Enumeration of red blood cells

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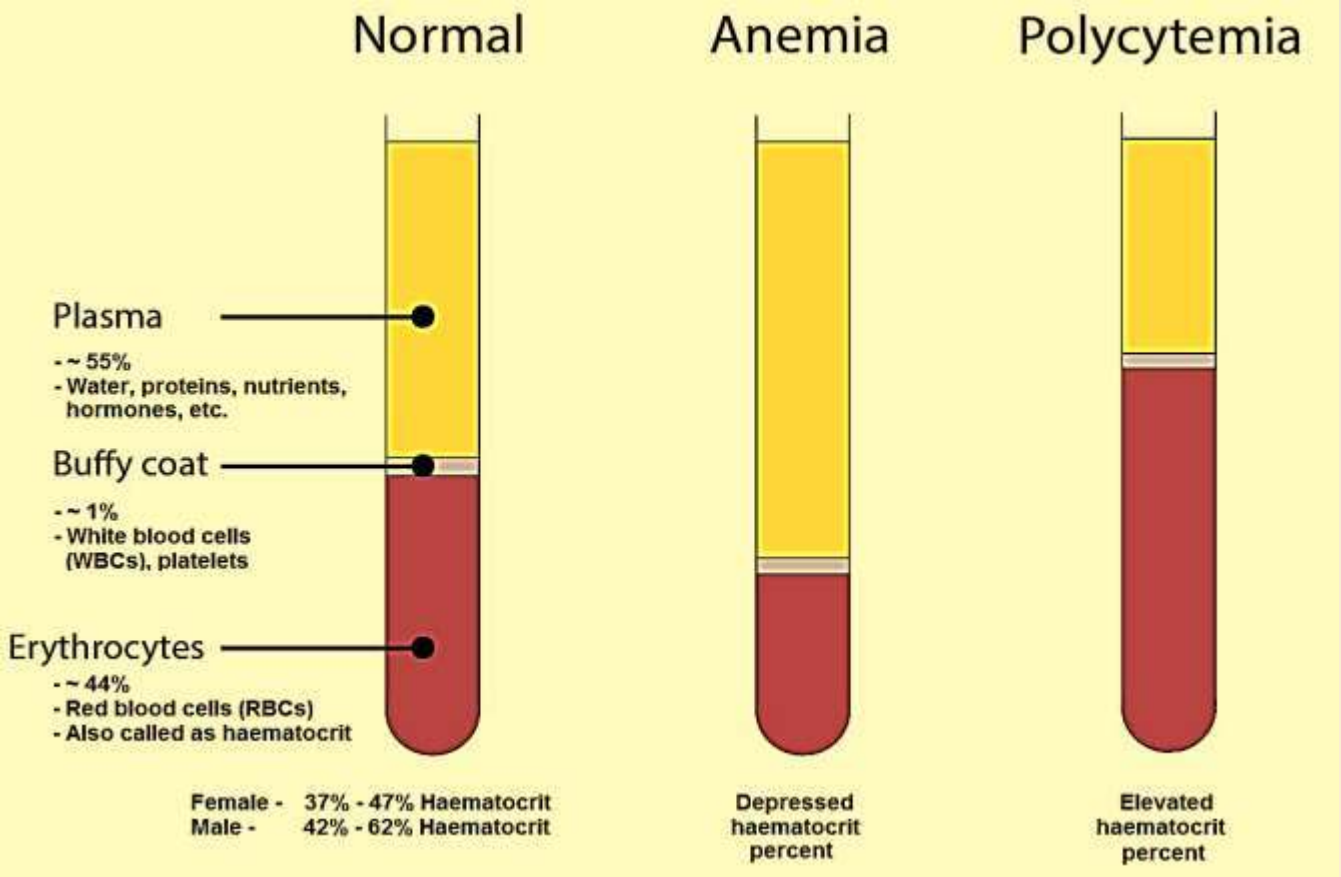
Introduction

- Study of blood is related to haematology. Haematology is, simply, defined as the science or study of blood, blood-forming organs and related disease.
- The tests related to the blood, blood cells and blood chemistry is known as haematological profile or complete blood profile (CBC) that includes haemoglobin estimation (Hb), RBC, WBC, Platelets (PLT), Total leukocytes (TLC), Lymphocytes, Monocytes, Eosinophiles, Basophils, etc ., in per micro-litre volume of blood.
- Haemoglobin is estimated as gram per decilitre (gm/dl).
- Blood is specilalized body fluid and it is considered as connective tissue due to presence of matrix (plasma acts as matrix).
- Basically, it is composed of 90% of water and rest 10% is plasma proteins and various blood cells, like red blood cells (RBCs), white blood cells (WBCs), and platelets (PLT).
- WBCs are of many types, e.g. lymphocyte, monocytes, neutrophils, basophils and eosinophils.

