

Practical Physiology

Red Blood Cells (Erythrocytes) count

Hemo-cyto-meter:

Hemocytometer consists of:

1. Improved Neubauer chamber
2. Two pipettes

a. WBC diluting pipette:

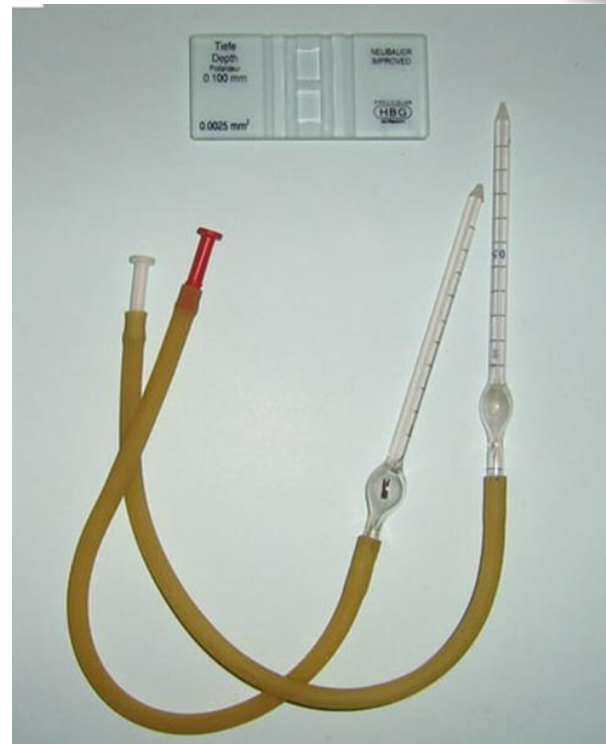
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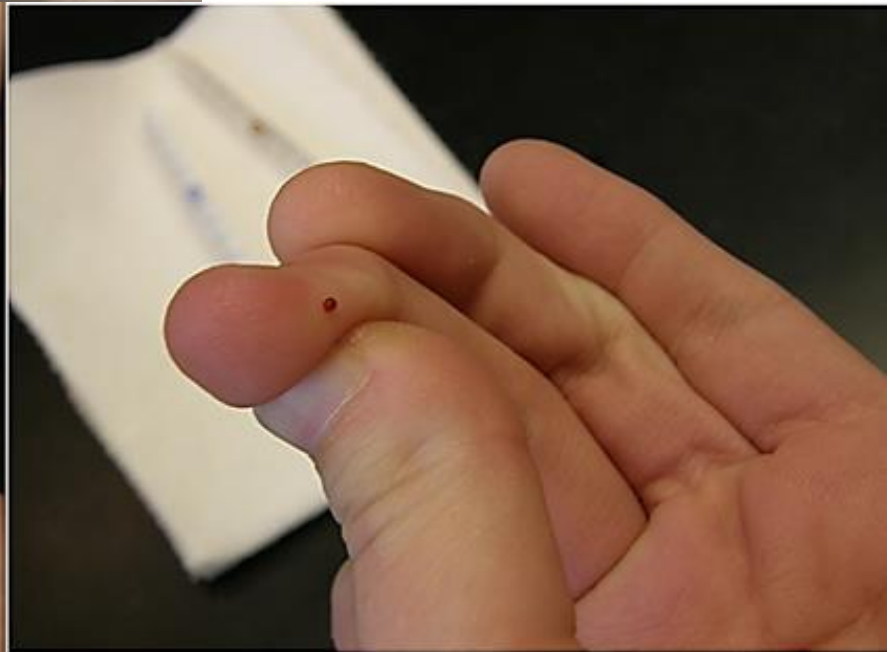
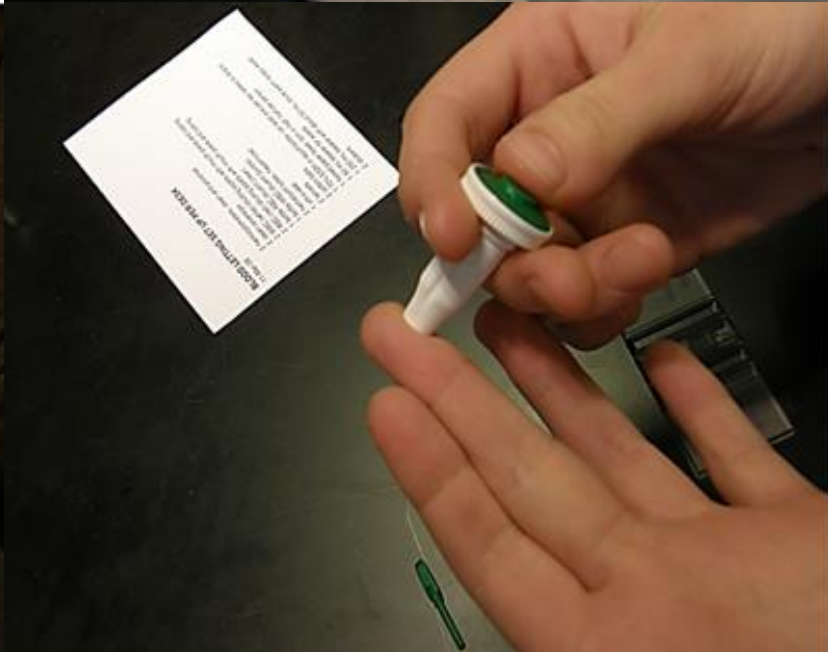
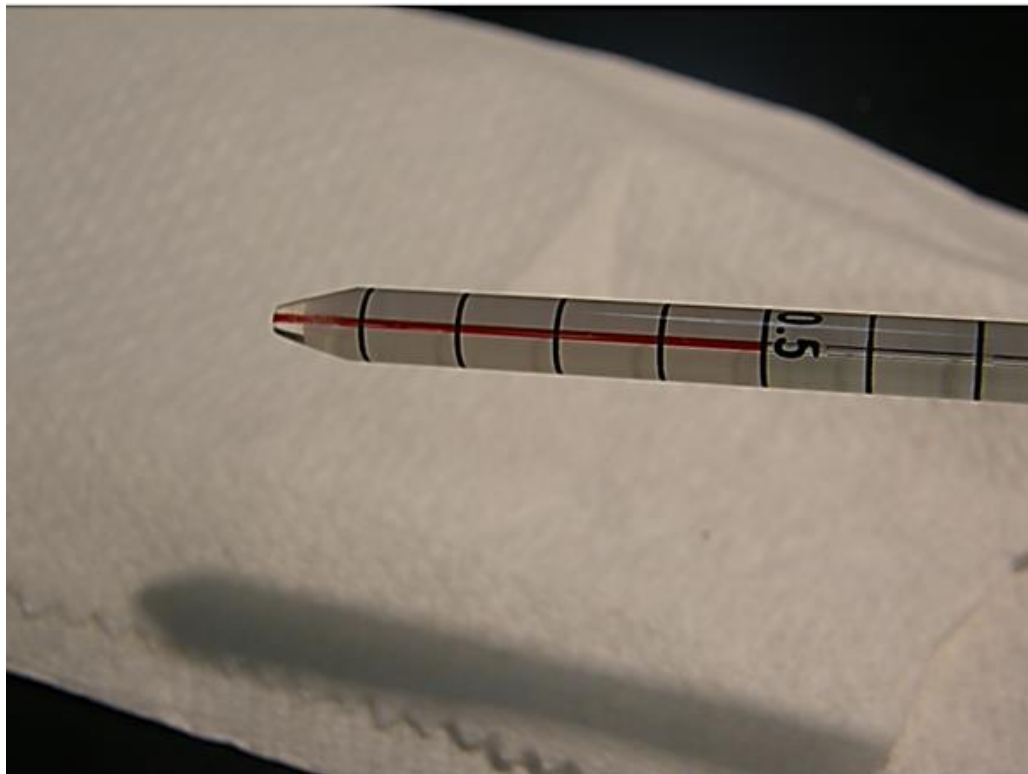
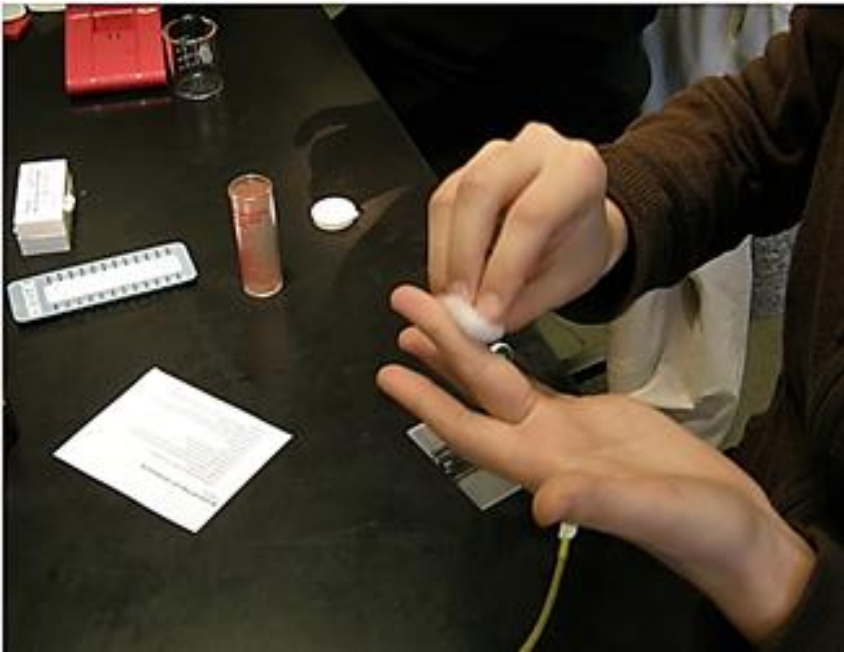
1. It has the numbers 0.5, 1, and 11
2. It has a white bead
3. White mouth piece

b. RBC diluting pipette:

Characterizes by:

