Pre-pharmacy

PHYSIOLOGY

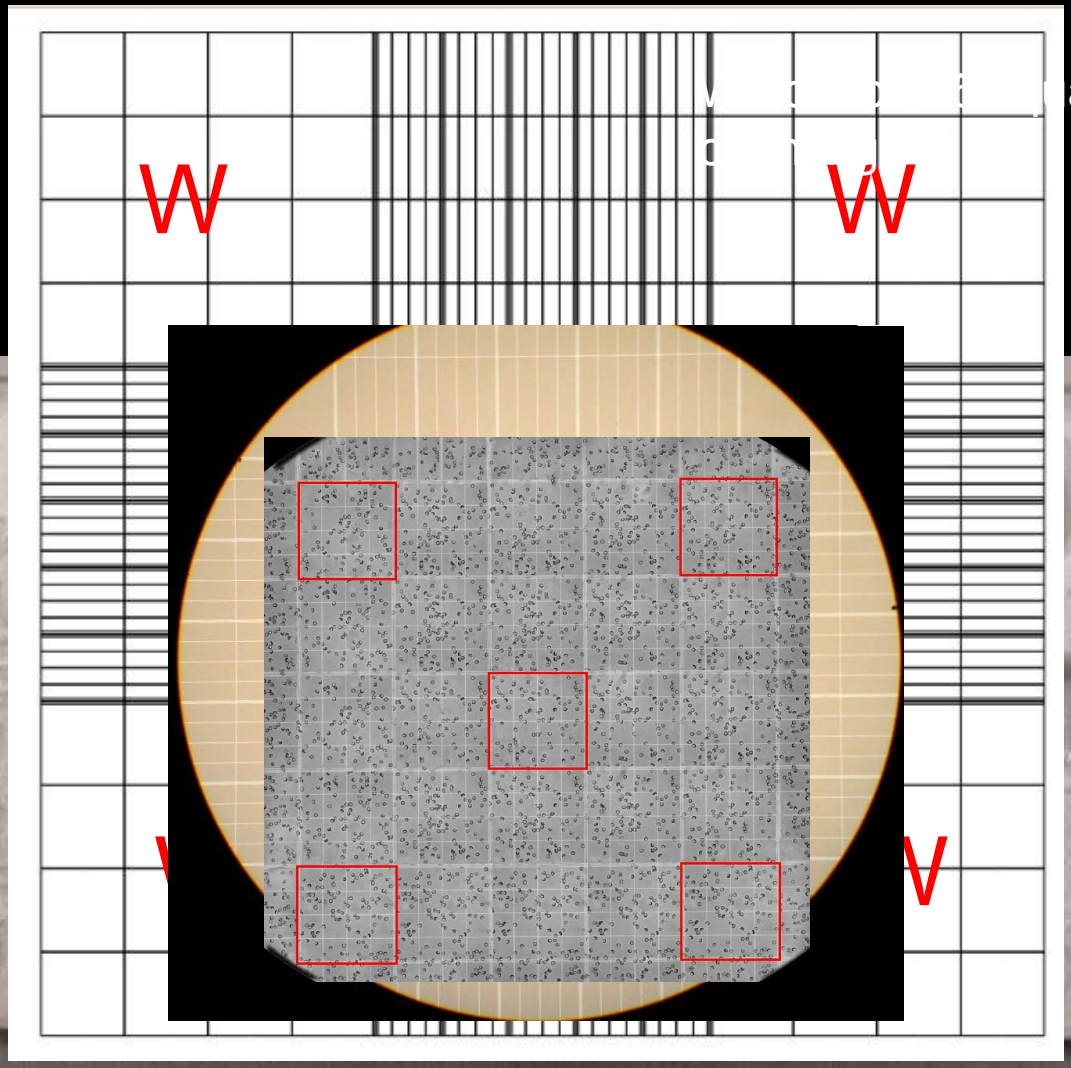
1- RBC’s count by haemocytometer

- Aim:
  - The number of RBC’s is counted by haemocytometer in a given volume.

- Counting slide:

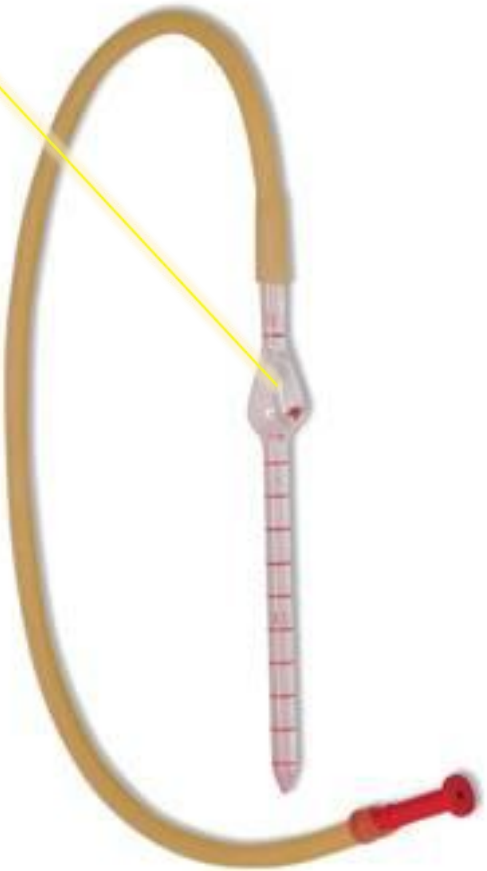
Is divided into “16” big squares separated by triple lines, each big square separated into “16” small squares .

ares for

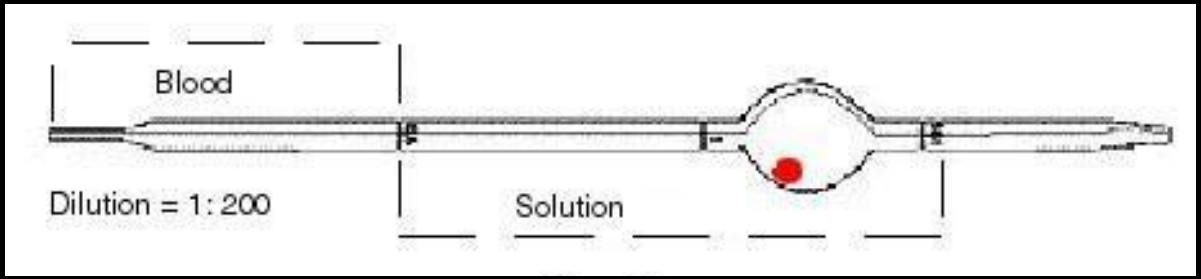


Red cell pipette:

Red bead

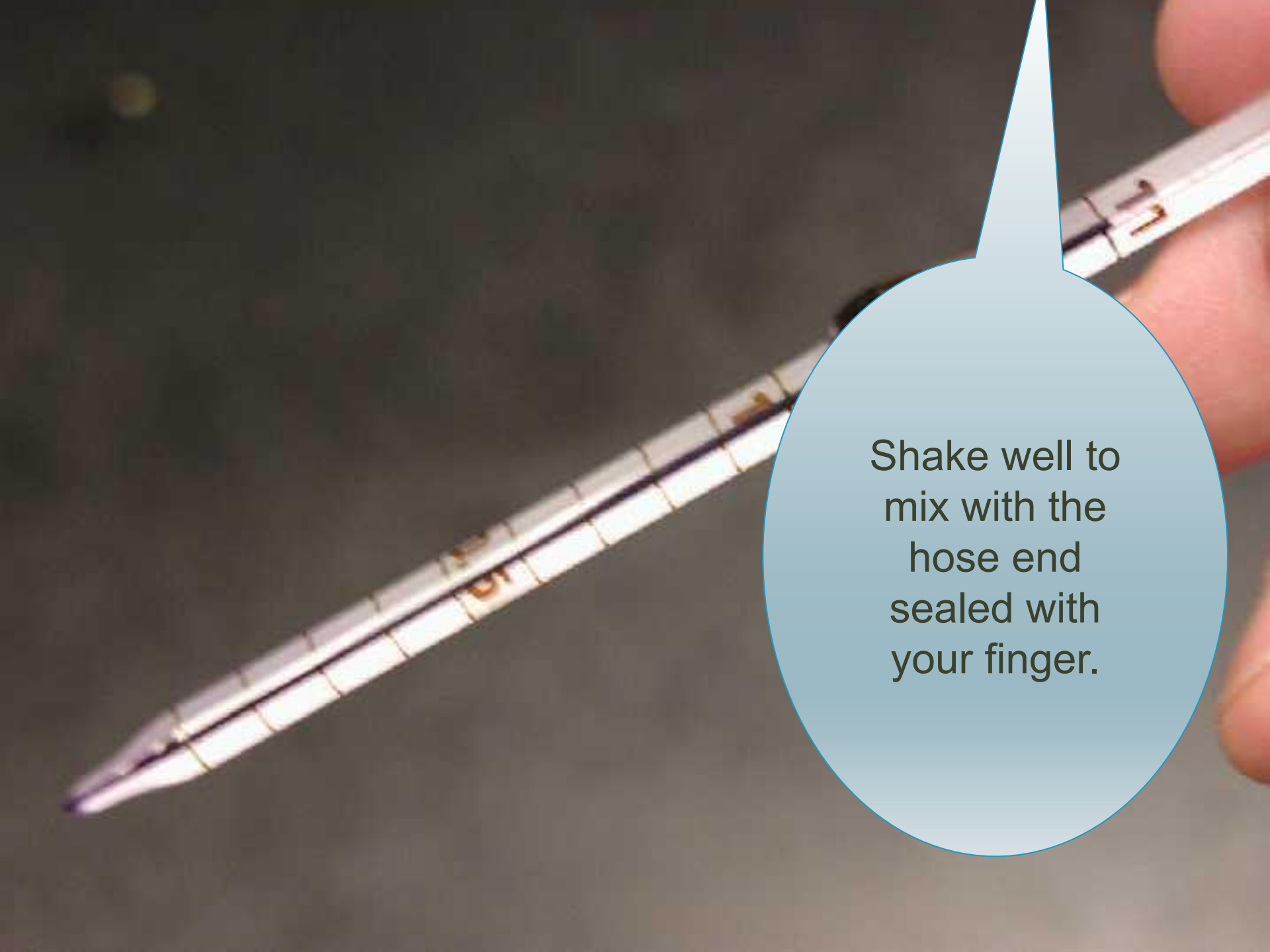


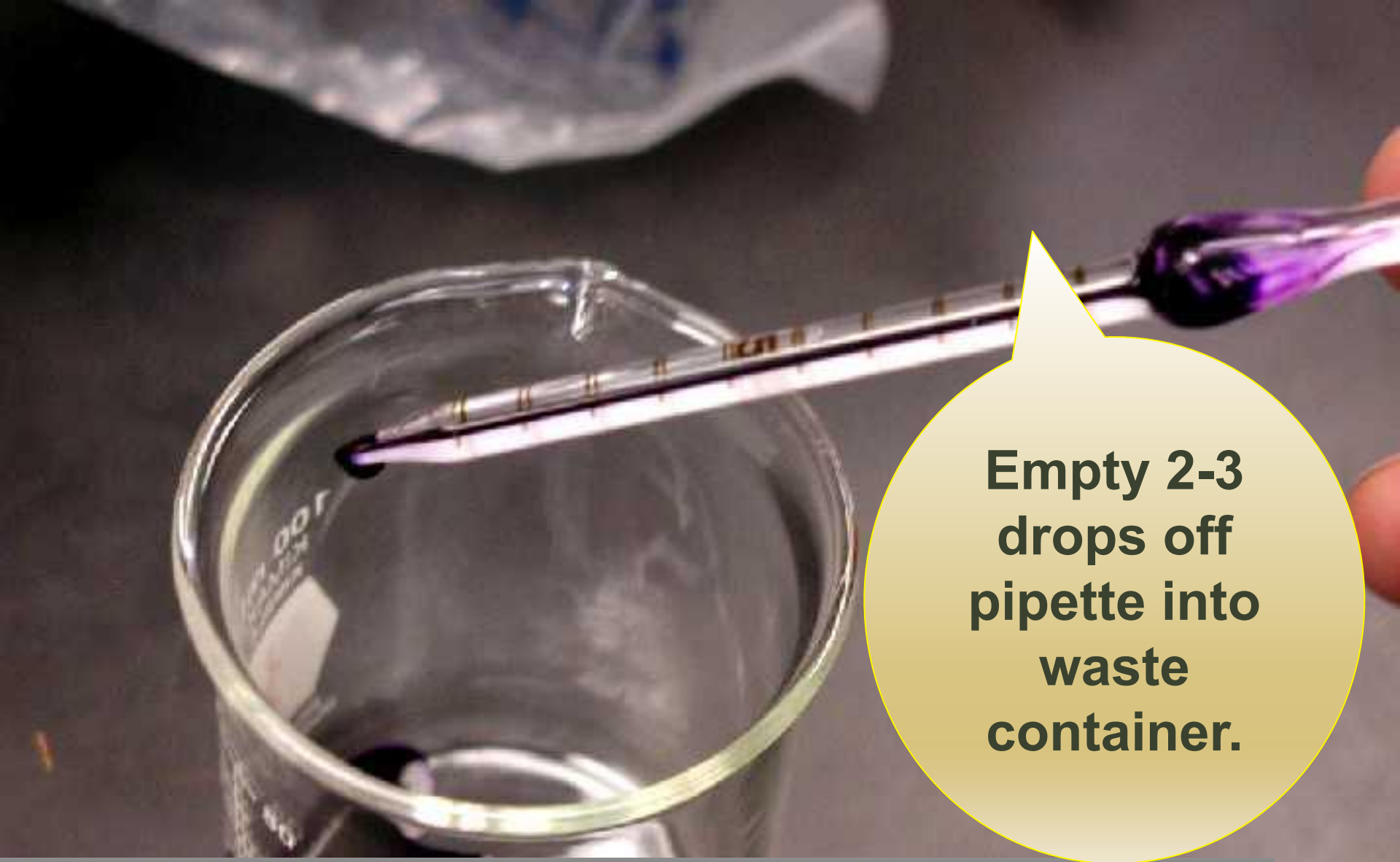
Blood is drawn to 0.5 mark, excess blood is wiped off.
Then fill slowly by isotonic solution (0.9% Nacl), till 101 mark.
Then mix the content by shaking and rubbing, 3 drops are expelled.
Then we fill the counting chamber, and let the cells settle for counting for 3 min.