Composition of blood

When blood is centrifuged in centrifuge machine, distinct bands can be observed.



Composition of blood

Plasma

- It is a pale yellow sticky liquid which is about 55% of the blood's volume.
- It is composed of 92% water, 8% dissolved proteins, and rest glucose, amino acid, CO_2, hormones, antibodies
- It carries .dissolved materials such as glucose, amino acids, minerals, vitamins, salts, CO_2, urea, hormones, and heat.
- After the removal of clotting factor, rest of plasma is known as serum, which is straw colour liquid.

Red Blood Cells

- These are tiny biconcave disc-shaped anucleated cells.
- They also do not contain any organelles including mitochondria.
- These cells are rich in haemoglobin, an iron containing biomolecule that can bind oxygen and is responsible for blood's red colour.
- Thus, they function as principal mean of delivering oxygen (O2) to the body tissue via blood flow through the circulatory system.

Composition of blood

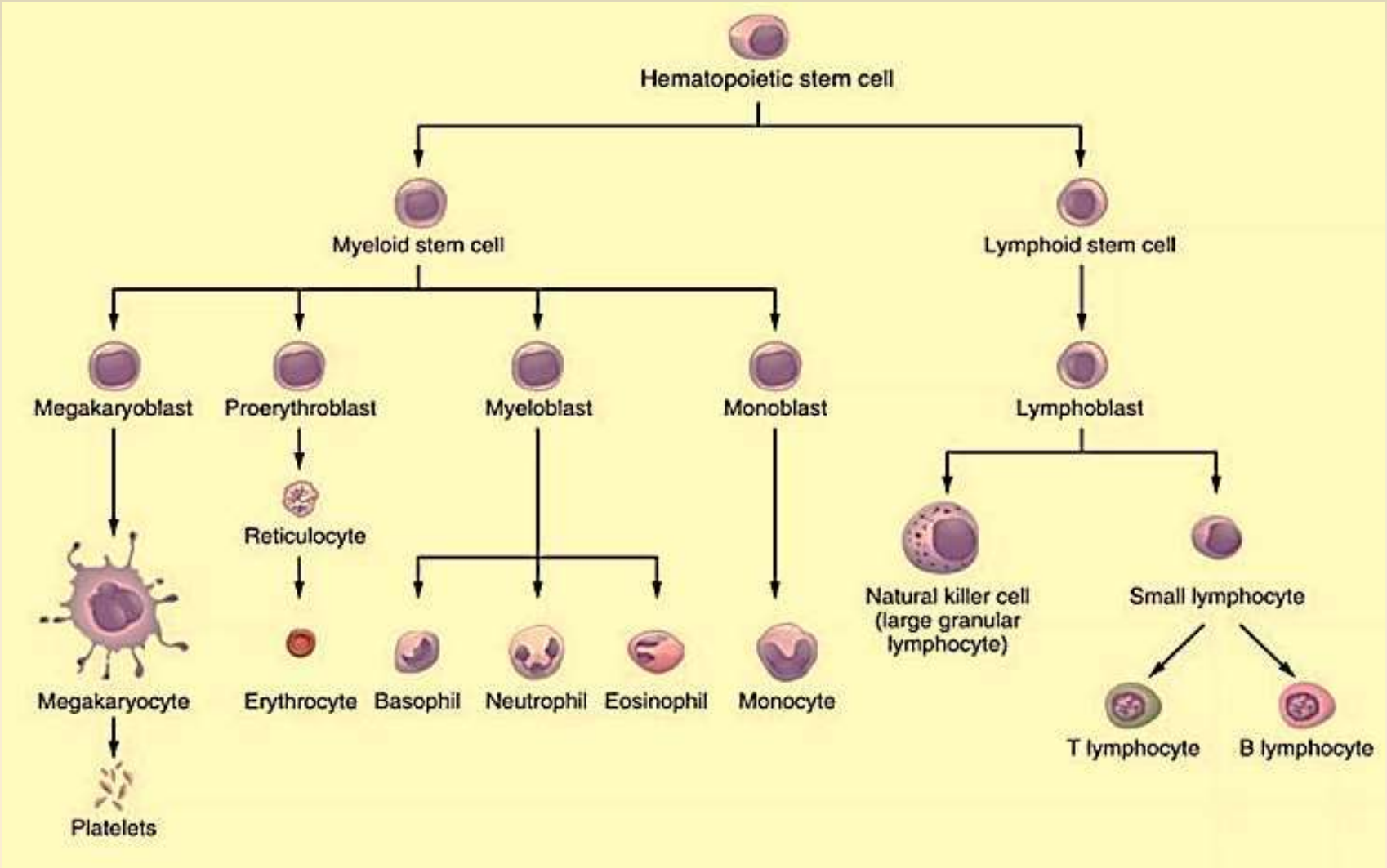
White Blood Cells

- They are also called and leukocytes which are colourless.
- Unlike to RBCS, they possess a nucleus and other subcellular organelles.
- Their main function is to defense against pathogens.
- Thus, some of them act as phagocytes, called as neutrophils, macrophages, dendritic cells, etc., some secretes antibodies and other active biomolecules, called lymphocytes.

Platelets

- They are also called as thrombocytes.
- They also lack nucleus and other subcellular organelles.
- They are tiny fragments of large bone marrow cells.
- They carry specialized blood clotting factors, which are released upon injury.