1. It has the numbers 0.5, 1, 101
2. It has a red bead.
3. Red mouth piece





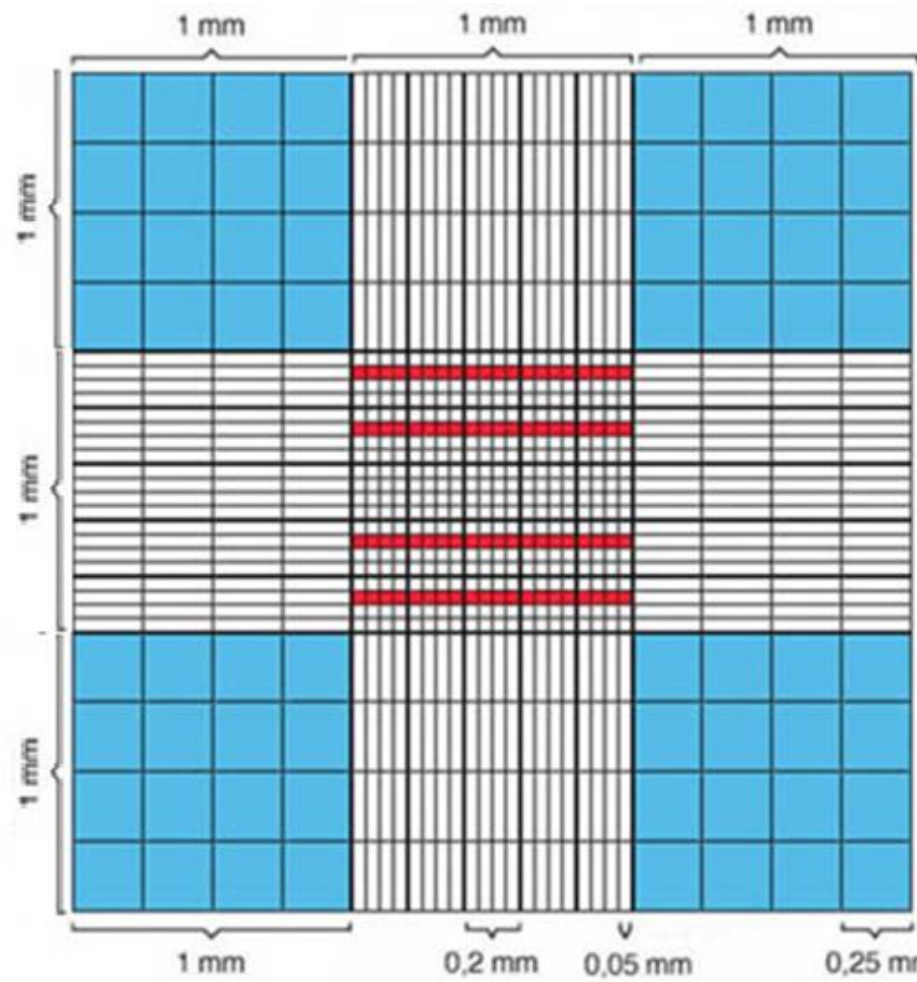
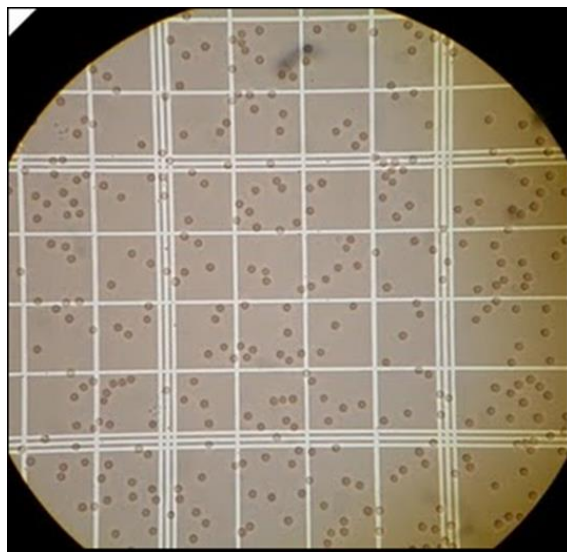
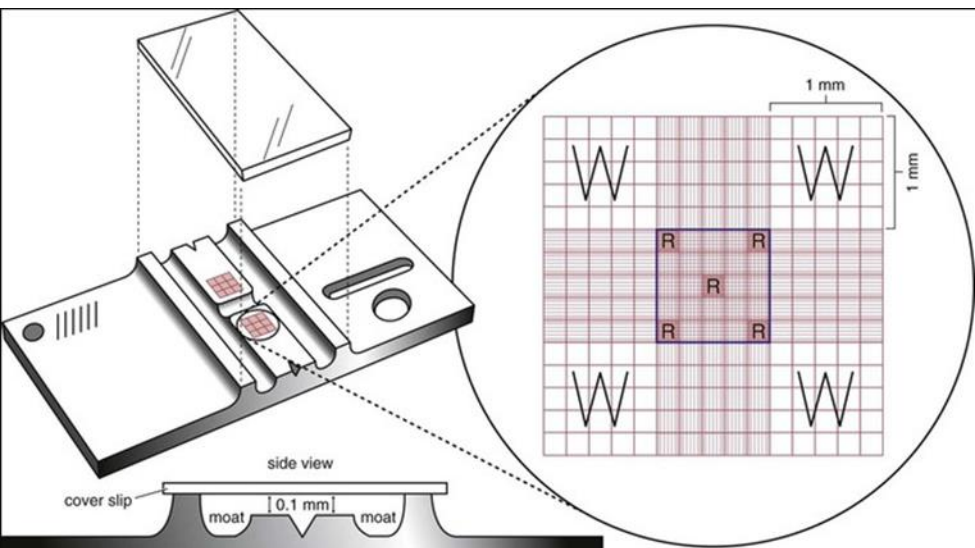
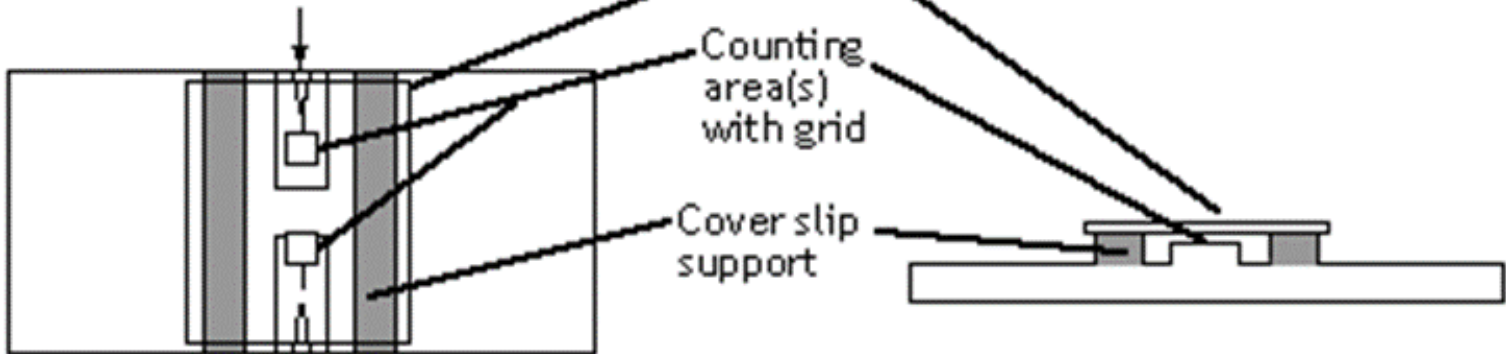
Hemocytometer

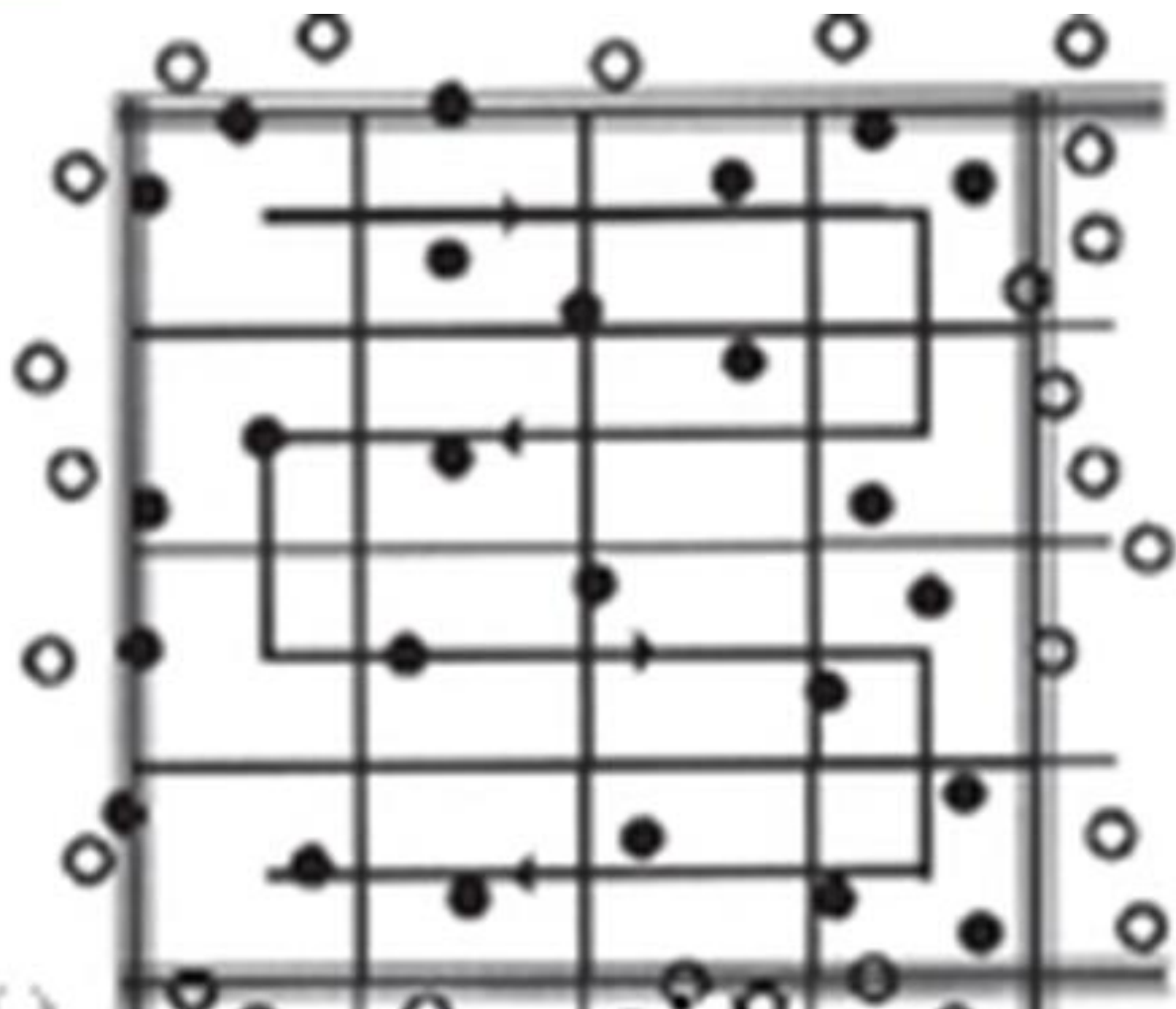
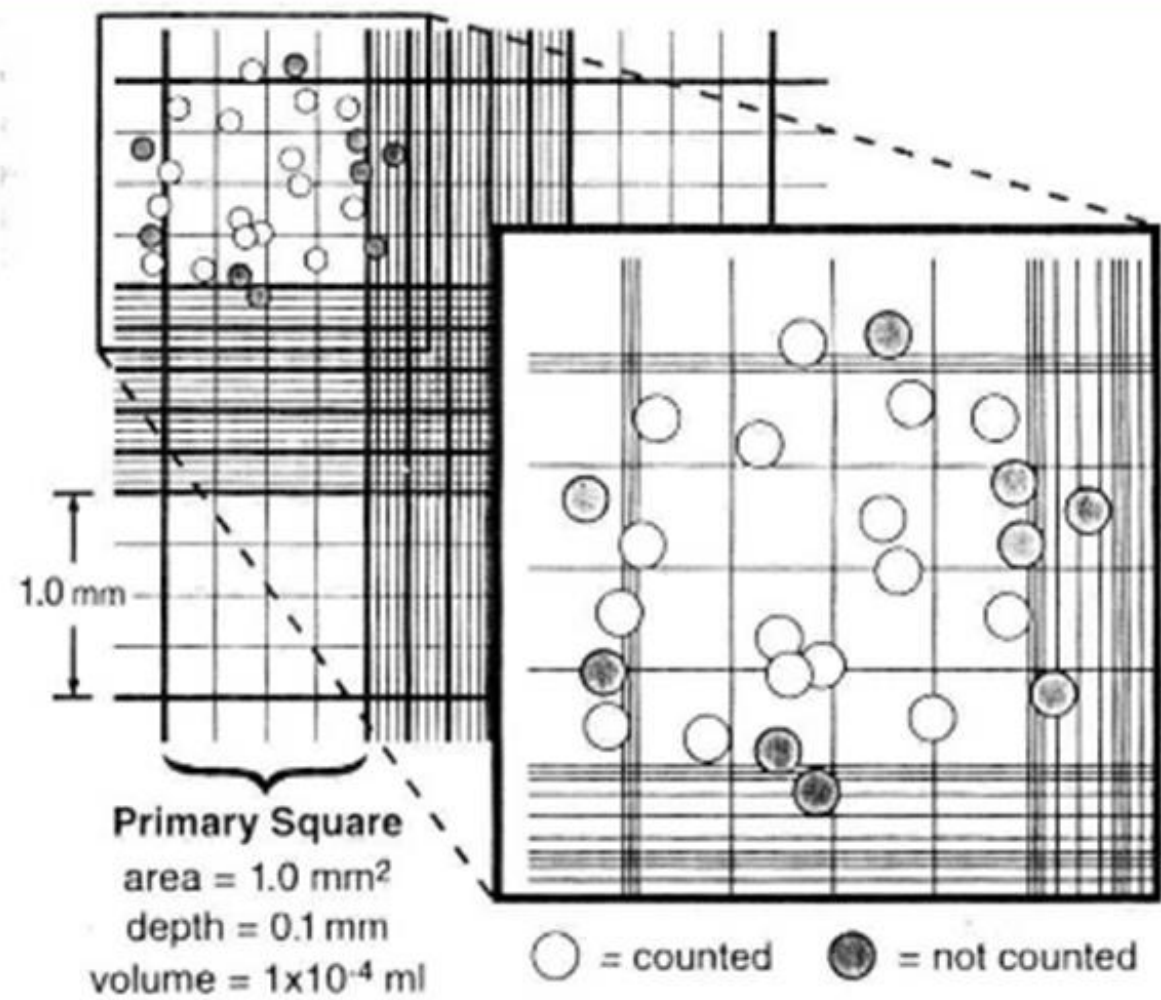
Sample Introduction point