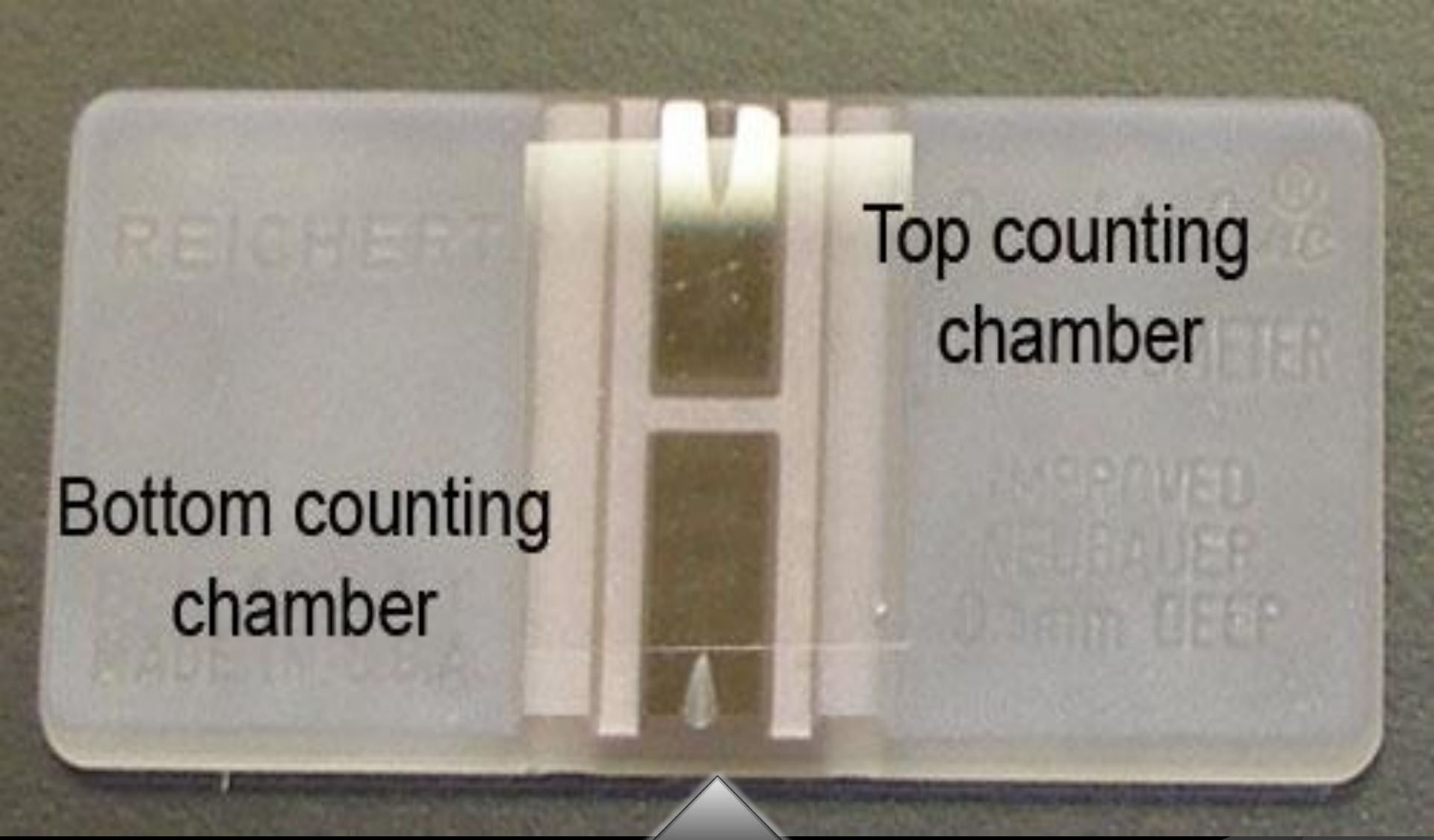
0.5

101

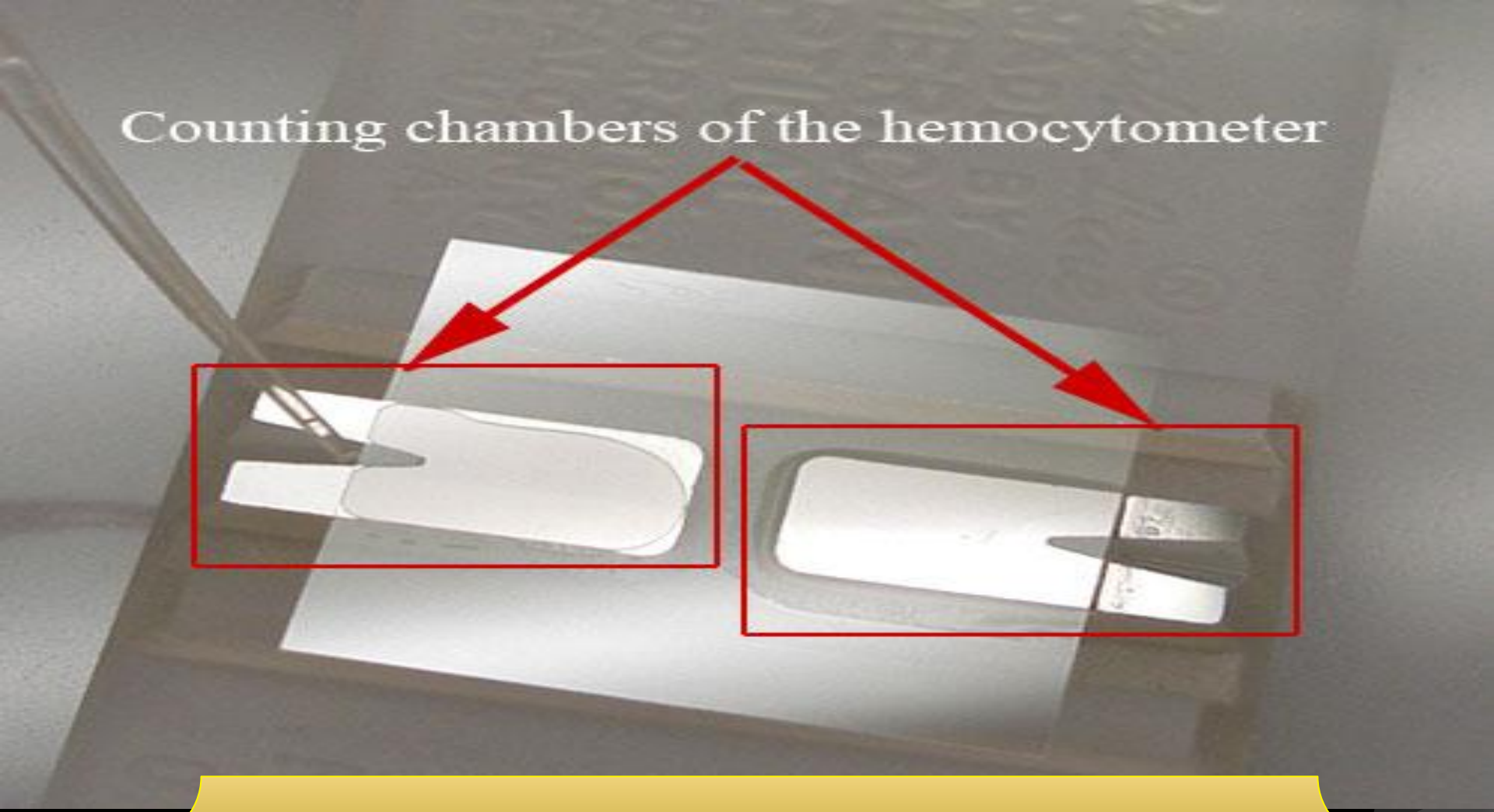

Shake well to mix with the hose end sealed with your finger.



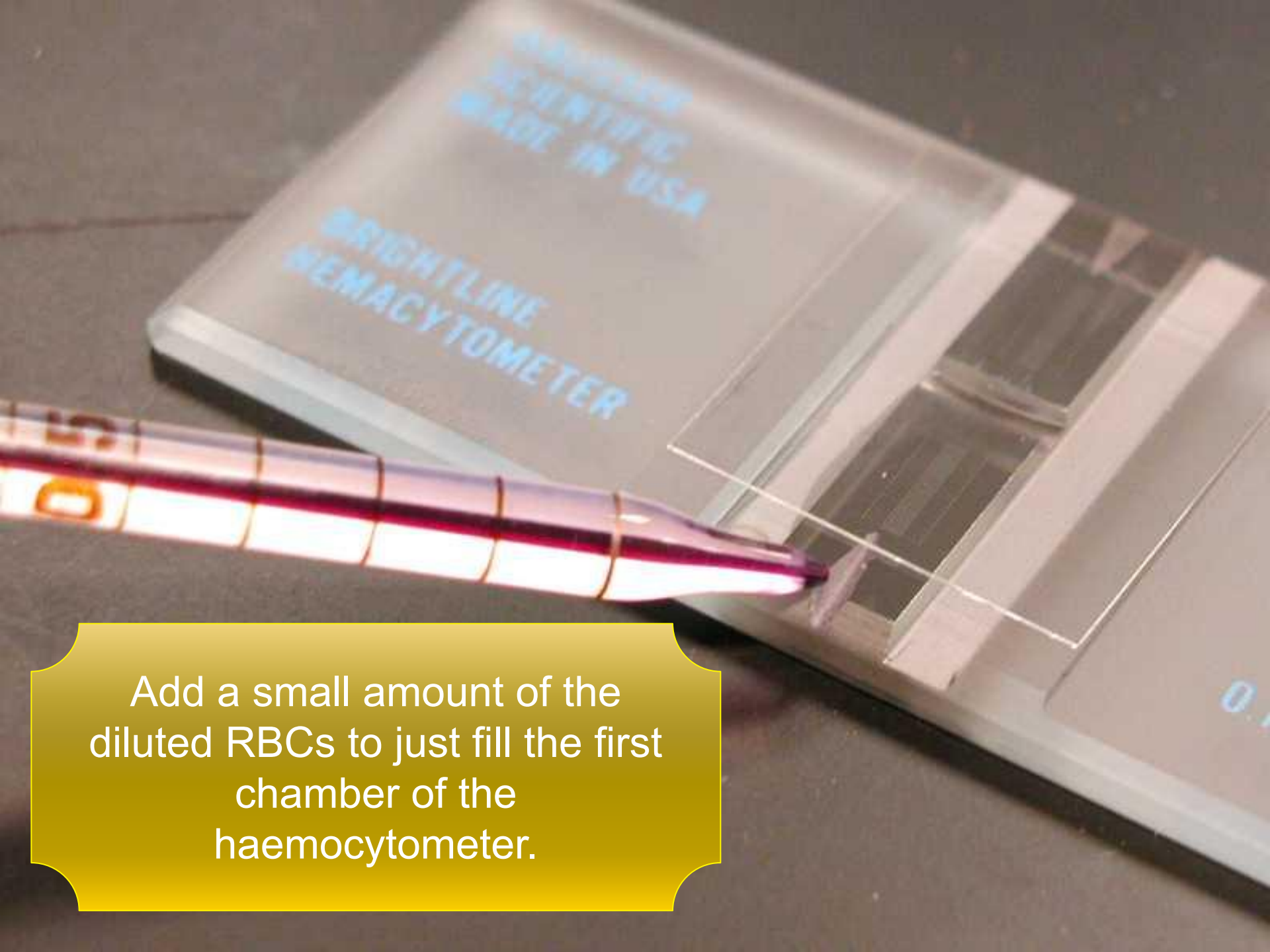
Unmixed cell free fluid in the capillary portion of the pipette