Formation of blood cells



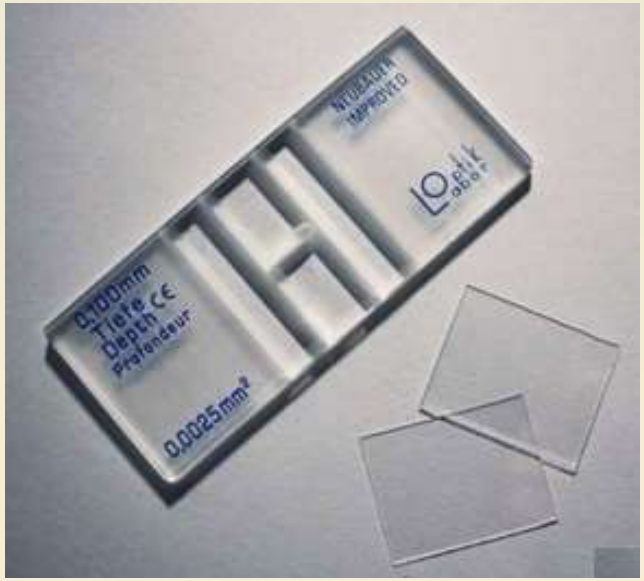
Red Blood Cells or RBCs

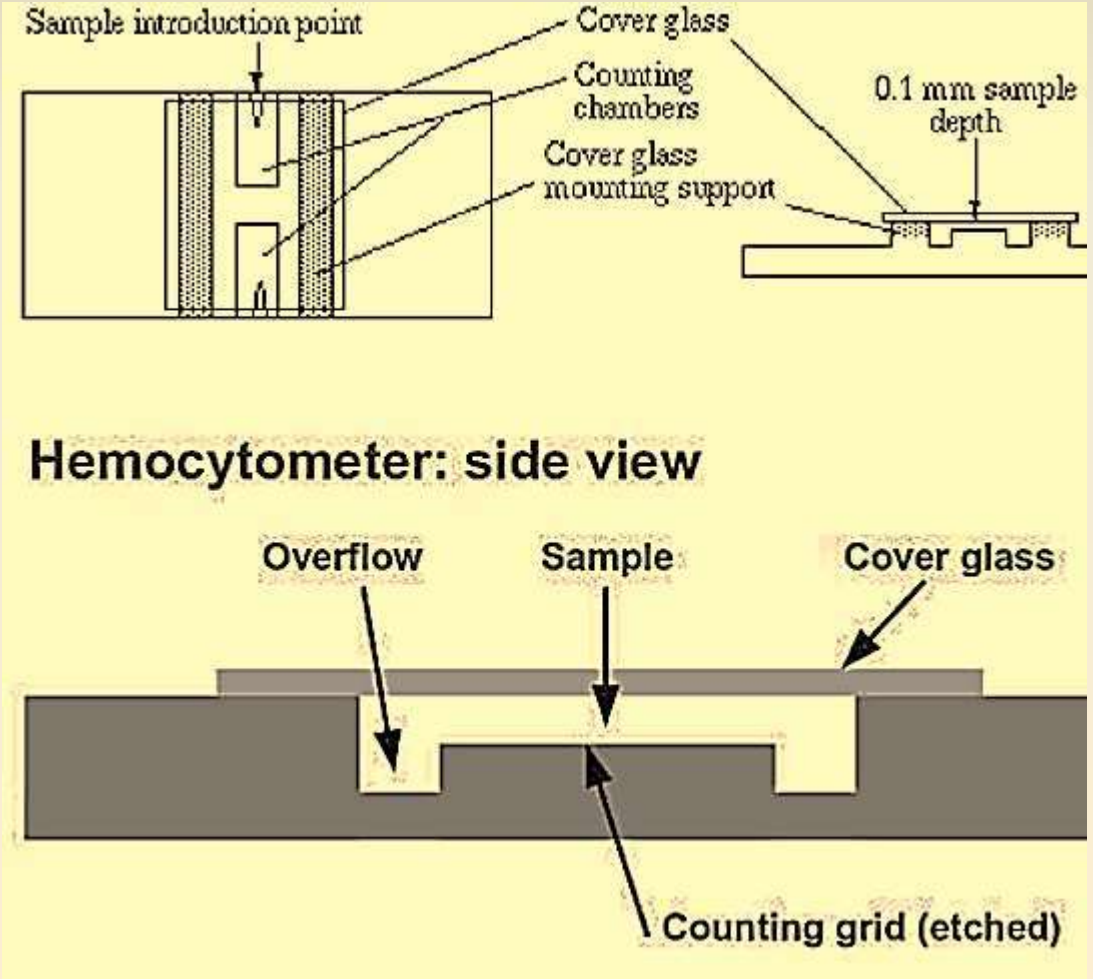
Red Blood Cell Count

- To know whether an individual has normal blood profile, or anaemic or polycythemic, complete blood count is necessitated.
- RBCs is either counted manually or through machine.
- RBCs are manually counted with the help of specialized apparatus known as Haemocytometer.
- A haemocytometer is a counting chamber invented by Louis-Charles Malassez.
- It includes:
  - ✓ A Neubauer's slide
  - ✓ Cover slip
  - ✓ RBC pipette
  - ✓ WBC pipette