Cover slip

Counting area(s) with grid

Cover slip support





RBC's/mm³ = number of cells in 5 squares (80 small square) X VCF X DF

The dilution factor (DF) = total volume / sample volume

$$= 101-1/0.5=200$$

Volume correction factor (VCF) = 1 mm³ /total volume of the five medium squares

The total volumes of the five medium squares =

Volume of each small square x total number of small squares:

Volume of each small square = (width x length x height)

$$= 1/20 \text{ mm} \times 1/20 \text{ mm} \times \text{depth } 1/10 \text{ mm} = 1/4000 \text{ mm}^3$$

Total number of small squares = 5 x 16 = 80 (each of the five medium squares has 16 small squares)

Then the total volumes of the five medium squares = $1/4000 \times 80 = 1/50 \text{ mm}^3$

So the volume correction factor = $1(1/50) = 50$

Example:

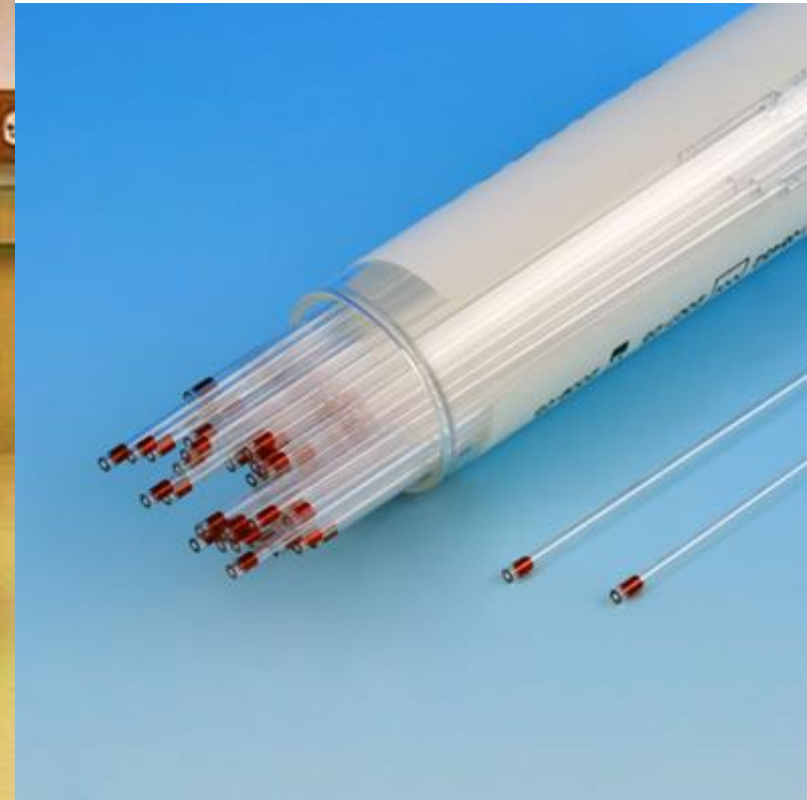
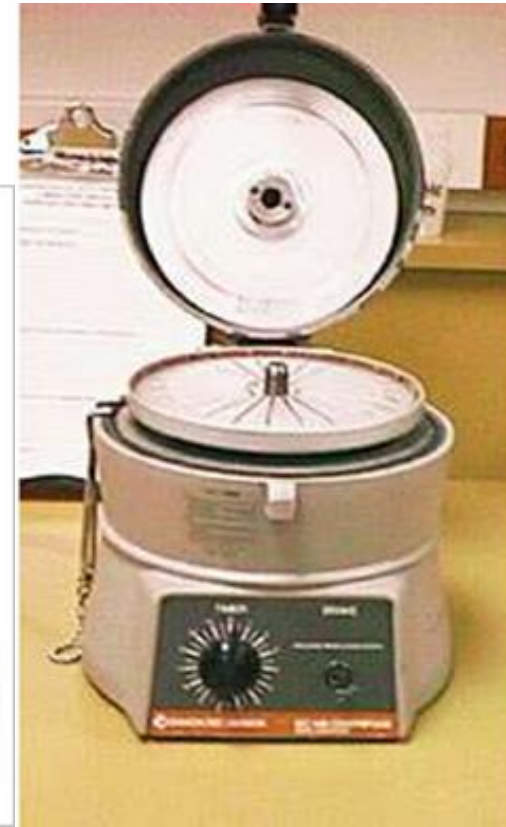
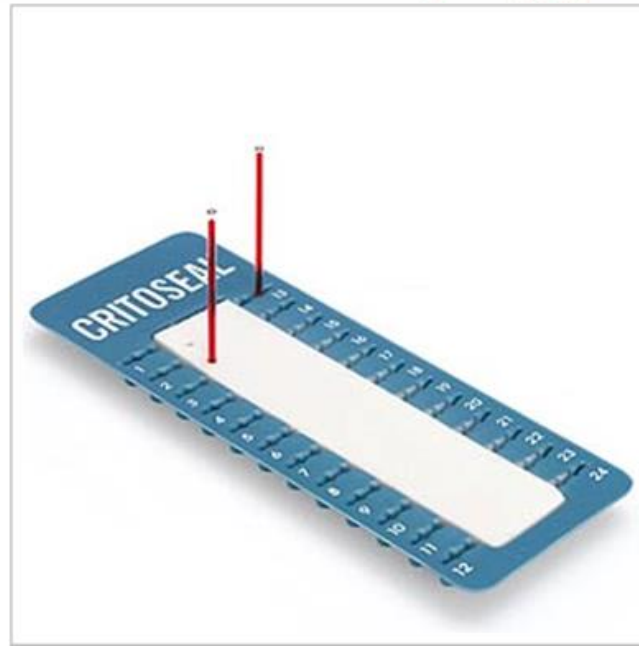
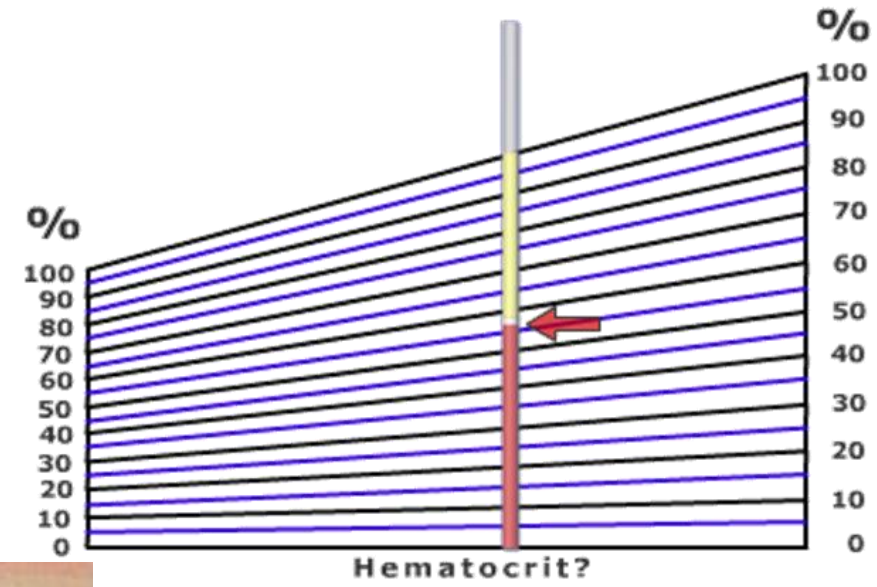
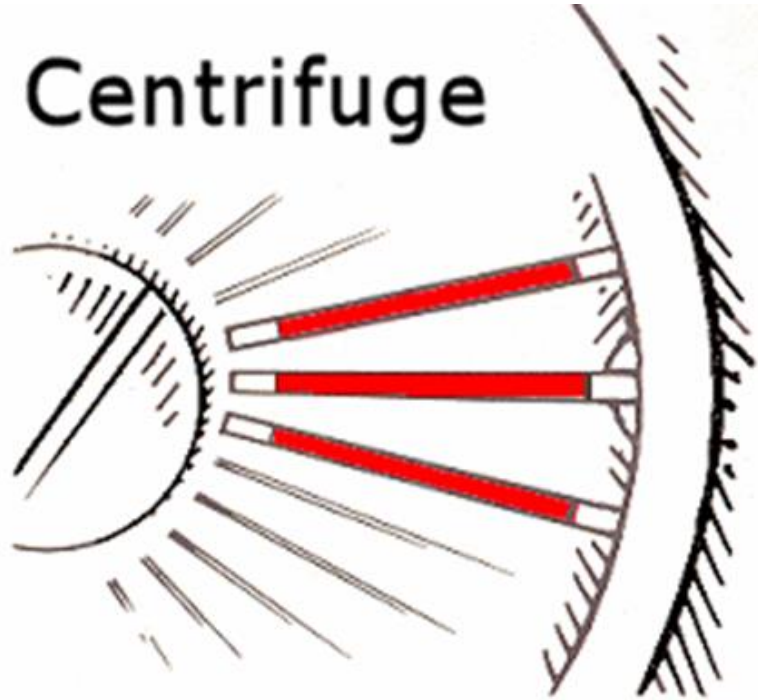
If total number of RBC counted in the five medium squares is = 423