A special device will be used for counting procedures called haemocytometer



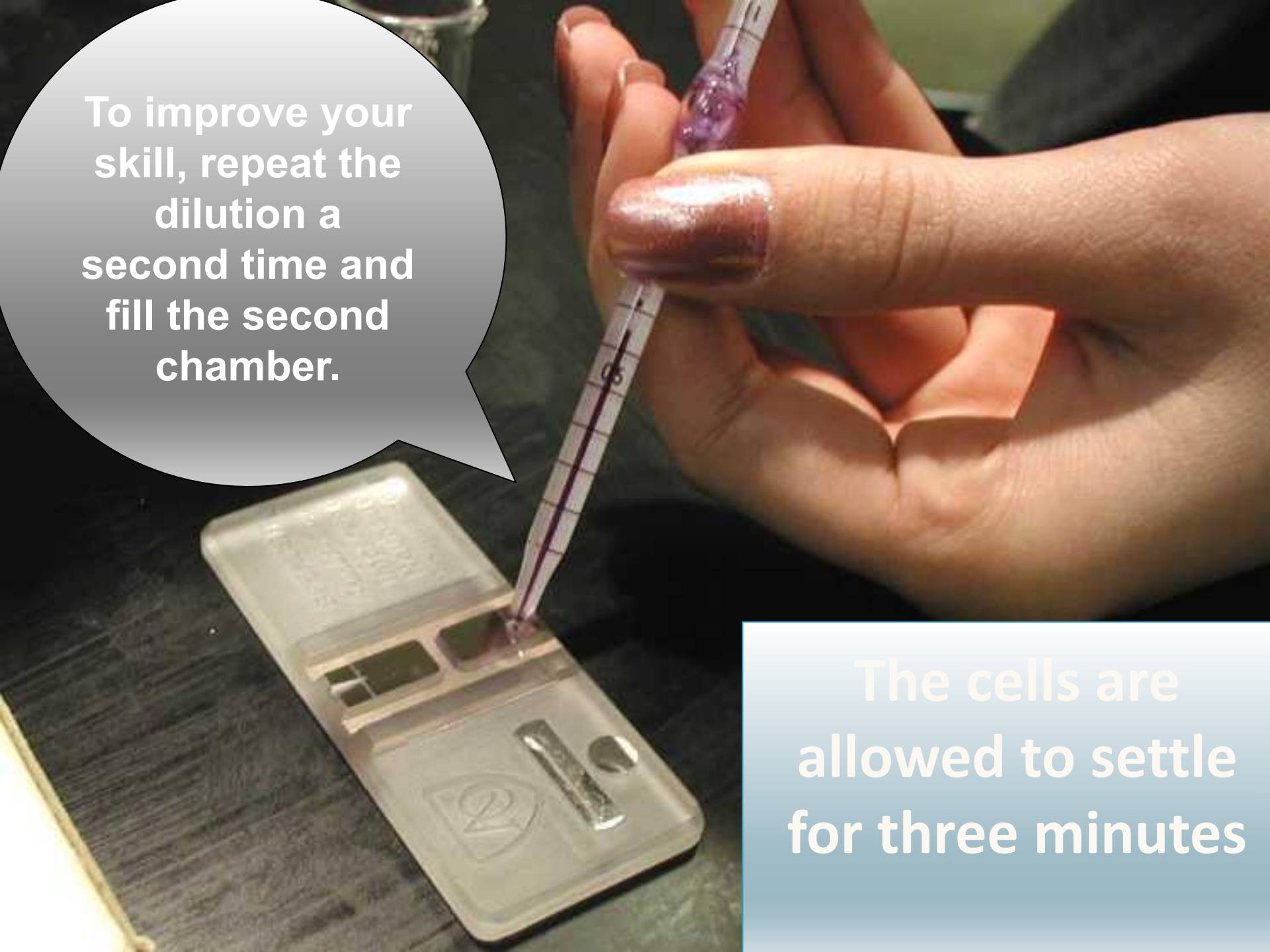
Carefully adjust the haemocytometer on the microscope and cover



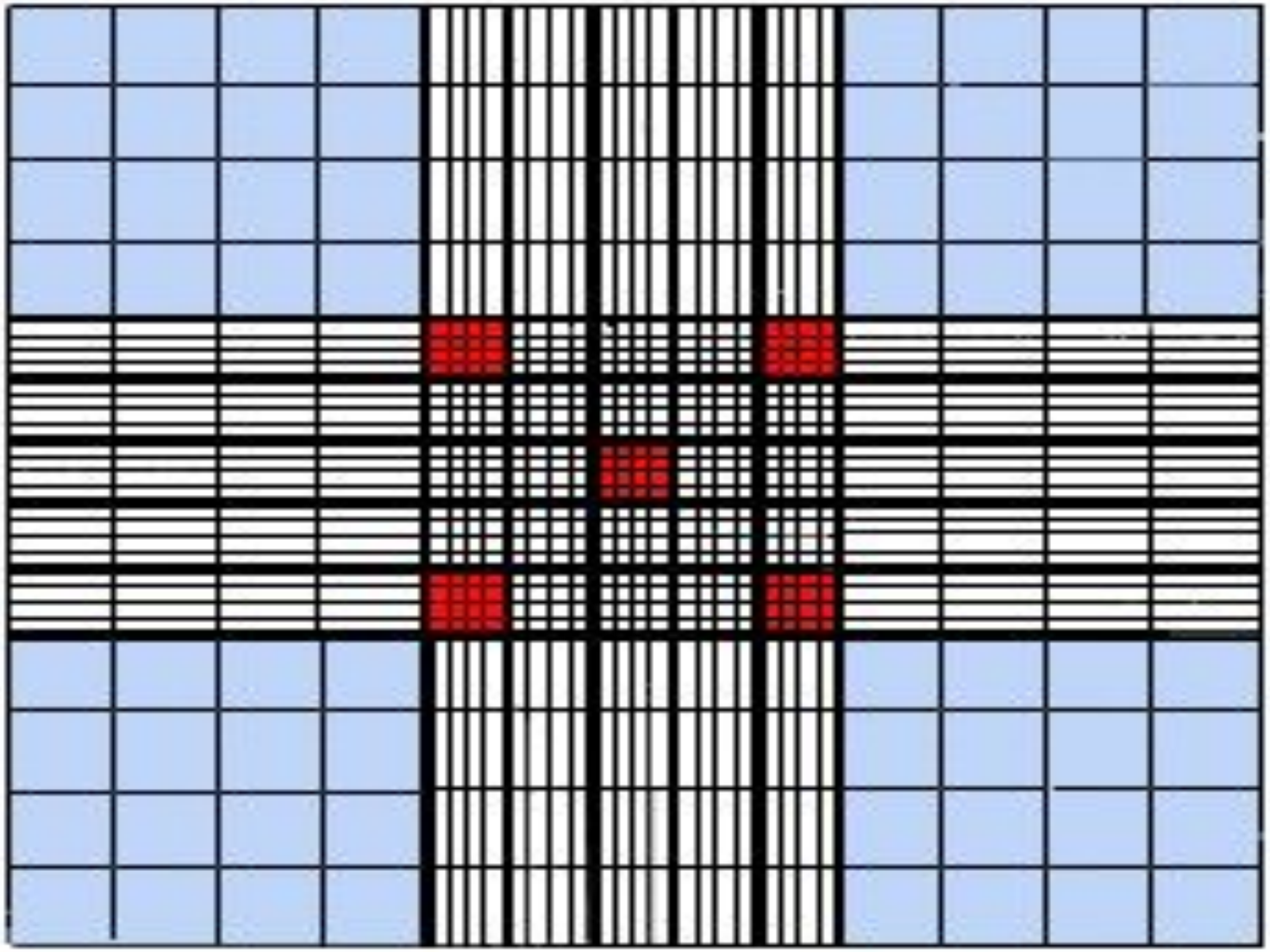
Add a small amount of the diluted RBCs to just fill the first chamber of the haemocytometer.

Notice that

- **It should flow in to fill the chamber by capillary action.**
- **Do not over fill.**


To improve your skill, repeat the dilution a second time and fill the second chamber.
The cells are allowed to settle for three minutes

areas of the grid where WBC are counted



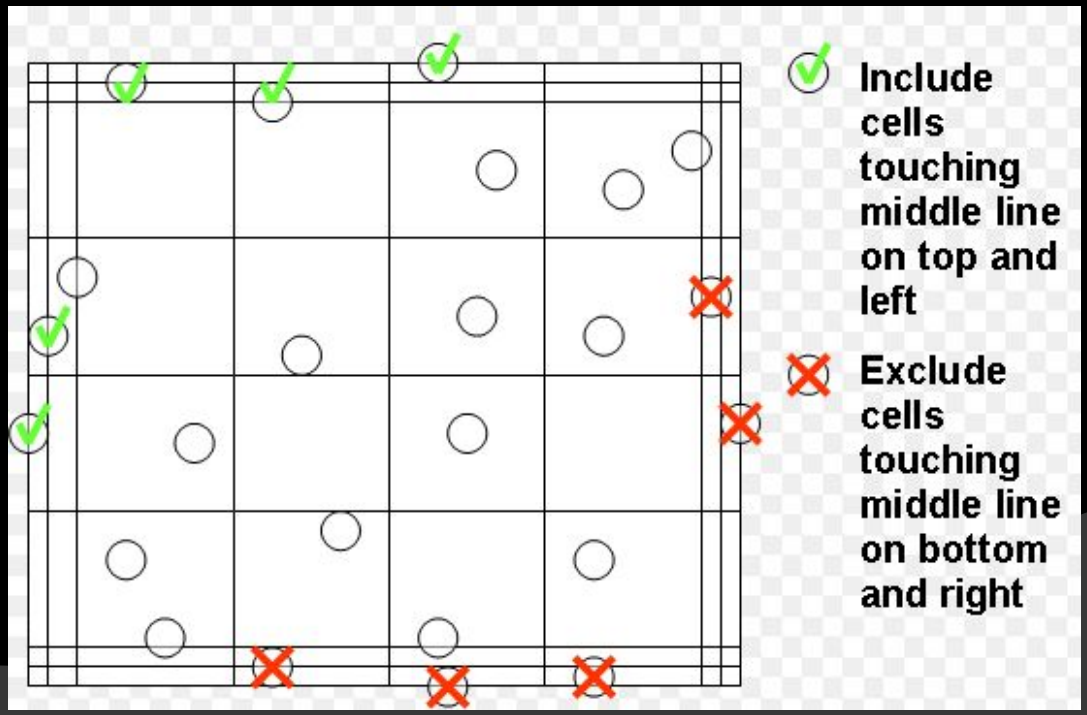
areas of the grid where RBC are counted

- 4 or 5 groups of the 16 squares are counted using the high objective
- All the cells within the squares and those touching the upper and right hand are counted.

Counting chamber

It is the space formed between the cover placed on the counting slide and its surface.

Note:



To avoid repetition!

Calculations

- Calculations:

$$\bar{x} = \frac{\sum x}{n} = \frac{x_1 + x_2 + x_3 + x_4}{4}$$

- No, of RBC's / small square = $\frac{\bar{x}}{16}$
- Volume of small square = $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} = \frac{1}{4000}$
- $\frac{\bar{x}}{16} \longrightarrow \frac{1}{4000}$

  $? \quad 1ml$
- No, of RBC's = $\frac{\bar{x}}{16} \times 4000 \times 200$

Diluting factor

- Normal value:
  - ♂ = 4.8 -5.6 million cell/ mm3
  - ♀ = 4.6 – 5.2 million cell / mm3

2- colorimetric determination of “Hb” by haemometer

- Aim:
  - Determination of amount of “Hb” by change in color using haemometer.
  - Hb → hemoglobin : it’s formed in the bone marrow, and consists of:

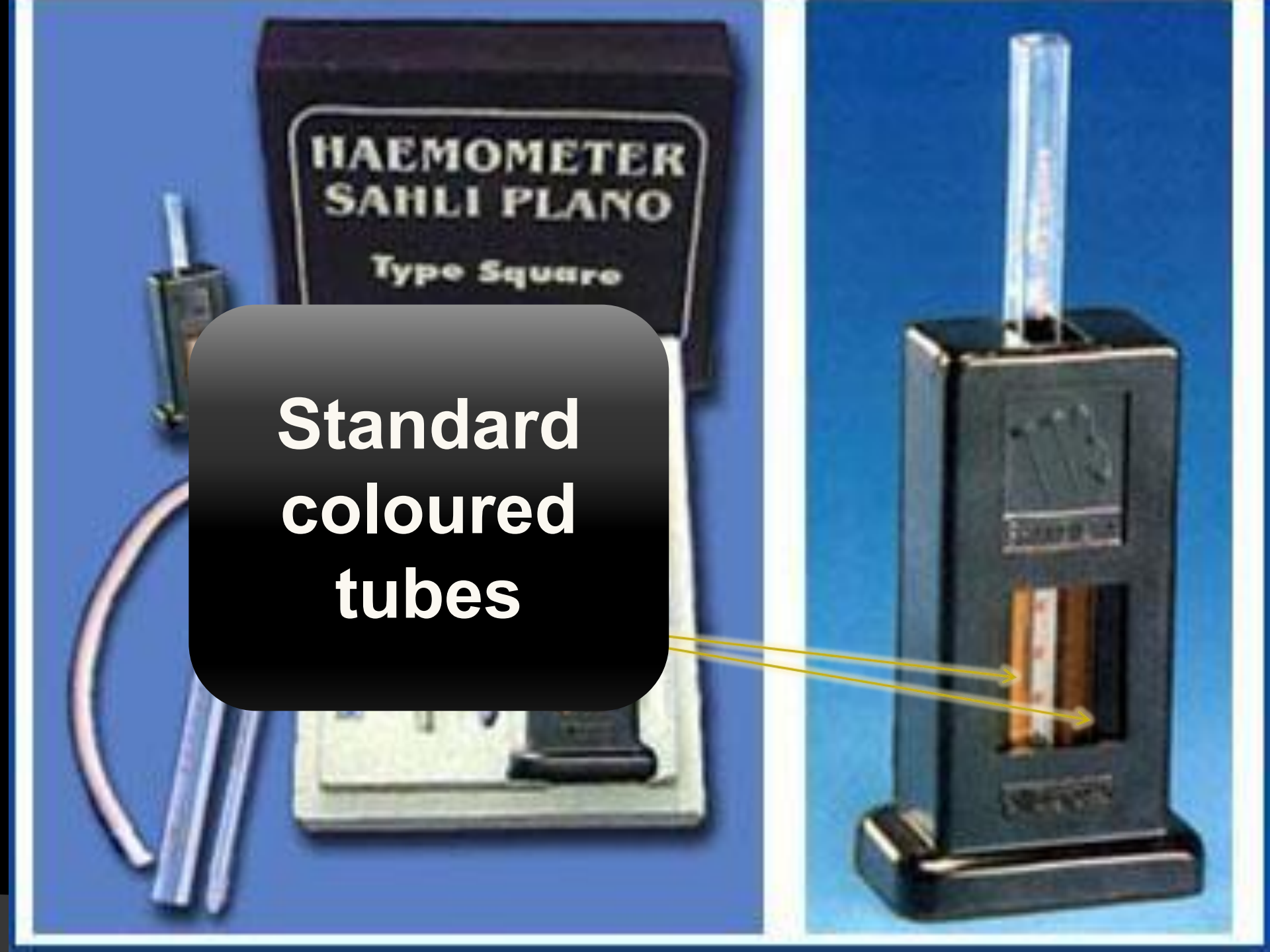
    → “haem + globin”