Red Blood Cell Count

Neubauer's slide

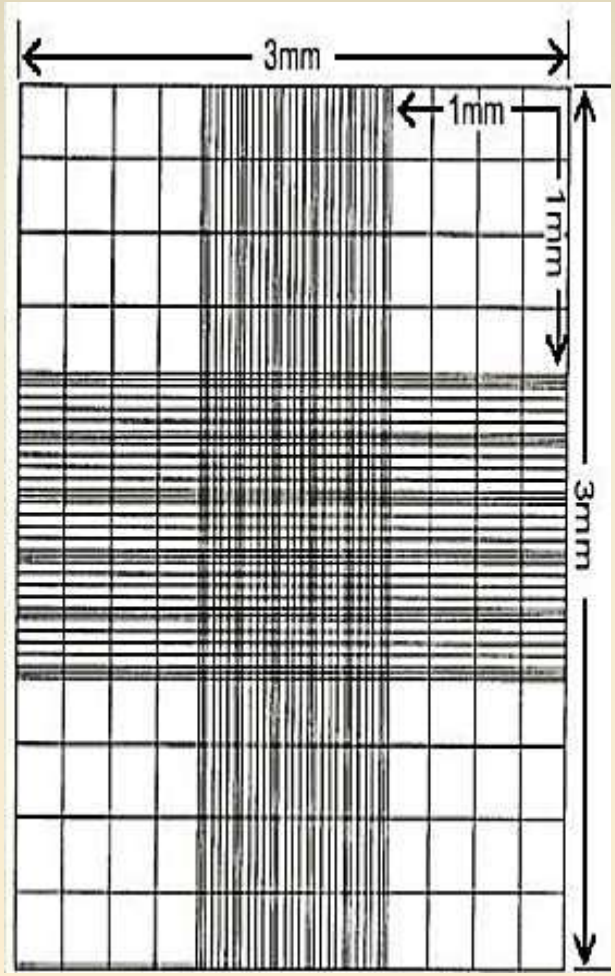




Red Blood Cell Count

Neubauer's slide

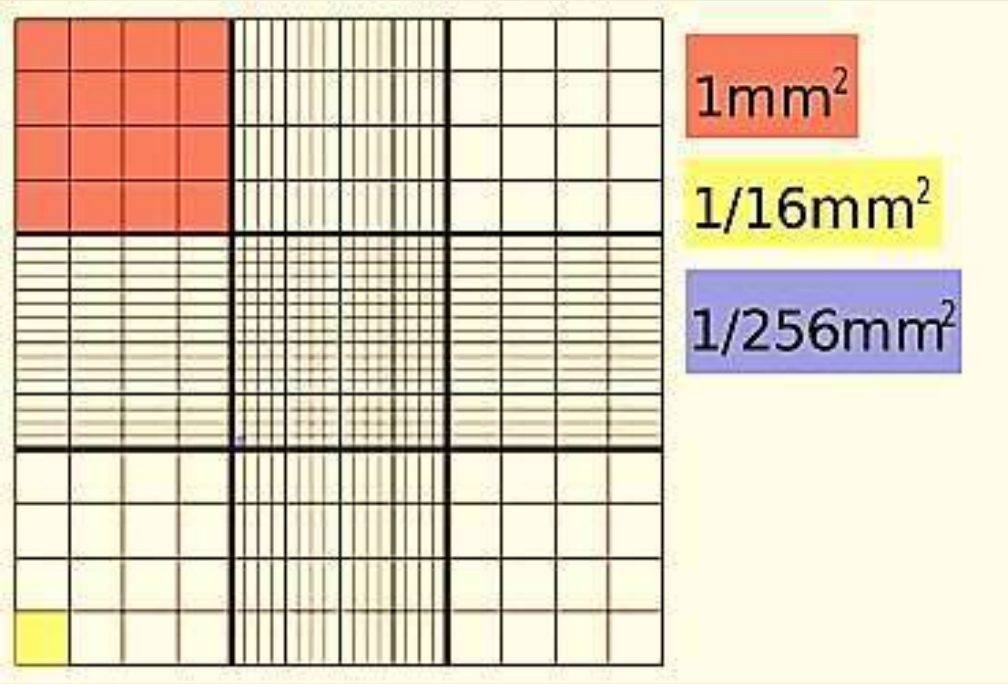
- It is a thick glass slide.
- In the center of the slide, there is an **H – shaped** groove.
- On the two sides of the central horizontal bar, there are scales for counting the blood cells
- The depth of the scales is $\frac{1}{10}mm$ or 0.1mm.
- Each scale is 3mm wide and 3mm long, i.e. 9mm^2.