Then the number of RBC in 1 mm³ blood = $423 \times 200 \times 50 = 4,230,000$

Hematocrit or Packed cell volume (PCV)

Procedure:

Centrifuge



Estimation of blood hemoglobin

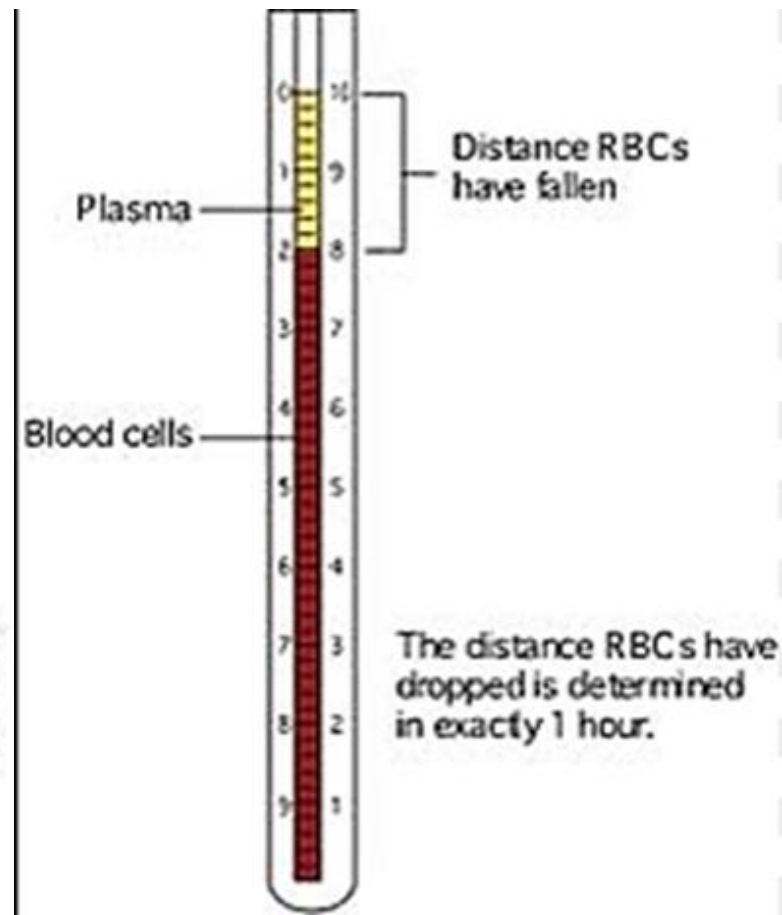
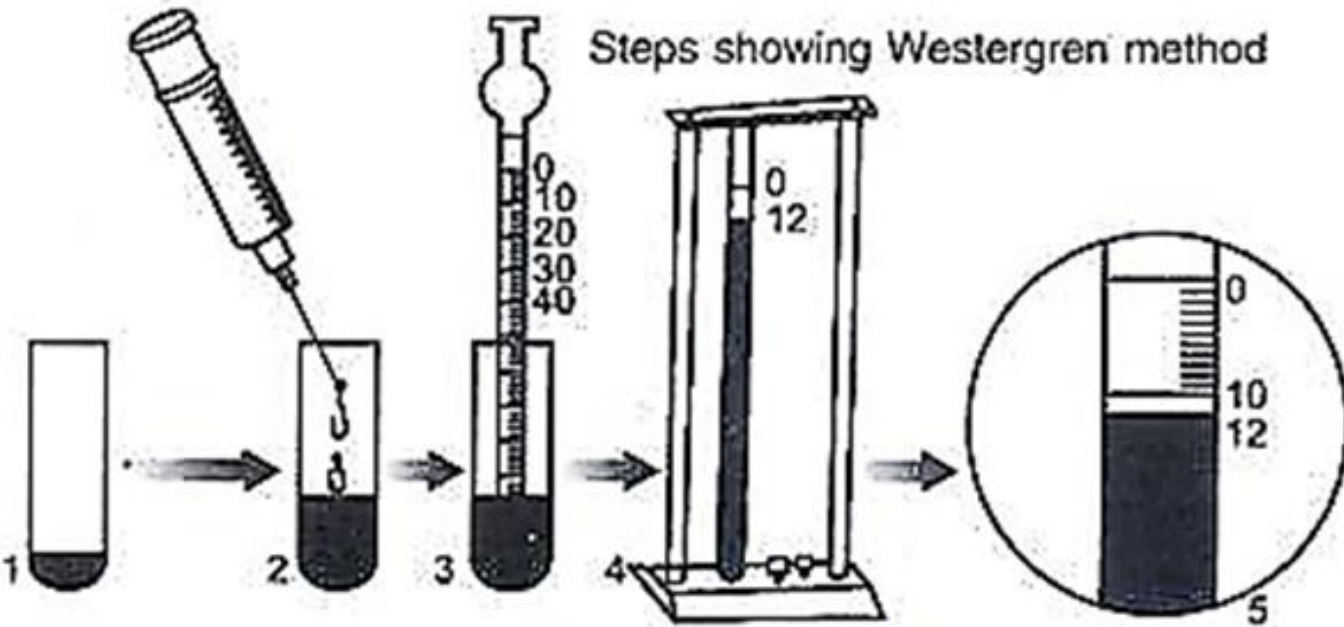
Practical part:

Sahli's Method of Hemoglobin Determination:



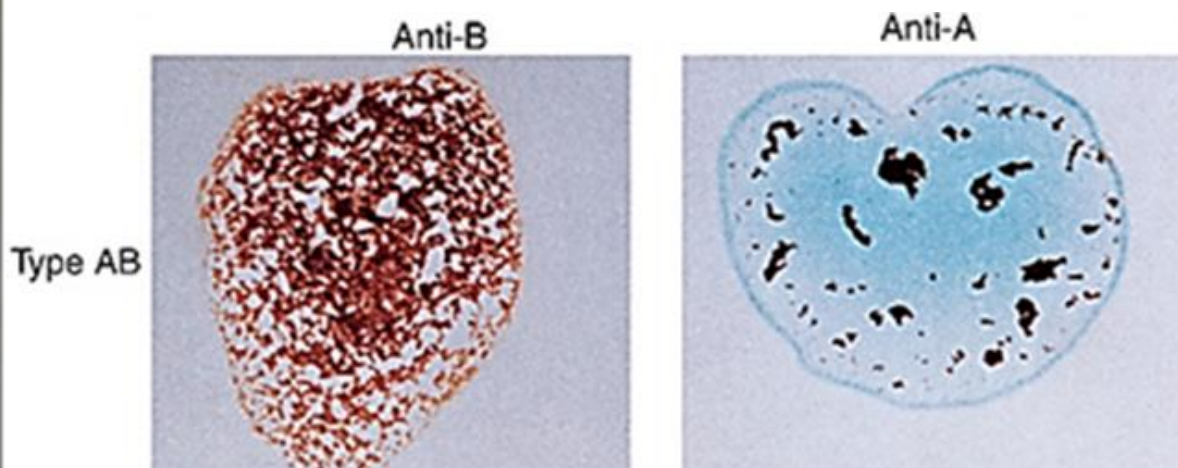
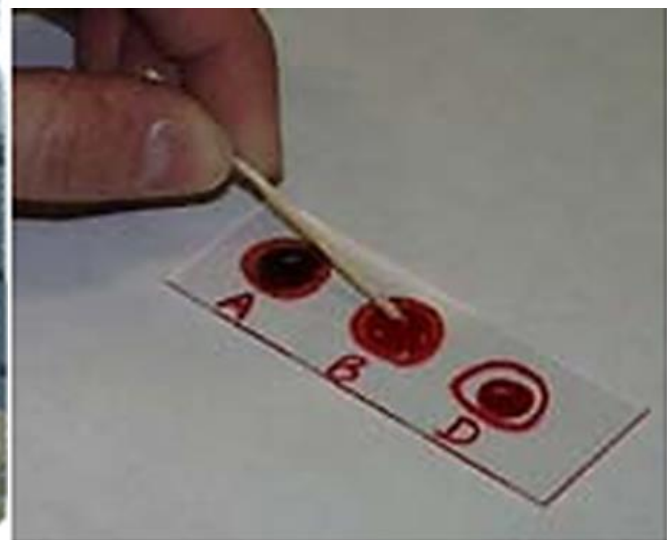
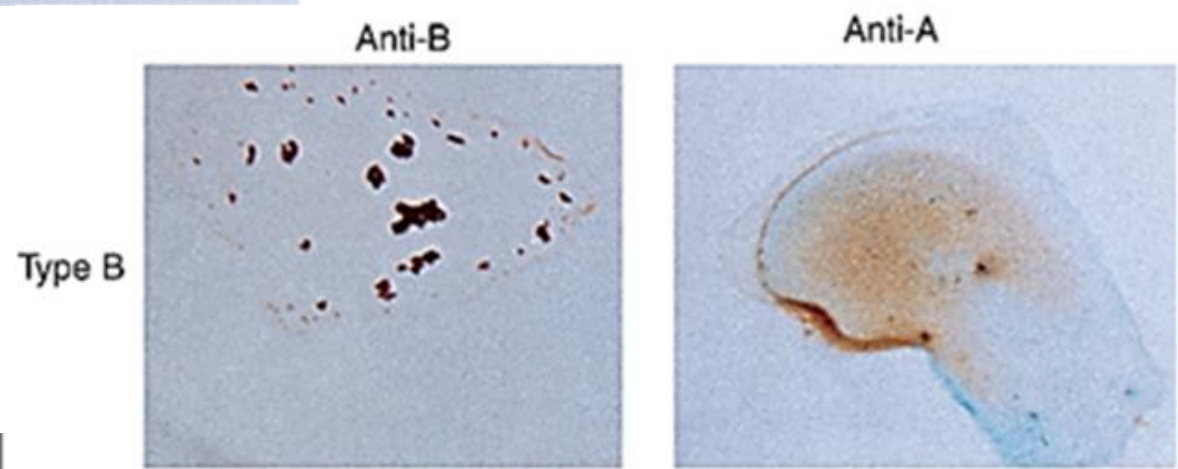
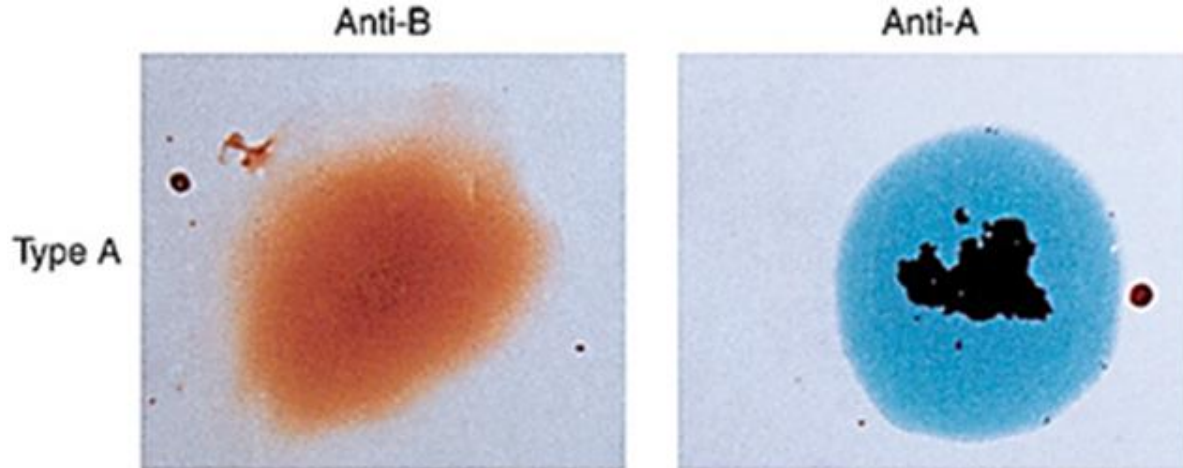
Erythrocyte sedimentation Rate (ESR)