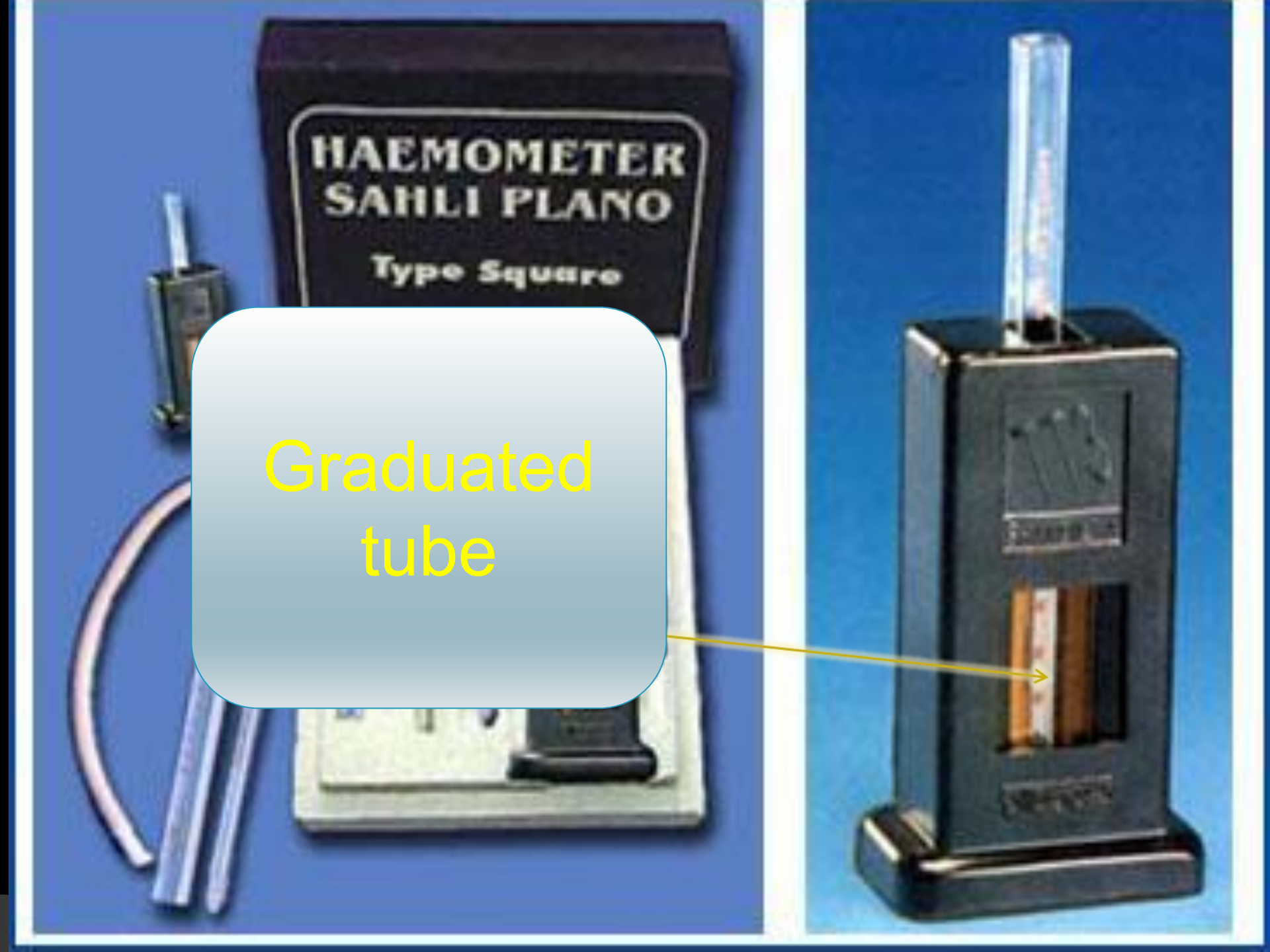
    - Haem : Iron + protoporphrin
    - Globin : Amino acid + ribonucleic acid

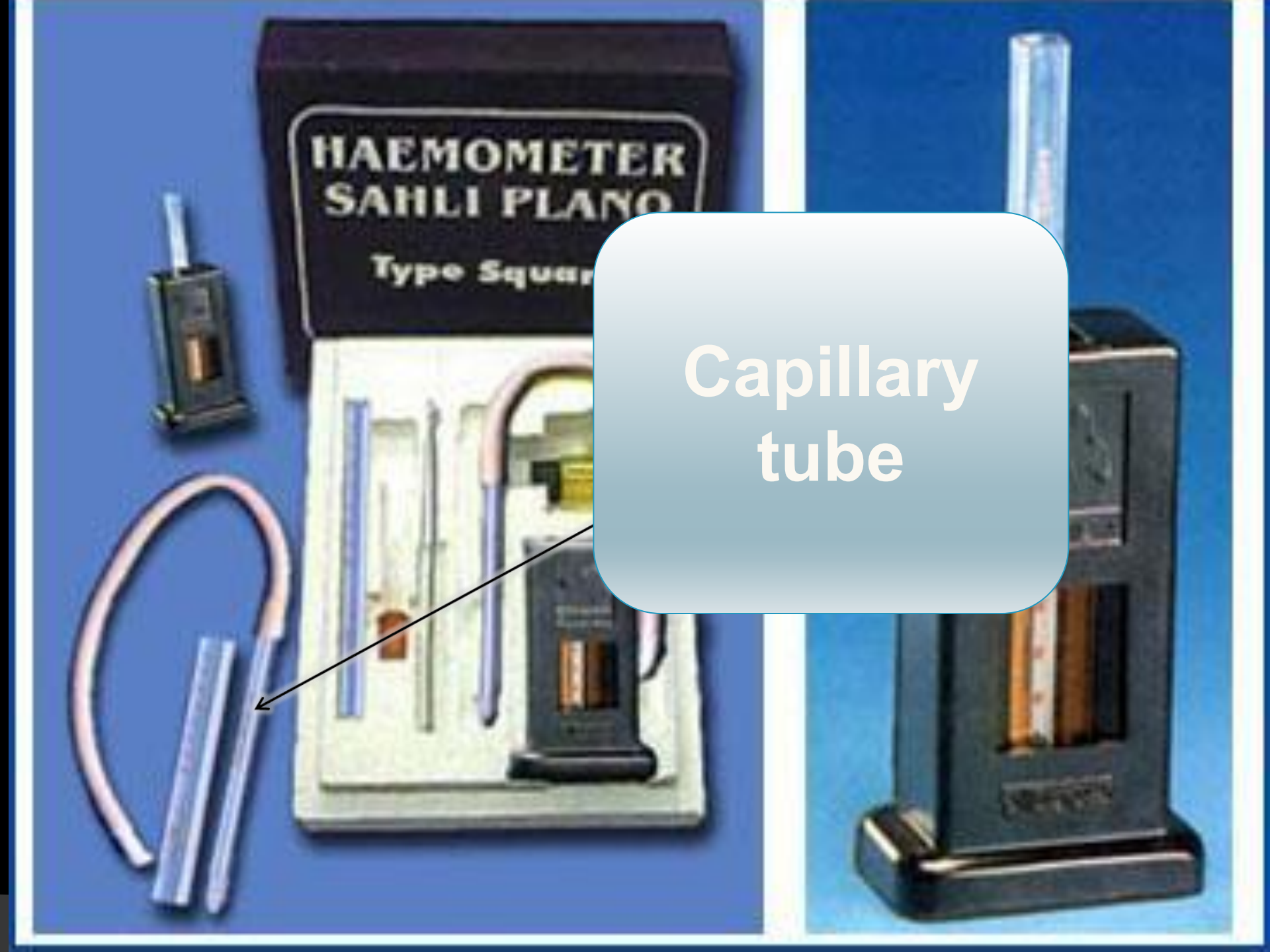
- Principle:
  - The haemolysis of RBC's by using the acid "HCl" to get a free "Hb" in the medium .
  - During this the color changes form

red brown

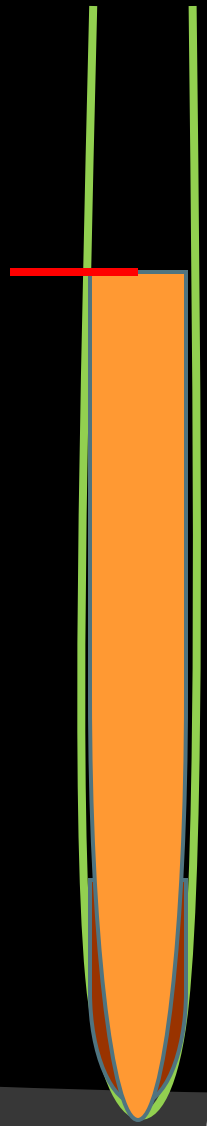
- Haemometer:
  - Consists of:
    - “2” standard colored tubes
    - Graduated tube
    - Capillary tube


HAEMOMETER
SAHLI PLANO
Type Square
Standard coloured tubes


HAEMOMETER
SAHLI PLANO
Type Square
Graduated tube


HAEMOMETER
SAHLI PLANO
Type Squar
Capillary tube

Procedure:



Graduated tube “GT”

Take blood till the 0.2 mark in the capillary tube.

Place 5 drops of 0.1 HCL.

Immediately blow the blood from the capillary tube into the “GT”

A brown color is formed

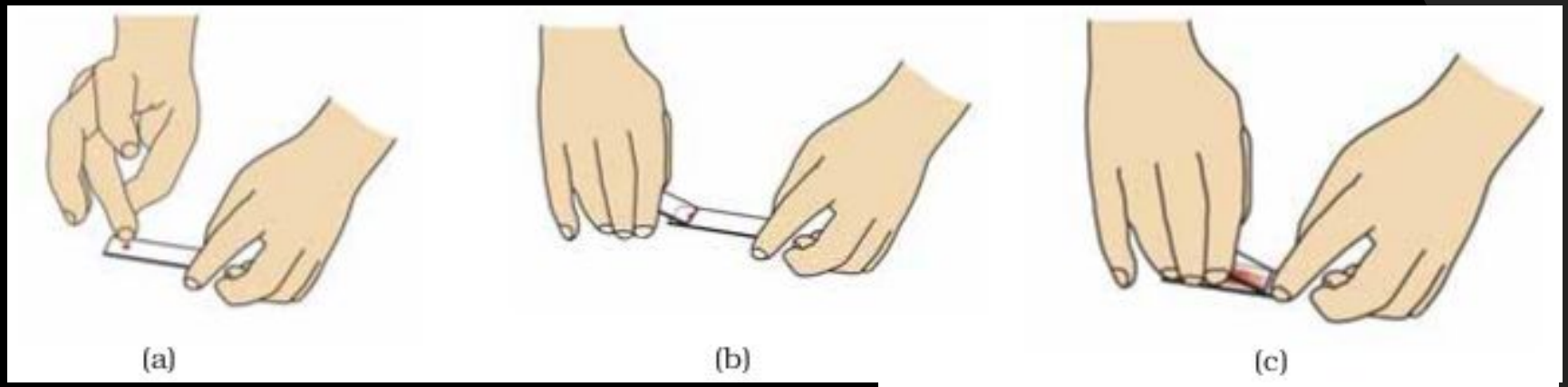
Shake well and add distilled water drop by drop and mix well,
When the color is matched with the standard, read the result from the scale graduation.

- Normal value:
  - ♂ = 93 – 118 %
  - ♀ = 83 – 107 %
  - 1 gm ⟶ 6.9 %

3- Blood film

- Principle:
  - A small drop of blood is placed near the end of a clean glass slide. By using a second slide as a spreader, the blood is streaked to a thin film and allowed to dry. It's then stained.
- Aim:
  - It is a basic and essential test in the morphologic examination and evaluation of haemologic disorders.