- The whole scale is divided into 9 large squares.
- Each large square is 1mm long and 1mm wide, i.e. 1mm^2.
- The large squares are further divided in 3 directions; 0.25 x 0.25 mm (0.0625mm^2), 0.25 x 0.20mm (0.05mm^2) and 0.20 x 0.20mm (0.04mm^2).



Red Blood Cell Count

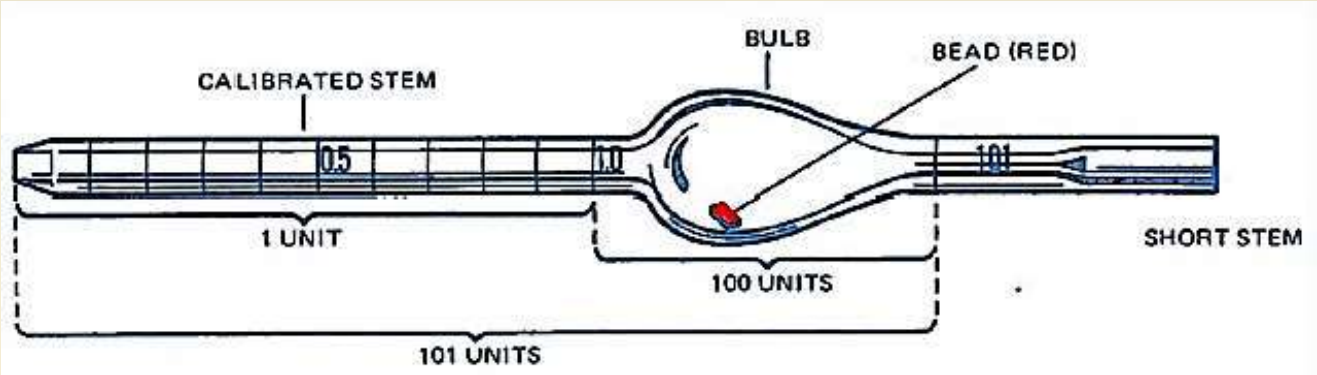
Neubauer's slide

- The central square is further subdivided into 0.05 x 0.05mm (0.0025mm^2) squares.
- The raised edges hold the coverslip 0.1mm off the grid giving each square a defined volume.