Practical part:



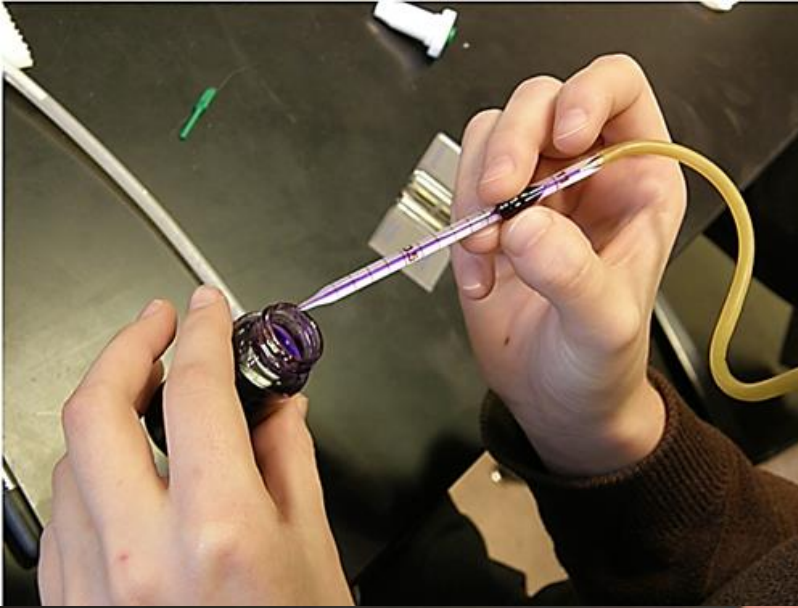
Blood Typing

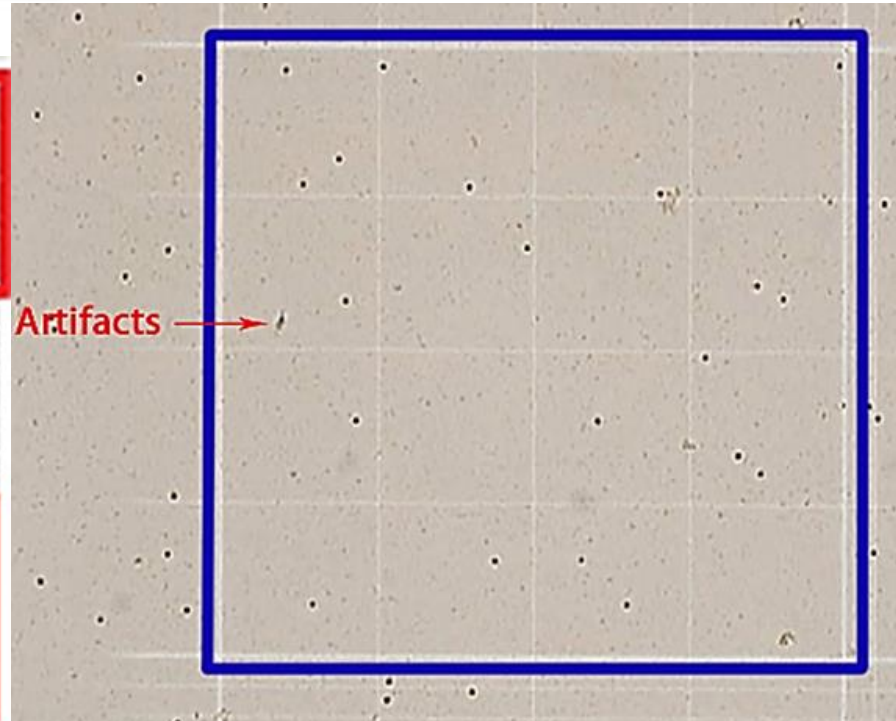
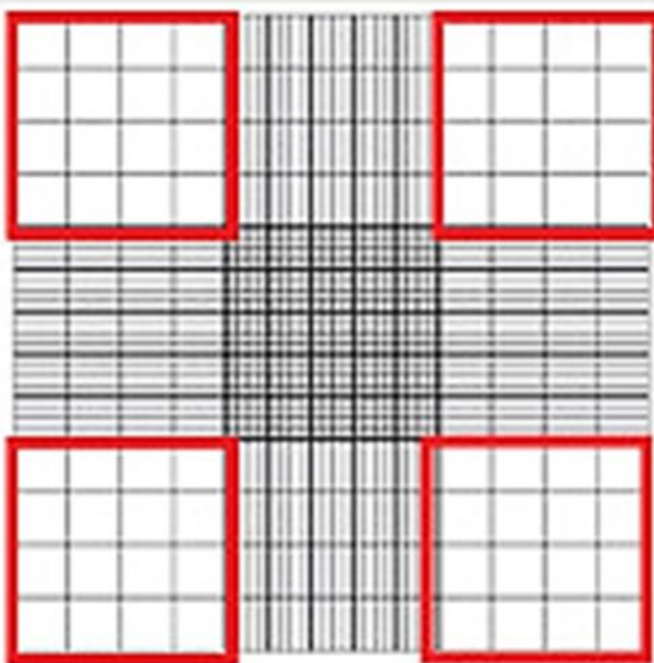
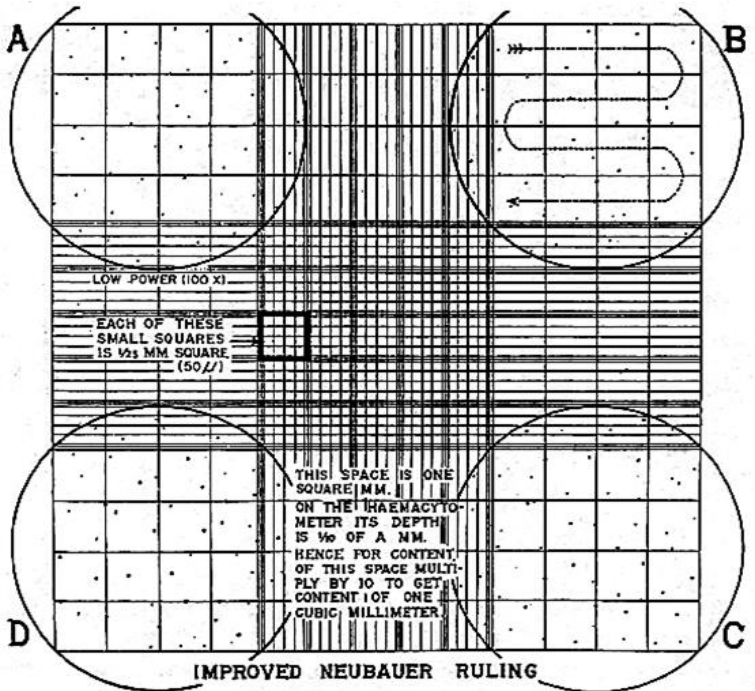
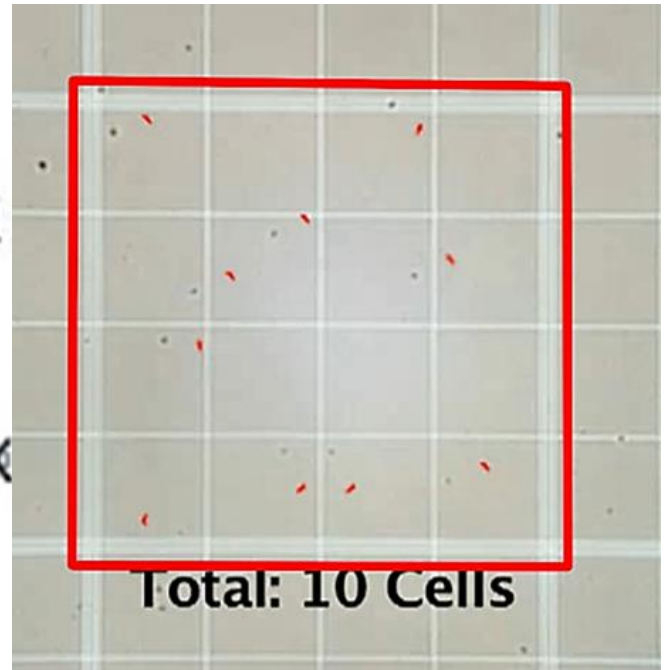
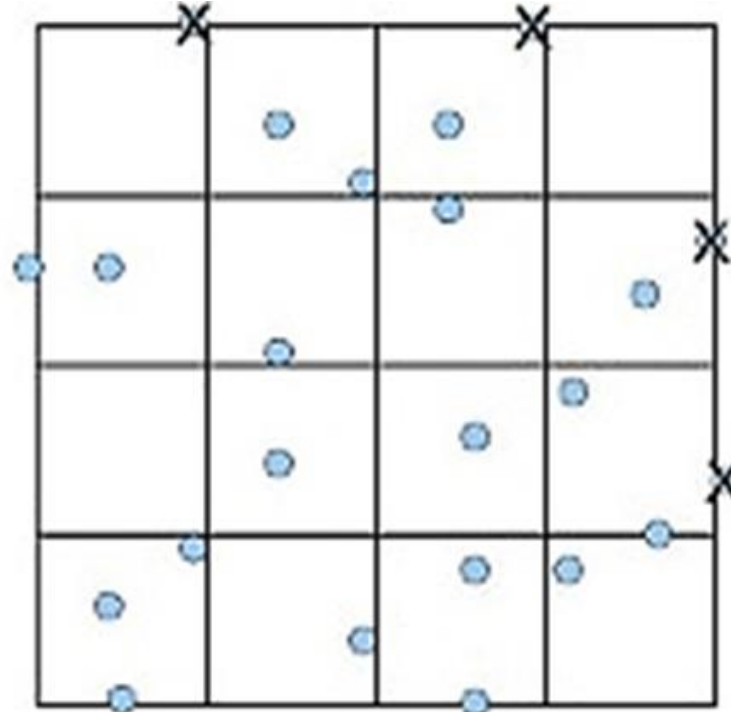
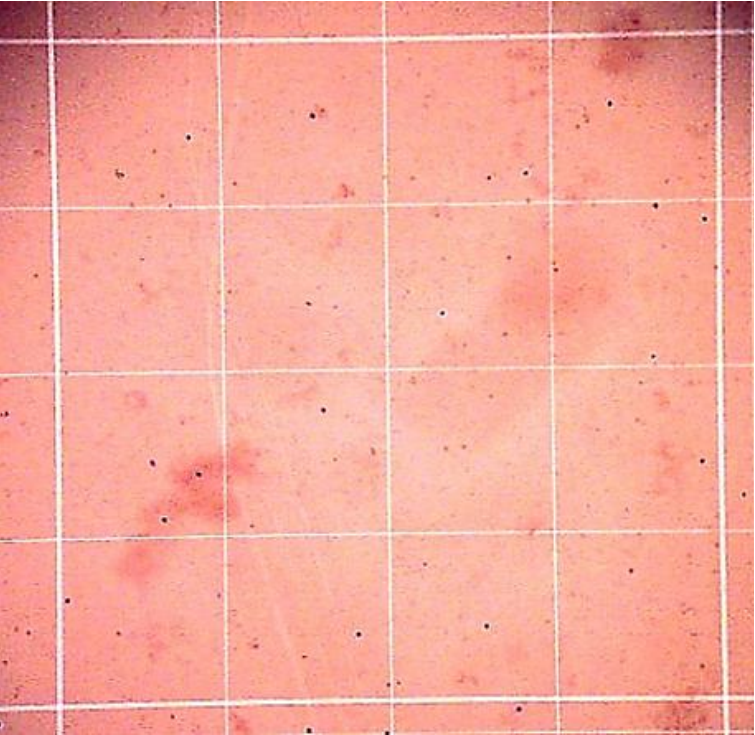
Practical part:



Leukocytes (White Blood Cells) count

Practical part:





Calculation:

(1) Routinely, blood is drawn to the 0.5 mark and diluted to the 11 mark with WBC diluting fluid. All the blood is washed into the bulb of the pipet (which has a volume of 10). Therefore, 0.5 volumes of blood are contained in 10 volumes of diluting fluid. The resulting dilution is 1:20. (These figures are arbitrary and refer strictly to dilution and not to specific volumetric measurements.)

(2) The depth of the counting chamber is 0.1 mm and the area counted is 4 sq mm (4 squares are counted, each with an area of 1.0 sq mm therefore, $4 \times 1.0 \text{ sq mm} =$ a total of 4 sq mm). The volume counted is: area x depth = volume.

Four sq mm x 0.1 mm = 0.4 cu mm

(3) The volume correction factor = $1 / 0.4 = 2.5$

If there are n cells in 0.4 mm³ diluted blood, then the number of cells in 1 mm³ blood

= n x dilution factor x volume correction factor

= n x 20 x 2.5

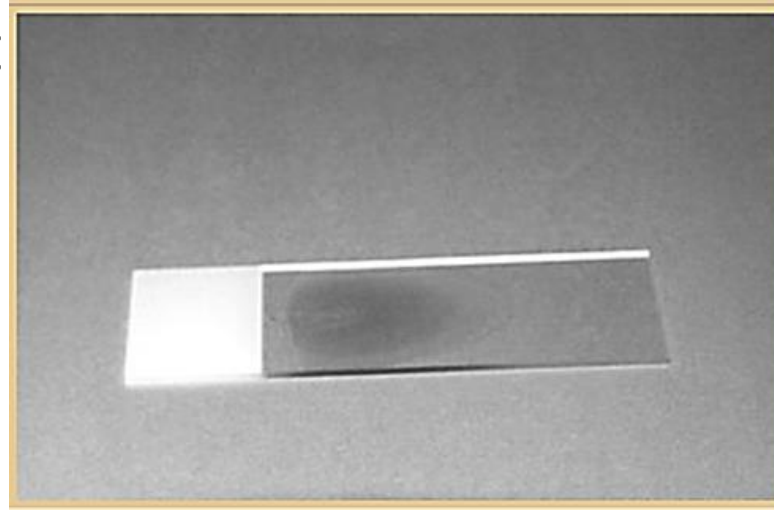
= n x 50

Example:

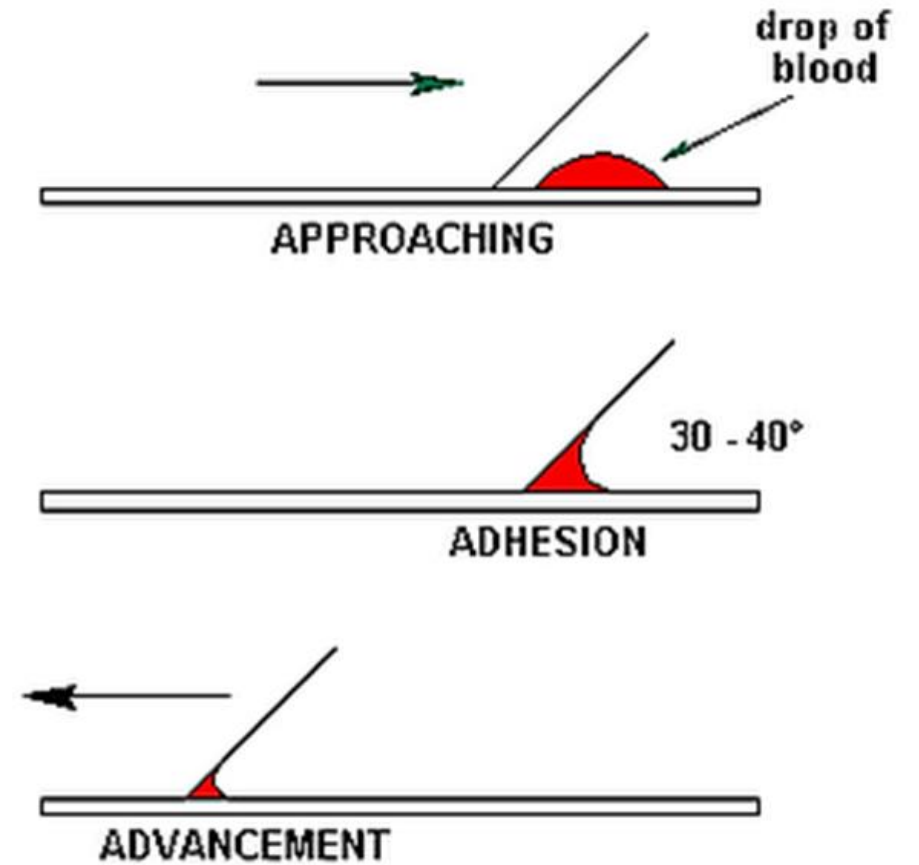
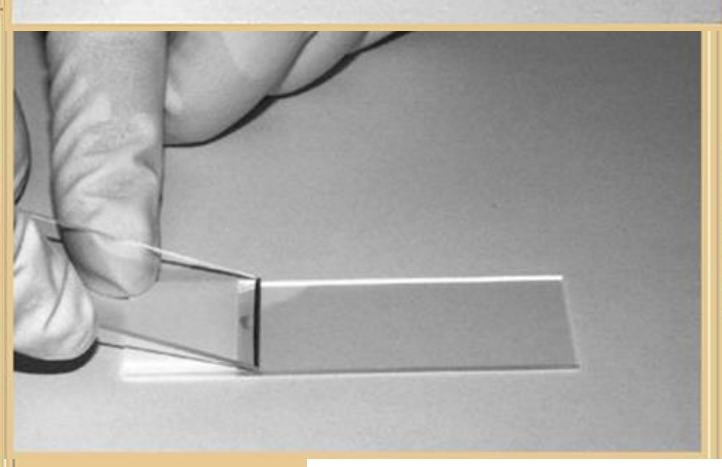
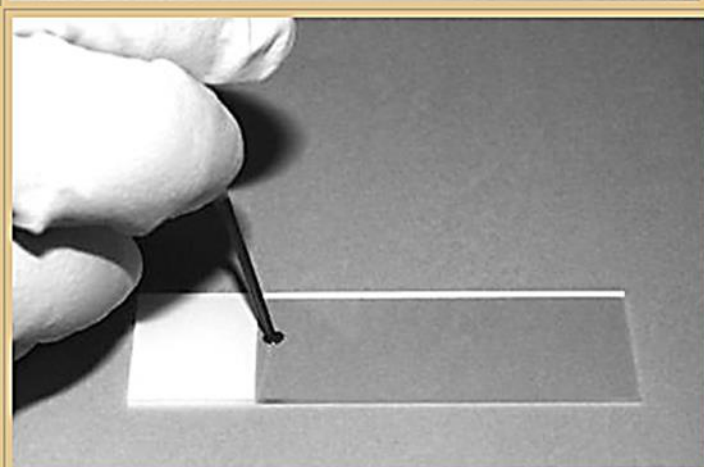
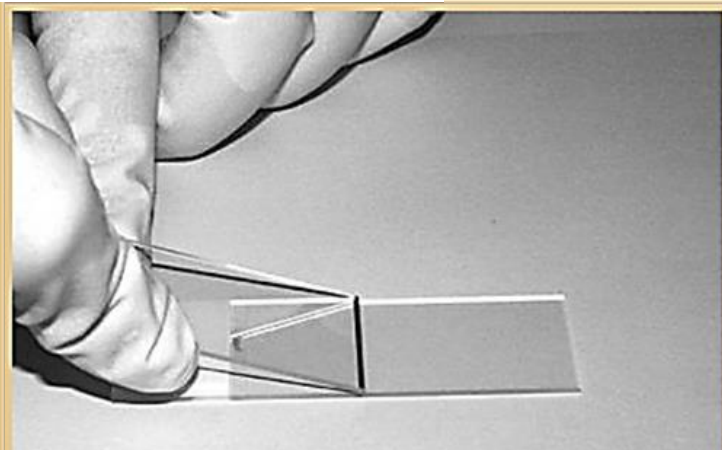
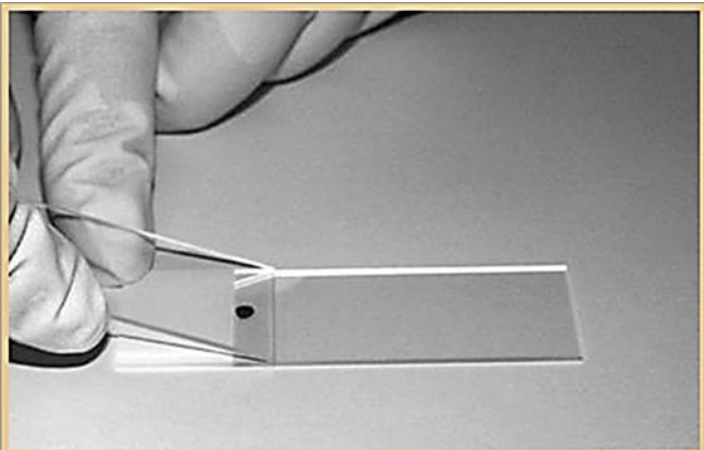
If the total number of WBCs counted in the four large corner square is = 120.