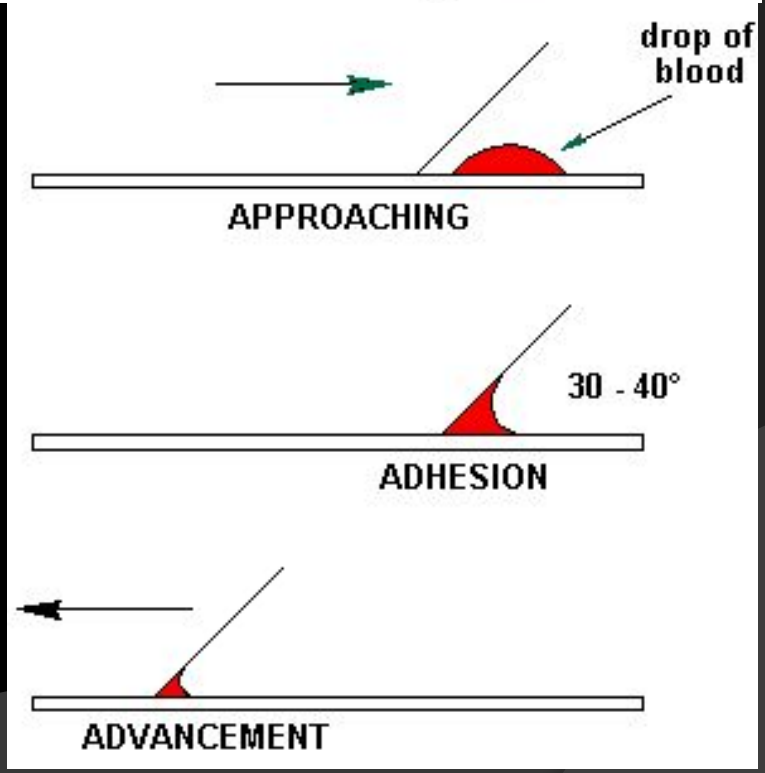
Method:



A finger puncture in made, and a small drop of blood is placed on the end of a slide.

Spreader slide is held 30-40 .. We approach the drop of blood , then we push smooth and tight towards the opposite side.

Let the blood to air dry , then stain.



NOTE THAT:-

- The thickness of the film can be varied by:-
  1. the spread with which the slide is pushed
  2. The angle of the spreader

Thinner blood film

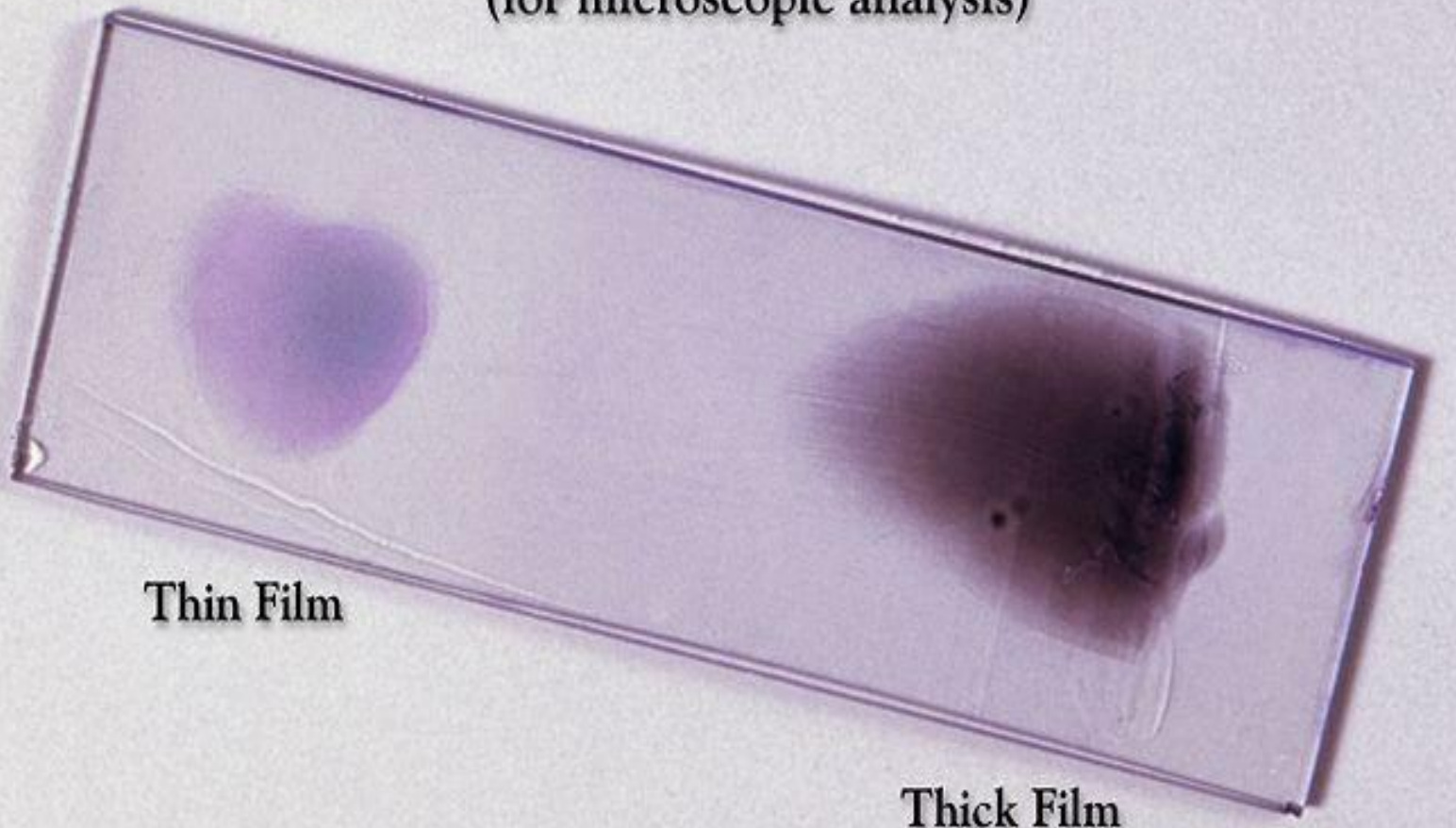
Speed

Angle

Blood Films

Properly Prepared Smears

(for microscopic analysis)

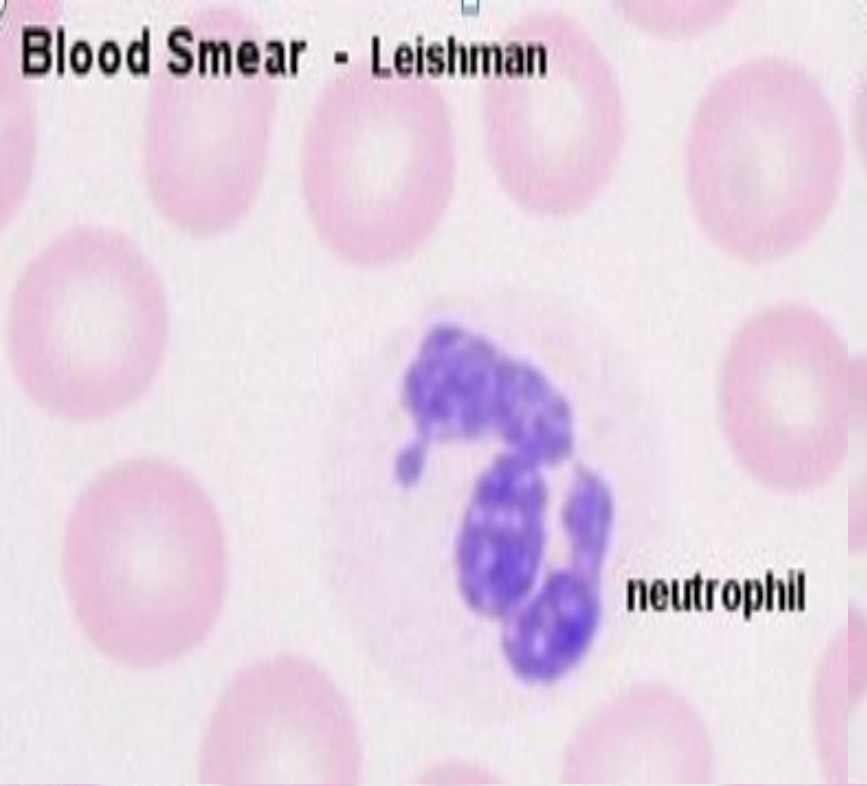


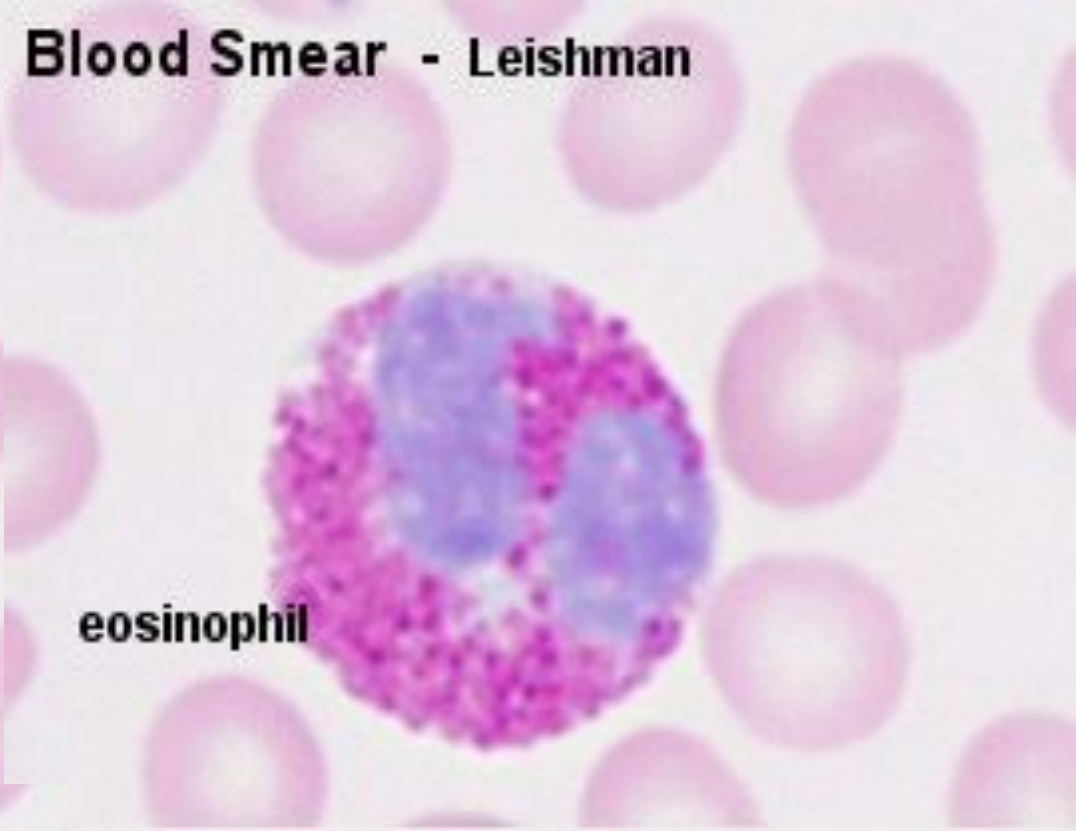
- Stains used:

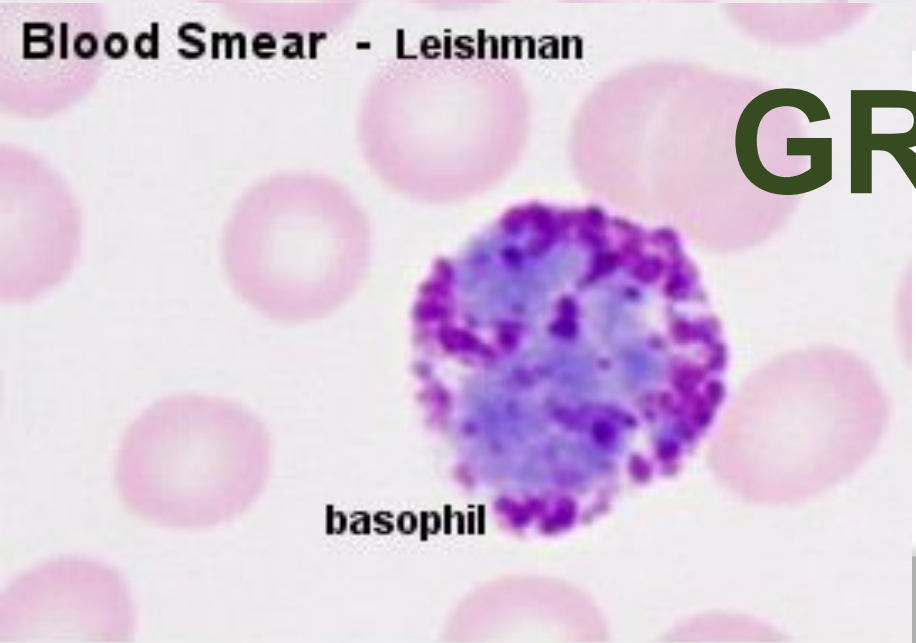
Leishman Stain : and it consists of

  - Methylene blue :
    - It stains nuclear DNA
  - Eosin in methyl alcohol :
    - Eosin stains the more basic compounds as “Hb” with “pinkish” color
    - Methyl alcohol acts as a “Fixative”