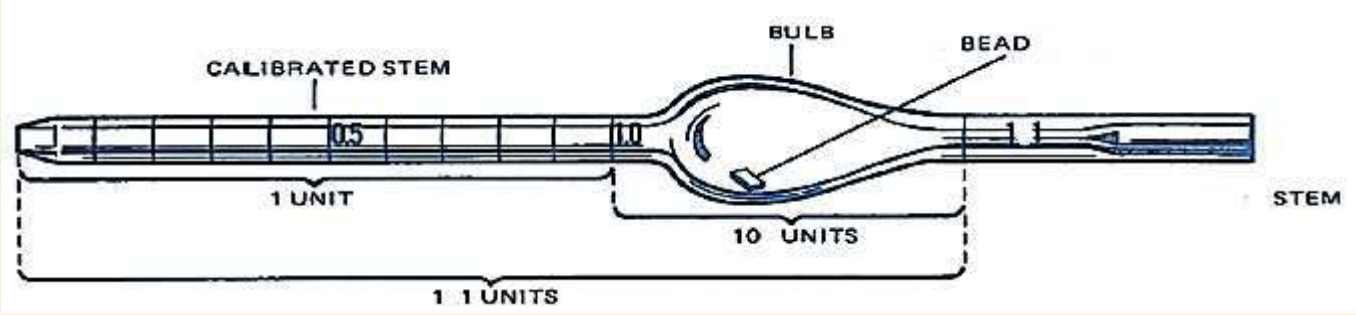


Red Blood Cell Count

RBC Pipette



WBC Pipette



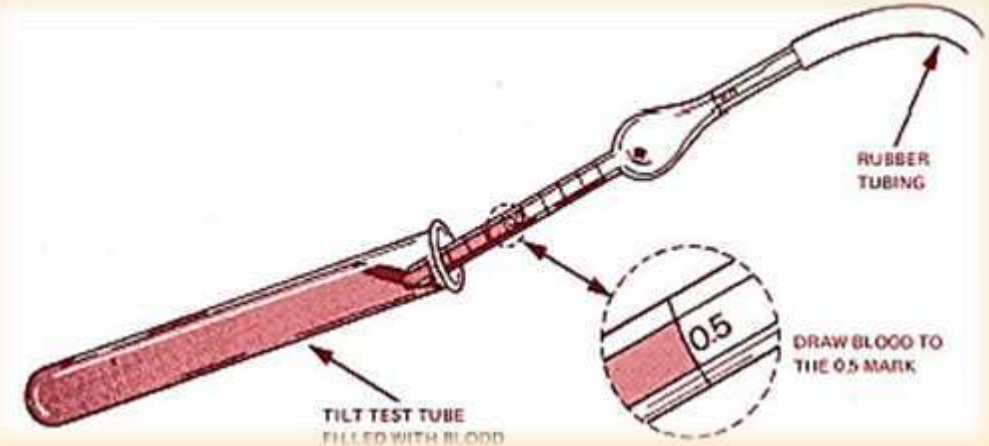
Red Blood Cell Count

Dilution factors

<u>For cells counting</u>

Blood is filled till mark 0.5 and fluid is then filled till mark 101 or 11.

Both are thoroughly mixed.

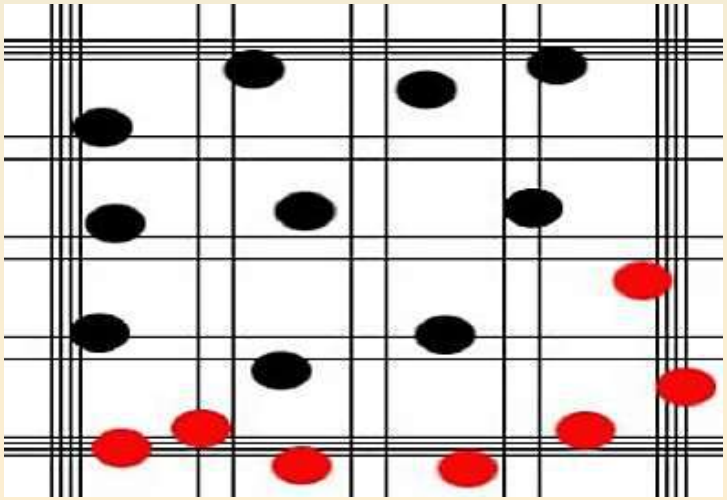


Red Blood Cell Count

Methodology

- Clean the Neubauer chamber and the cover slip with 70% ethanol.
- With the microscope, using a 4x objective, identify the nine main squares of the chamber delimited by three lines each as shown in the following image.
- Now changes to 10x objective and focus one of the 9 main squares.
- The counting is performed in the area delimited by three lines.