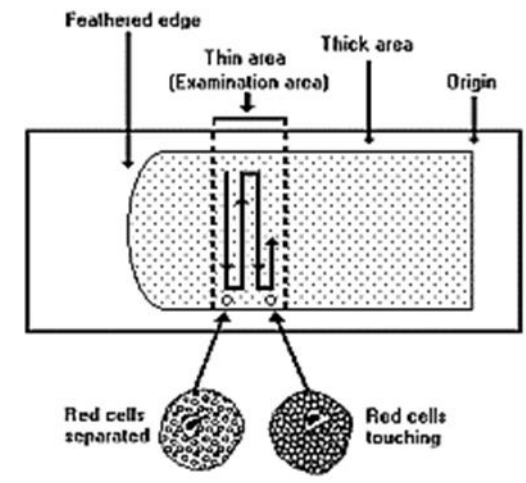
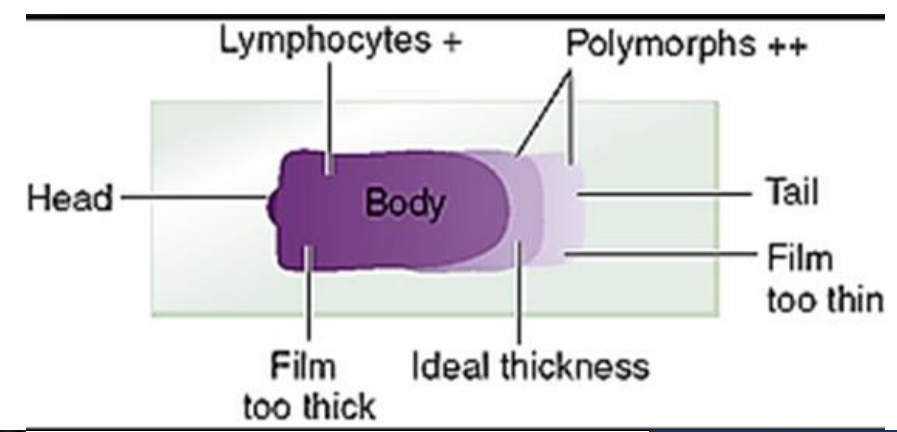
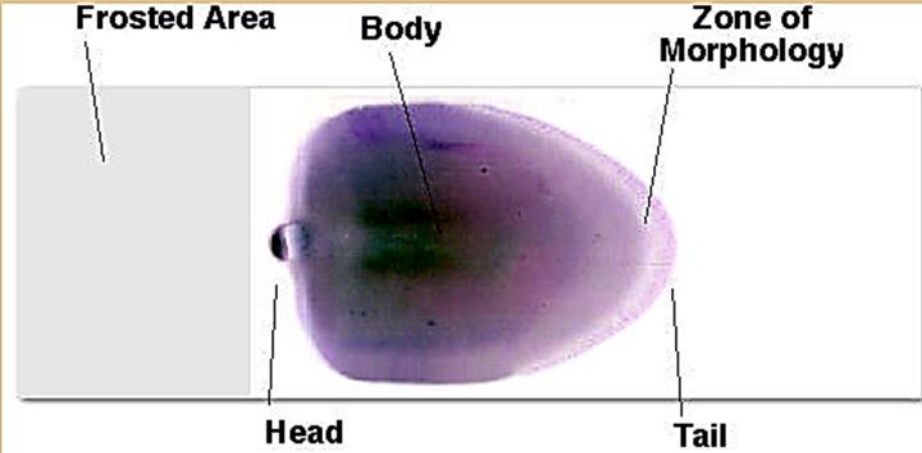
Then the number of WBCs in 1 mm³ blood = $120 \times 50 = 6000$ cell.

- Leukocytes (WBC) differential count
- Practical part:



Step 6. End result. A glass slide with a well-formed blood film. After drying for about 10 minutes, the slide can be stained manually or placed on an automated slide stainer.





Estimation of clotting mechanism

Clotting mechanism:

Practical Part:

Blood coagulation tests:

Bleeding Time:

The bleeding time is the time taken from the puncture of the blood vessel to the **stoppage of bleeding**.

The bleeding time test is a useful tool to test for **platelet plug formation and capillary integrity**.

The bleeding time is more important than Clotting time.

Principle:

Bleeding time assays are used to evaluate

- ① **the vascular (effectiveness of vasoconstriction) and**
- ② **platelet responses** that are associated with hemostasis.

When a sharp-pointed knife is used to pierce the tip of the finger or lobe of the ear, bleeding ordinarily lasts for 1 to 6 minutes. The time depends largely on

- ① the **depth** of the wound
- ② the degree **of hyperemia** in the finger or ear lobe at the time of the test.

Lack of any one of several of the clotting factors can prolong the bleeding time, but it is especially prolonged by lack of platelets.

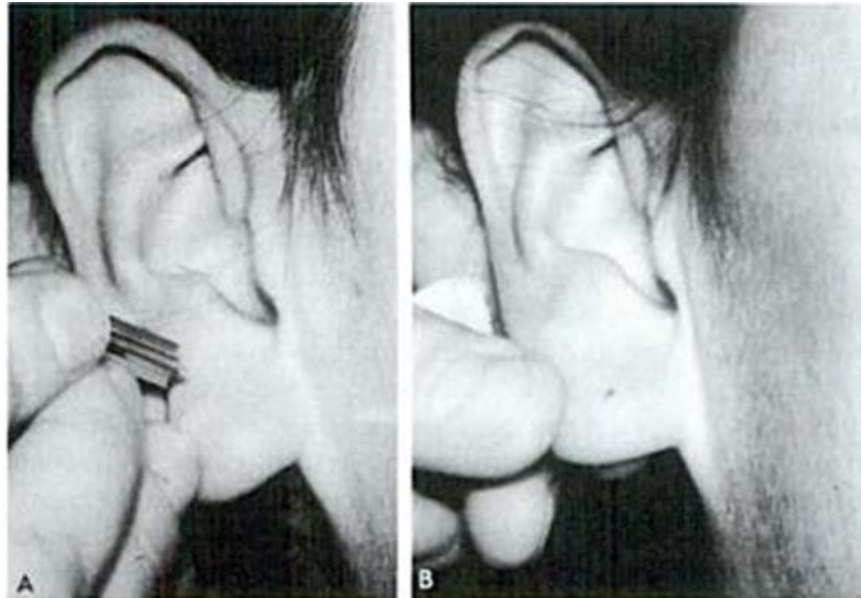
Method:

Bleeding time assay is referred to as the Duke method and in this assay bleeding should **cease within 1–5 minutes**.

A less invasive bleeding time assay involves the use of a lancet or special needle and a **3–4mm deep** prick is made on the fingertip or earlobe.

Bleeding times by Duke's method:

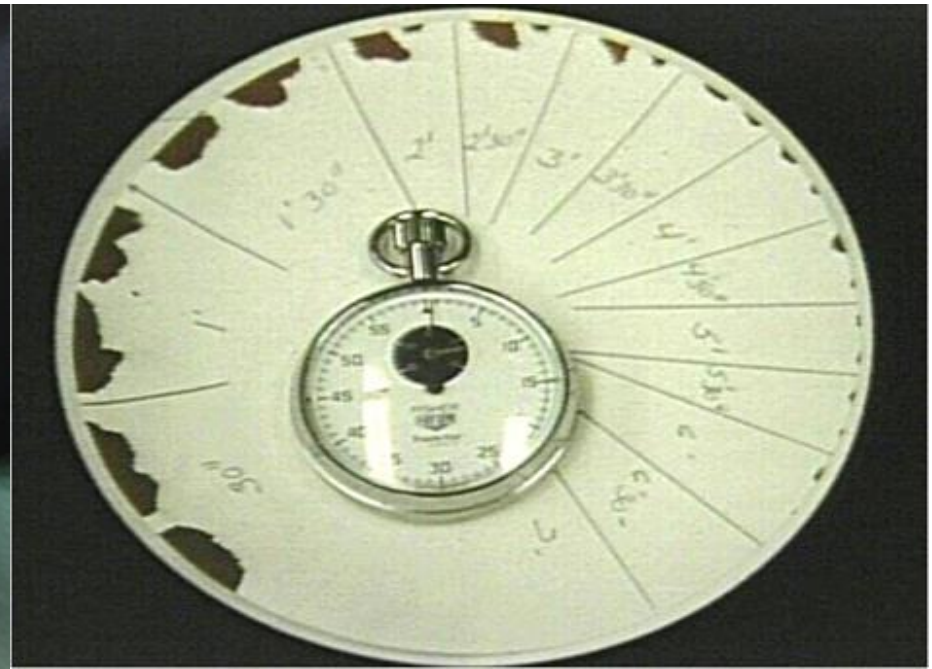
1. Clean and sterilize either the lobe of the ear or the tip of the little finger of your subject with 70% alcohol.
2. Allow the alcohol to dry and make a wound about 4mm deep in the ear lobe or the finger using a sharp lancet.
3. Remove the blood from the wound every 30 seconds on a clean piece of filter paper, using a different area of the paper each time.
4. Continue until the bleeding stops.
5. Count the spots of blood on the filter paper divided by 2 and that will give you the bleeding time in minutes. **The usual time is about 2-6 minutes.**



Bleeding times by Ivy method:

1. Clean the anterior surface of the forearm with spirit.
2. The **blood pressure cuff is placed on the upper arm and inflated to 40 mmHg.**
3. A lancet or scalpel blade is used to make a shallow incision that is **1 millimeter** deep on the anterior of the forearm.
4. The time from when the incision is made until all bleeding has stopped is measured and is called the bleeding time. Every 30 seconds, filter paper or a paper towel is used to draw off the blood.