- Examination of the Blood film:
  1. Evaluation of RBC's
  2. Evaluation of platelets
  3. Differentail leucocytic count

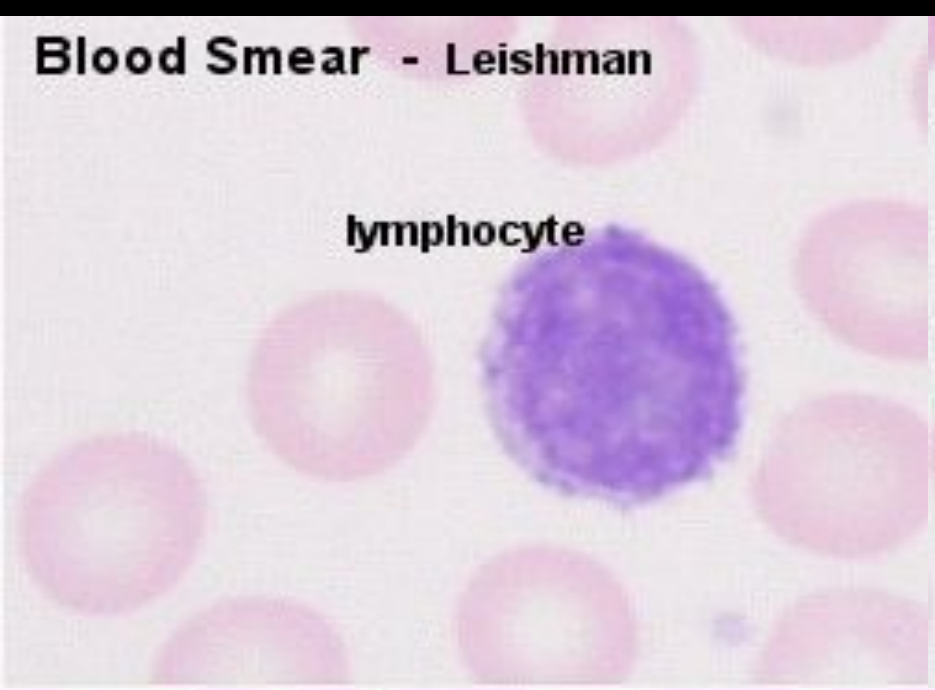


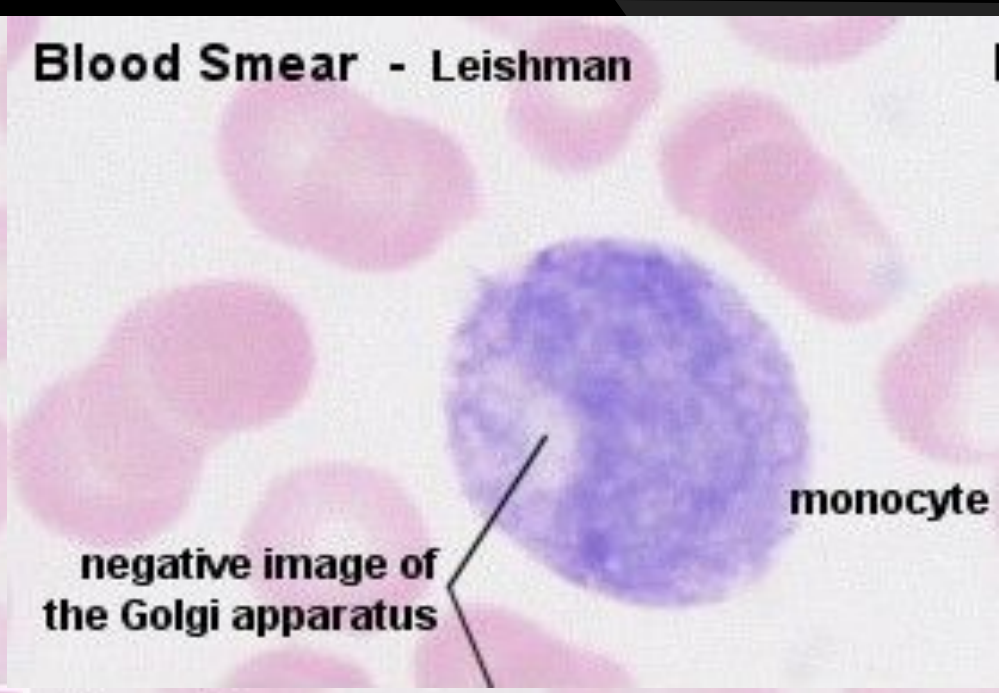


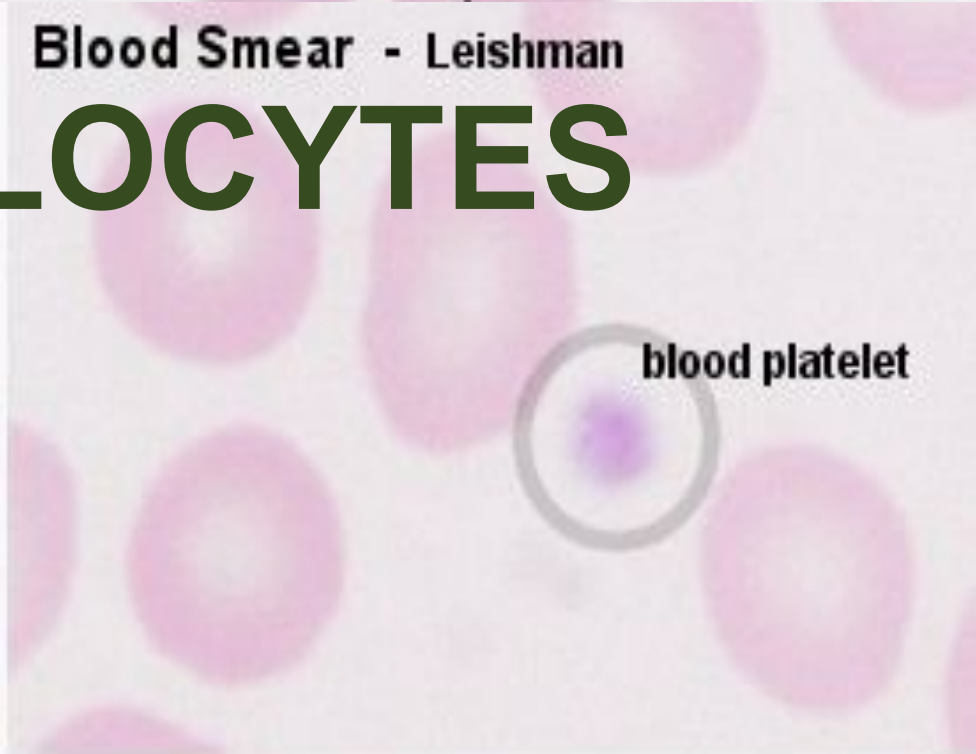


GRANULOCYTES

AGRANULOCYTES







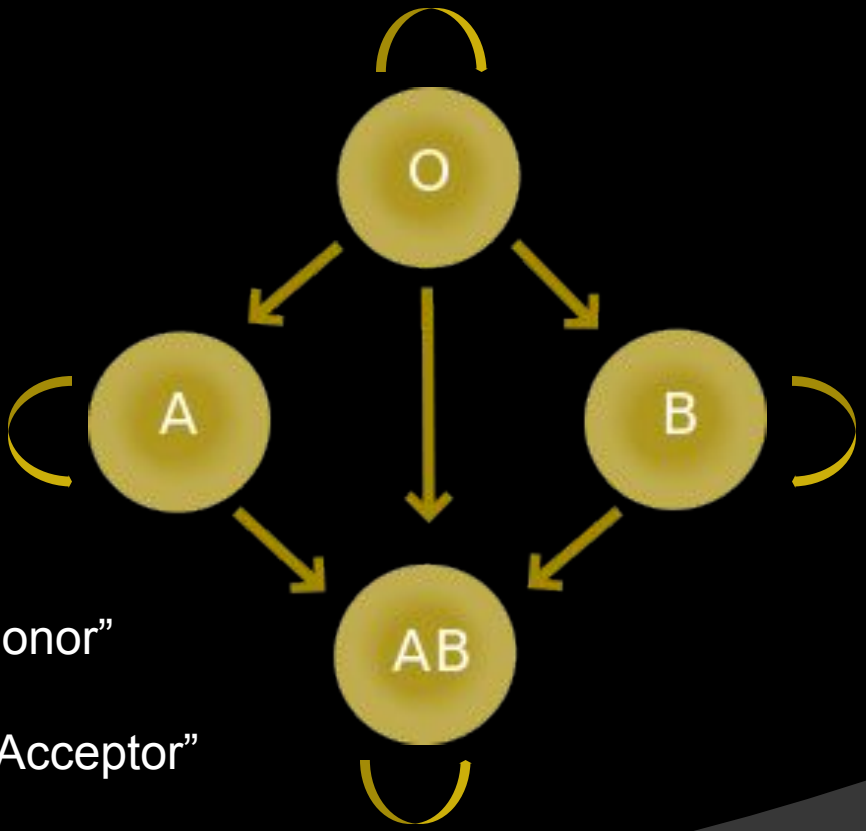
4- Determination of blood groups

- Principle :
  - The blood consists of plasma and cells (RBC's- WBC's- Platelets), the RBC's express specific Antigens on their membrane, "Agglutinogens" and the plasma contain Antibodies "Agglutinins)
  - Agglutination: it's a process in which the antigens on the RBC's are clumped by the their antibodies in the plasma.

The ABO Blood System

Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	 A agglutinogens only	 B agglutinogens only	 A and B agglutinogens	 No agglutinogens
Plasma Antibodies (phenotype)	 b agglutinin only	 a agglutinin only	NONE. No agglutinin	 a and b agglutinin

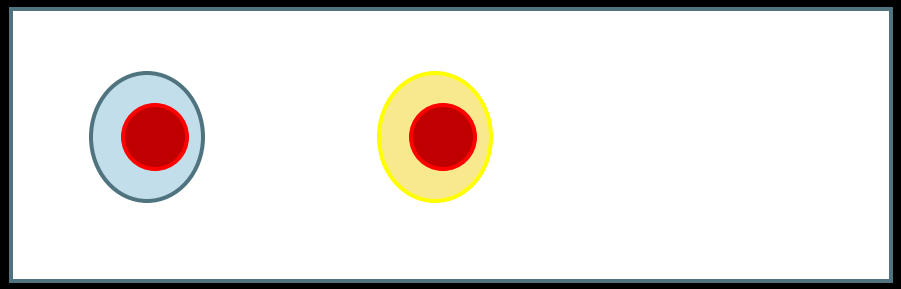
- This diagram shows the possible ways of blood transfusion without causing agglutination to the blood:

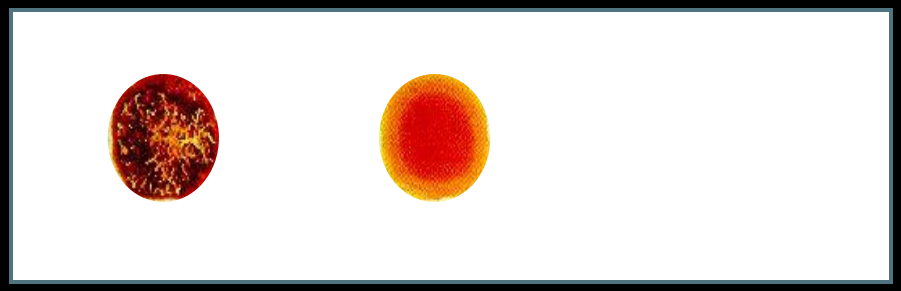


O: is a universal “Donor”

AB: is a universal “Acceptor”

Preparation of the slide:





Add 2 drops of blood to each!