- Cells that touch the upper an d left border are counted (black colour), while cells that touch the right and lower border are not counted..
- Area of square bounded by 3 lines is $0.04mm^2$.
- Area of small square within the large square is $0.0025mm^2$.



Red Blood Cell Count

Principle

- In order to facilitate RBCs count, a specified volume of blood is diluted with a specified volume of isotonic fluid, which must have property of an anti-cougulant, anti-haemolysis, anti-aggregation, anti-Rouleaux, and preserve RBC shape.

Reagents

- Diluting fluid used might be 0.85% sodium chloride (NaCl) in distilled water, Hayam's solution (Sodium sulfate – 10g, Sodium chloride – 2g, Mercuric chloride – 0.25g, DW – 100ml), Gower's solution (Sodium sulfate – 12.5g, Glacial acetic acid – 33.3ml, DW – 100ml), Citrate-formalin solution (Tri-sodium citrate & formalin).
- Hayam's solution may cause clumping of RBCs and Rouleaux formation in some cases, therefore generally it is avoided.

Sample

- Whole blood using EDTA or heparin as anticoagulant. However, capillary blood may also be used.

Red Blood Cell Count

Equipments

1. Pipettes – Thoma peipette (RBCs) or micropipette – 20 microlitre is the desired volume.
2. Improved Neubauer chamber with the cover slips.
3. Light microscope.
4. Clean gauze

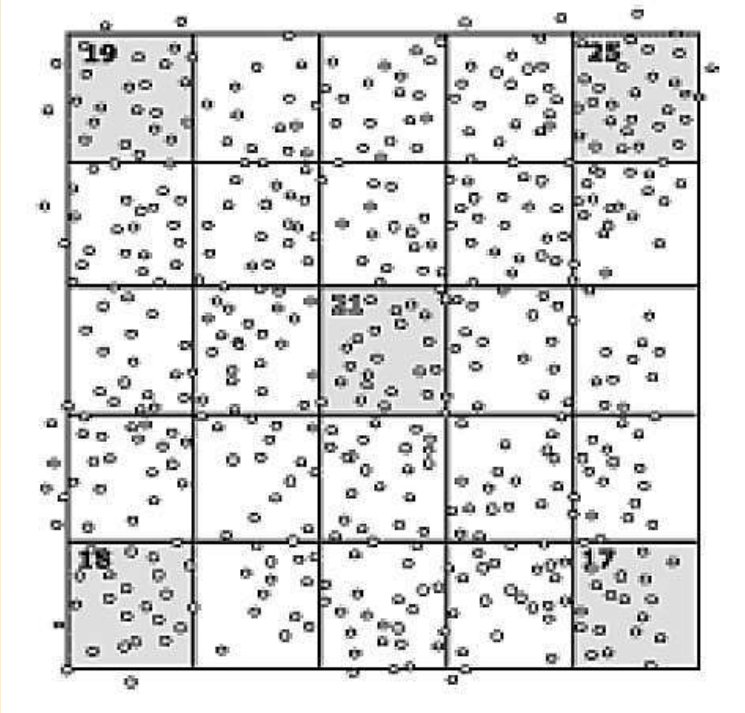
Procedure

1. Dilute the blood **(1:200 dilution)** – Draw the blood up to exactly the o.5 mark and dilute to the 101 mark or pipette 4.oml of diluting fluid into a tube and mix by pipetting 20 microlitre of well mixed anticoagulated whole blood.
2. Load the cleaned haematocytometer.
3. Place the haematocytometer on the microscope stage and focus the counting chamber with 10x objective lens on the large central square, which is ruled into 25 small squares, each of which is further divided into 16 smaller squares.