Normal BT by this method is 3-6 minutes.



Interpretation:

The bleeding time is affected (prolonged) by any defect in

- ① platelet function, by
- ② vascular disorders, and
- ③ in von Willebrand disease but is not affected by other coagulation factors.

Disorders that are commonly associated with an increased bleeding time include:

- ① Thrombocytopenia,
- ② Inherited plasma defect (e.g. disseminated intravascular coagulation (DIC) and factor V)
- ③ Abnormal bleeding times are also found in patients with Cushing syndrome, severe liver disease, leukemia, and bone marrow failure.
- ④ Vascular abnormality
- ⑤ Drug (e.g. aspirin and anti-histamine)
- ⑥ Thrombo-asthenia (platelet number is normal but function is abnormal)

Clotting time:

Clotting Time is the time taken from the puncture of the **blood vessel to the formation of a fibrin thread.**

Principle:

Whole blood, when removed from the vascular system and exposed to a foreign surface, will form a solid clot.

The time required for a sample of blood to coagulate in vitro under standard conditions is called "clotting time".

The whole blood clotting time is a rough measure of all **intrinsic clotting factors** in the absence of tissue factors.

Variations are wide and the test sensitivity is limited.

This procedure has been **replaced in most laboratories with the APTT.**

It is not sensitive to platelet abnormalities.

A prolonged clotting time immediately indicates impaired coagulation, **but a normal clotting time does not exclude many serious clotting defects.**

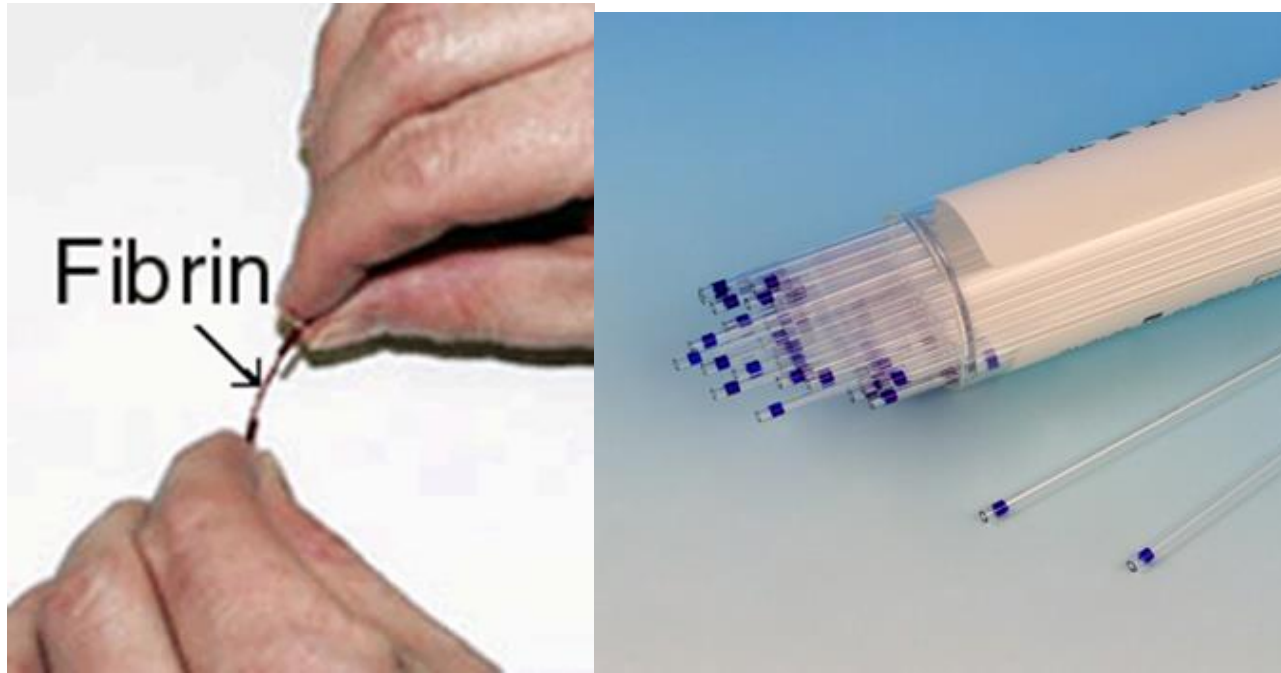
A. Capillary Glass Tube Method:

Method:

1. Clean the finger and sterilize it with 70% alcohol.
2. Obtain a capillary tube labeled “coagulation tubes” or “non-heparinized” tubes.
3. Prick your third fingertip and allow a large drop of blood to collect.
4. Hold the capillary tube horizontally, with one end in the drop of blood, and fill it quickly. Note the time at which the tube is filled.
5. Each 30 sec thereafter, break off about 1 cm of the tube (a paper towel can be used to shield the tube) and gently pull the piece away from the rest of the tube.
6. Continue breaking the tube and pulling away the broken segment until you see fibers or strands in the blood between the broken segment and the rest of the tube

The strand or fibers are evidence of clotting and, when they are observed, the time should again be noted. The interval between the time that the tube was filled and the time that coagulation was observed is known as the clotting time or coagulation time.

Normal clotting time by this **method 3-8 minutes**



B. Lee & White method:

Method:

The one most widely used is to collect blood in a chemically clean 8 mm diameter glass tube and then to tip the tube back and forth about every 30 seconds until the blood has clotted.

By this method, **the normal clotting time is 6 to 12 minutes**. Procedures using multiple test tubes have also been devised for determining clotting time more accurately.

Unfortunately, the clotting time varies widely, depending on the method used for measuring it, **so it is no longer used in many clinics**. Instead, measurements of the clotting factors themselves are made, using sophisticated chemical procedures.

Interpretation:

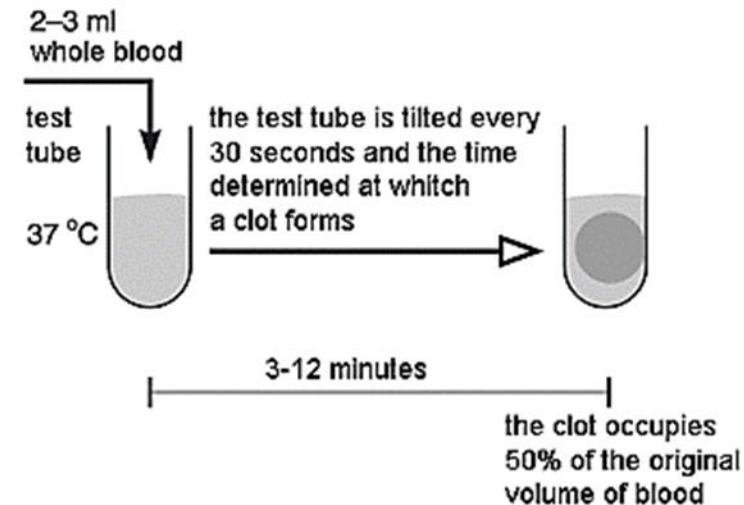
The clotting time is prolonged if there is deficiency in one of the coagulation factor of the intrinsic pathway

Causes of prolonged clotting time:

- ① Vitamin K deficiency as obstructive jaundice & prolonged use of antibiotics that will kill intestinal flora
- ② Liver cell failure
- ③ Hemophilia: factor II, VIII, IX, X and XI deficiency
- ④ Patients receiving dicumarol or heparin (anticoagulant drugs).

Thrombocytopenia bleeding time increased, clotting time normal.

Hemophilia bleeding time normal, clotting time increased.



Capillary Fragility Test:

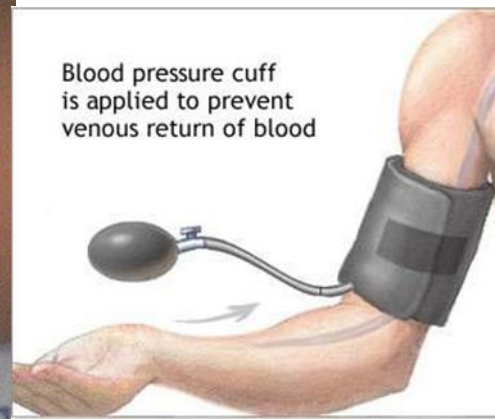
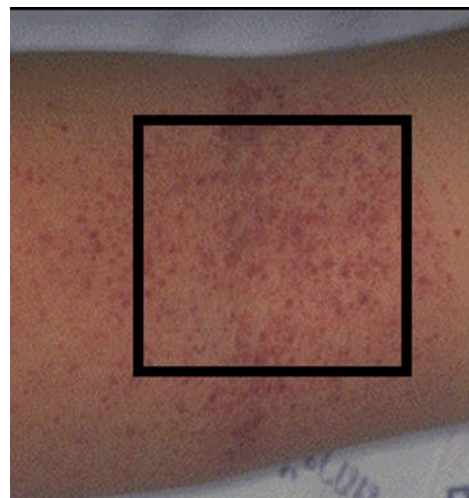
Capillary Fragility Test (also called the **Hess test** or **Rumpel-Leede** test or tourniquet test) is a medical test used to assess mechanical capillary fragility by raising pressure within them

Method:

1. A circle of 5 cm diameter is marked on the forearm.
2. Any purple spots present in the circle are marked with blue ink
3. Pressure is applied to the forearm with a blood pressure cuff inflated to between systolic and **diastolic blood pressure for 15 minutes.**
4. After removing the cuff, the number of petechial in the area under pressure is counted.

Normally less than 15 petechiae are seen.

15 or more petechiae indicate capillary fragility,



Interpretation:

Positive Capillary Fragility Test is seen in

1. Poor platelet function or number (thrombocytopenia)
2. Scurvy
3. Dengue fever

Interfering factors with this test are women who are premenstrual, postmenstrual and not taking hormones, or those with sun damaged skin, since all will have increased capillary fragility.