Add a drop of antibody A “blue”

Add a drop of antibody B “yellow”

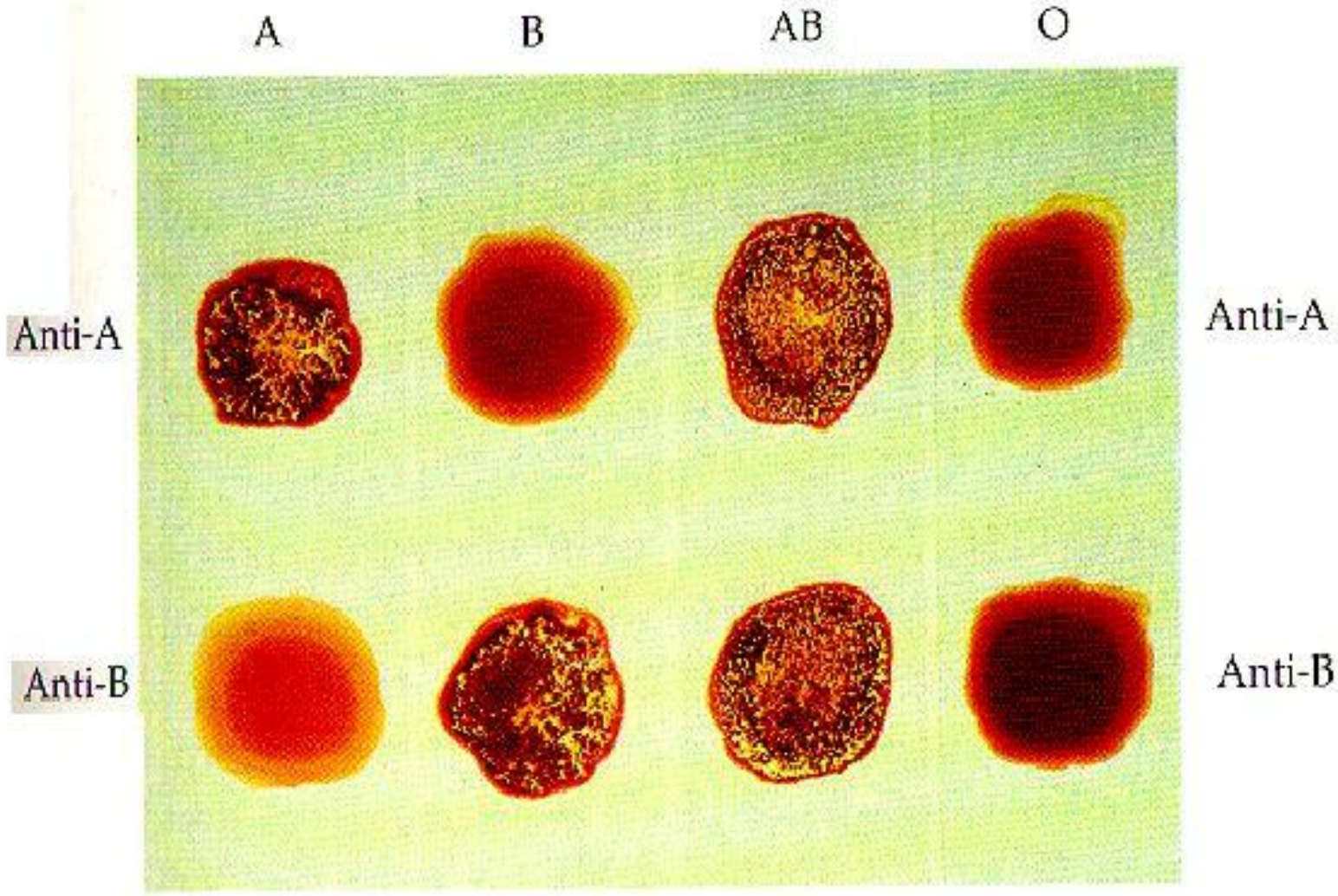
Shake the slide..

For example .. If the blood gp was **A** .

Agglutination

clear

ABO Blood Reactions



Haematocrite value

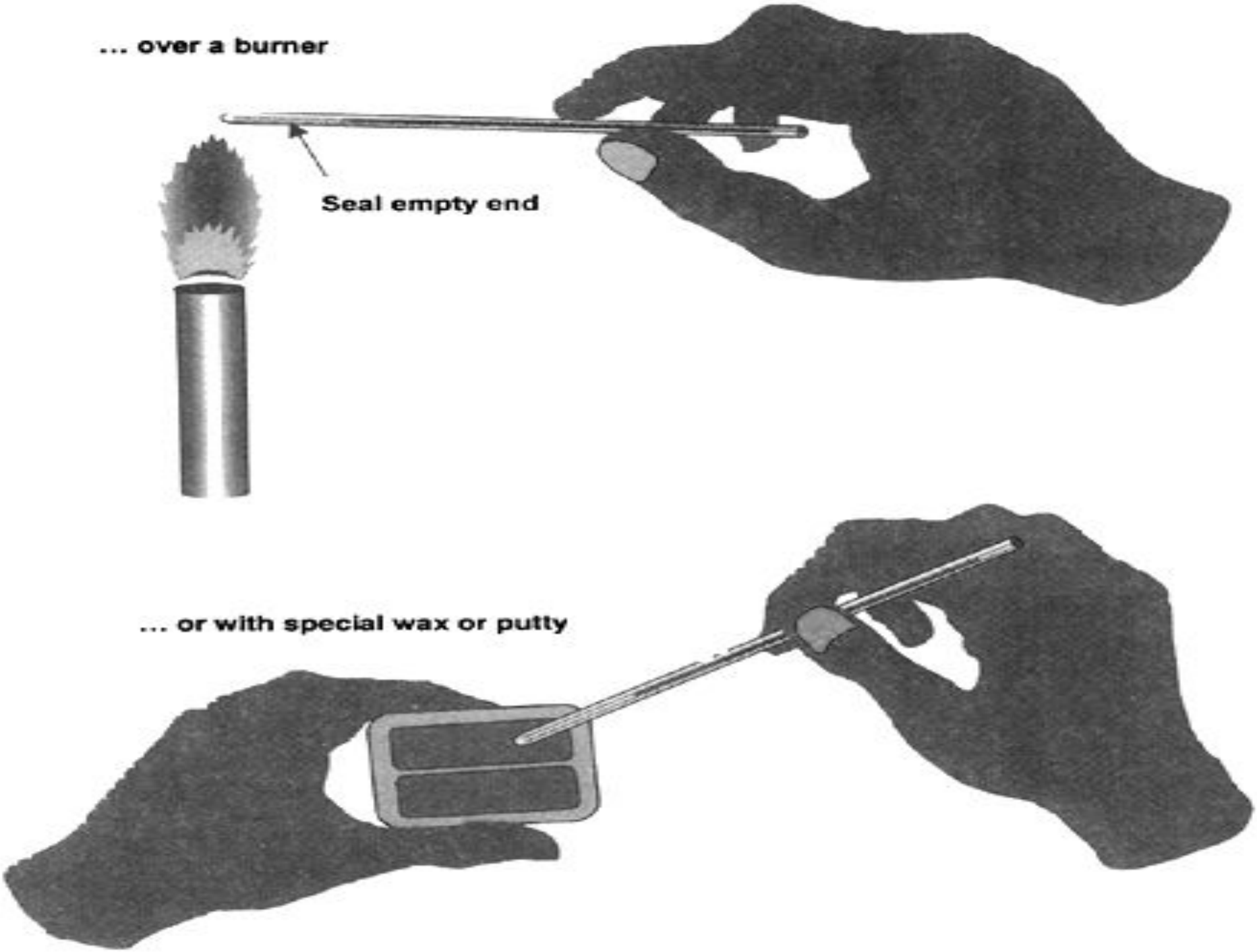
Define:-

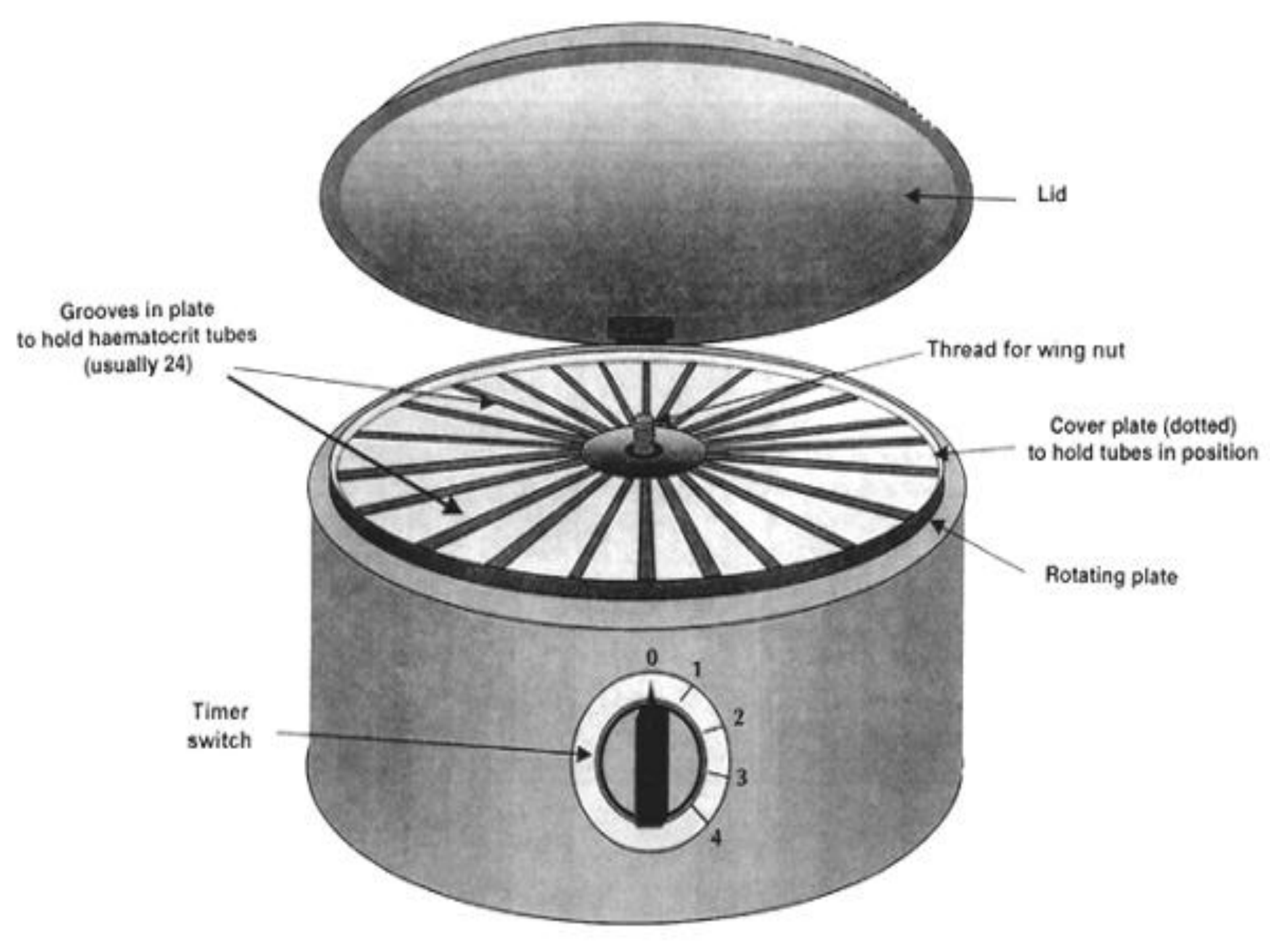
- **hematocrit (Ht)**, also known as **packed cell volume (PCV)** or **erythrocyte volume fraction** (EVF), is the volume percentage (%) of red blood cells in blood.

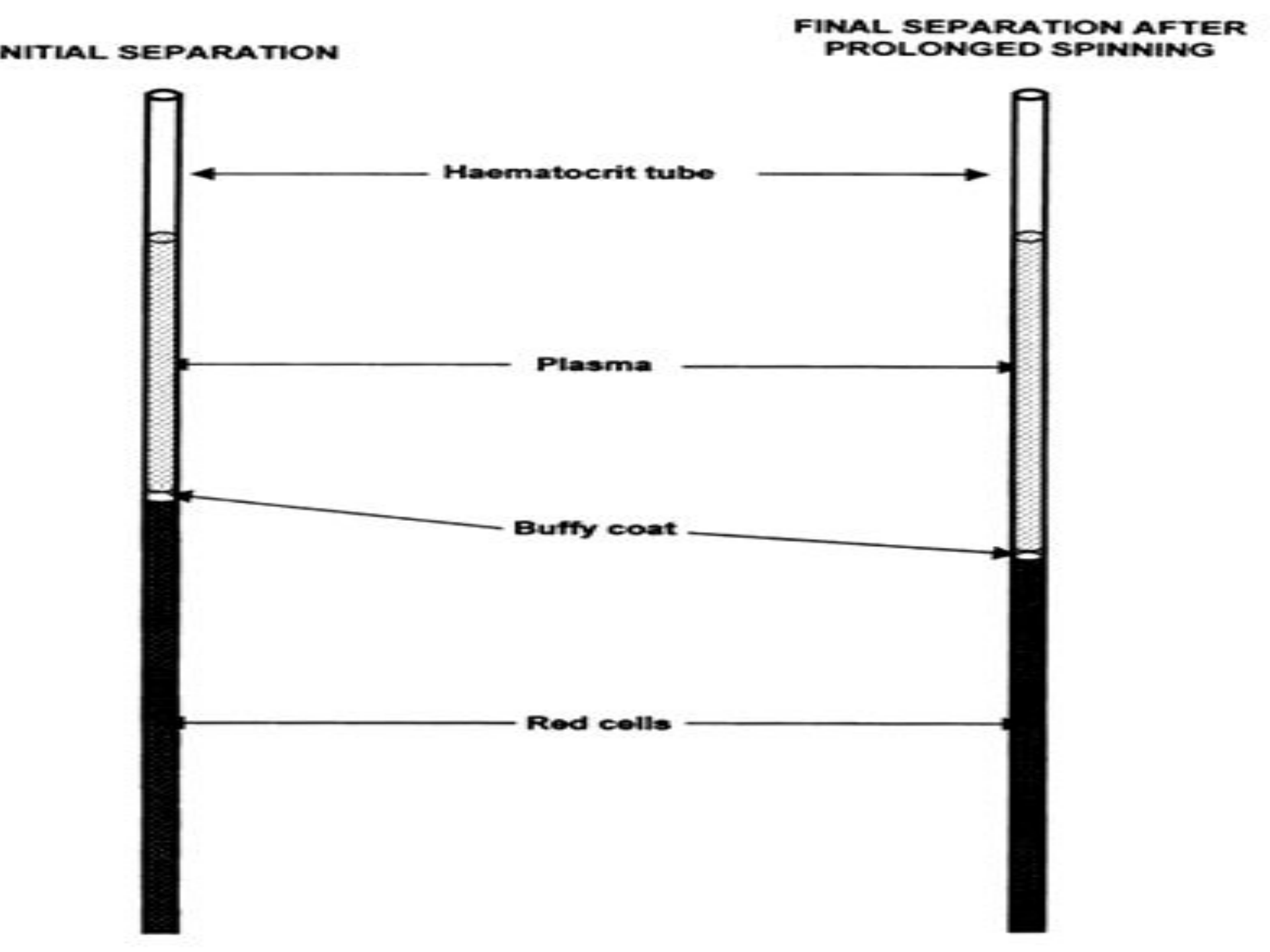
- It is normally about 45% for men and 40% for women

