correct.
2. No overflow in the moats.
3. No air bubbles and debris in the chamber area.
4. No scratches in the ruled area of the chamber.
5. Pipettes used must be clean and dry.

Thank you