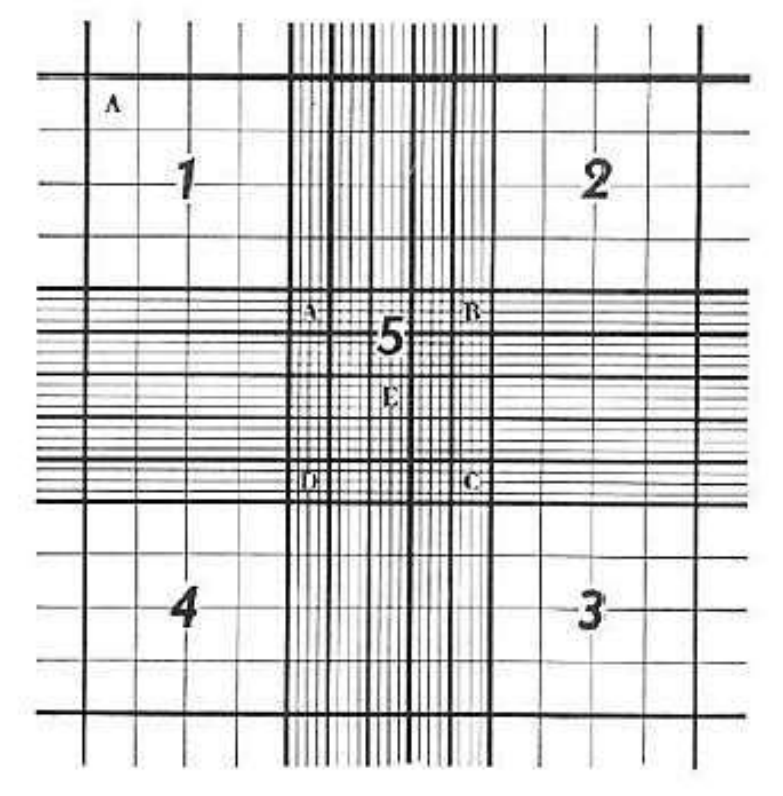
Red Blood Cell Count

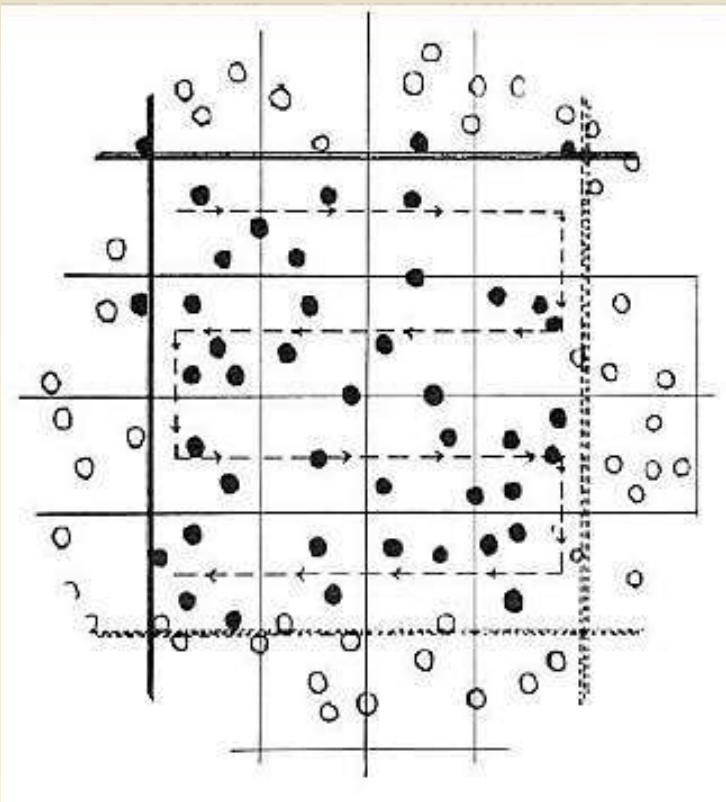
Procedure

4. Count RBCs in the 4 corner squares and 1 middle square out of 25 squares.
5. Switch to 40x objective lens, and start counting in the five designated squares.



Red Blood Cell Count





Red Blood Cell Count

Calculation

Total RBC count = N X Dilution factor X Volume correction factor

Where:

N = the total number of red blood cells counted in the counting chamber.

Dilution factor = 200 (since it is 1: 200)

Counted volume:

- Each counted square has a volume of 0.2 x 0.2 x 0.1 = 0.004.
- 5 squares volume = 5 x 0.004 = 0.02 cc
- Volume correction factor = 1/0.02 = 50

Therefore,

Total RBC = N x 200 x 50 = N x 10,000

Further reading

Bain B.J., Bates I., Laffan M.A. 2016. Dacie and Lewis Practical Haematolog. Elsevier Health Sciences, Philadelphia, USA

Singh T. 2017. Text and Practical Haematology for MBBS. Arya Publications, New Delhi, India