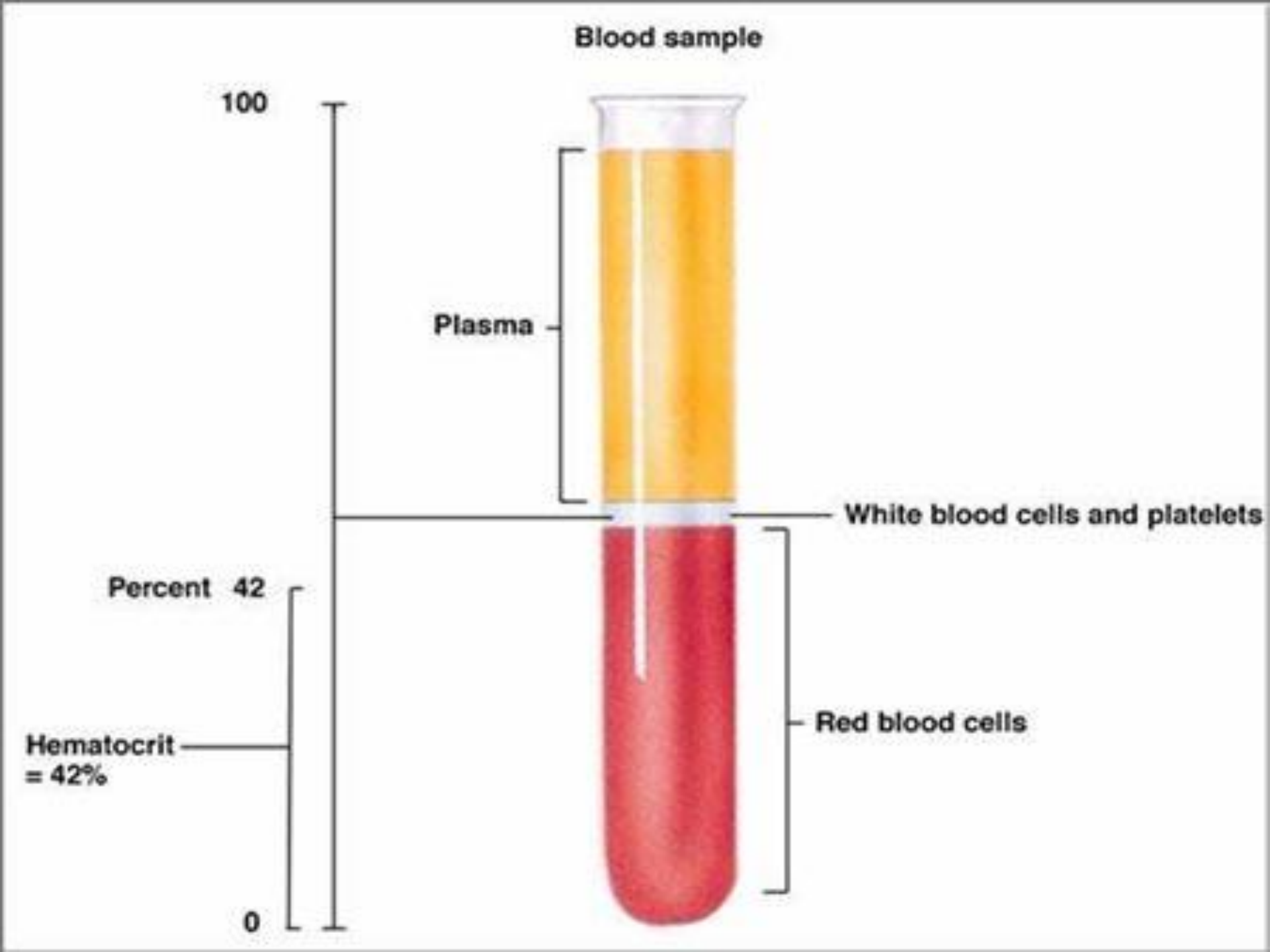
How to calculate Ht/PCV?

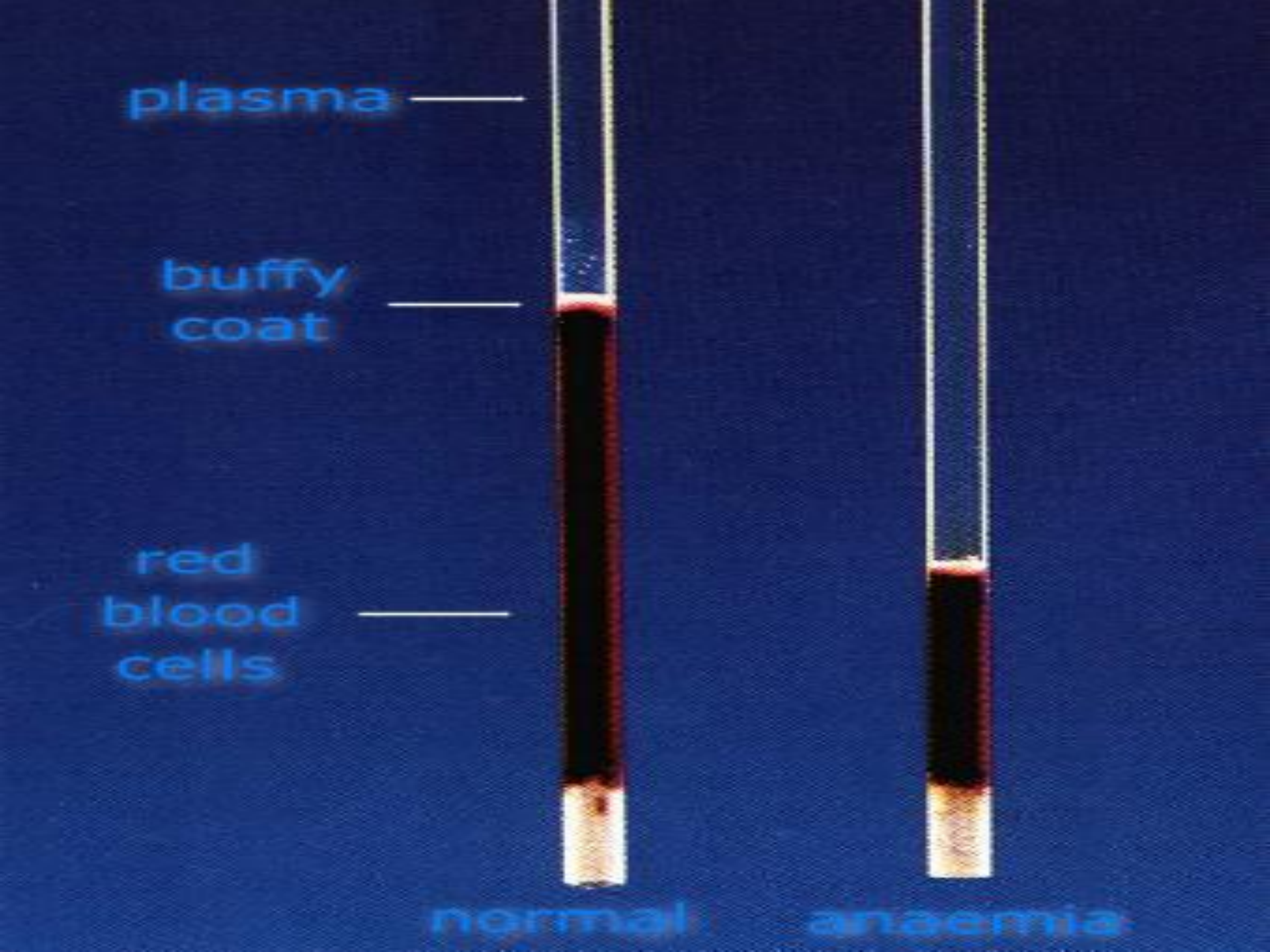
- The packed cell volume (PCV) can be determined by centrifugingThe packed cell volume (PCV) can be determined by centrifuging heparinizedThe packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes


... over a burner
Seal empty end
... or with special wax or putty


Lid
Grooves in plate
to hold haematocrit tubes
(usually 24)
Thread for wing nut
Cover plate (dotted)
to hold tubes in position
Rotating plate
Timer
switch
0
1
2
3
4


NITIAL SEPARATION
FINAL SEPARATION AFTER PROLONGED SPINNING
Haematocrit tube
Plasma
Buffy coat
Red cells


Blood sample
100
Plasma
White blood cells and platelets
Percent 42
Red blood cells
Hematocrit
= 42%
0


plasma
buffy coat
red blood cells
normal
anaemia

Rate of sedimentation

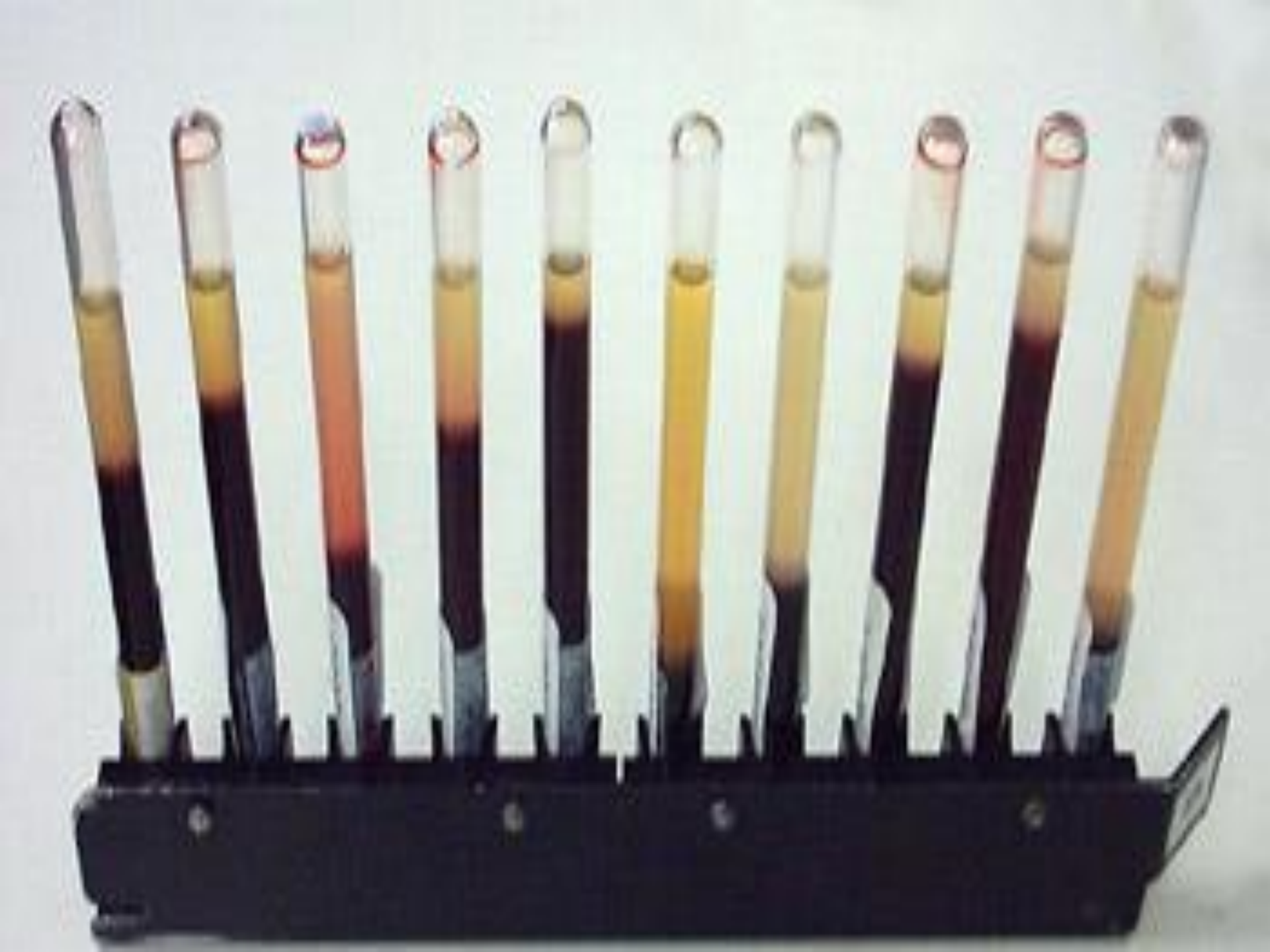
(ESR=Erythrocyte sedimentation rate)

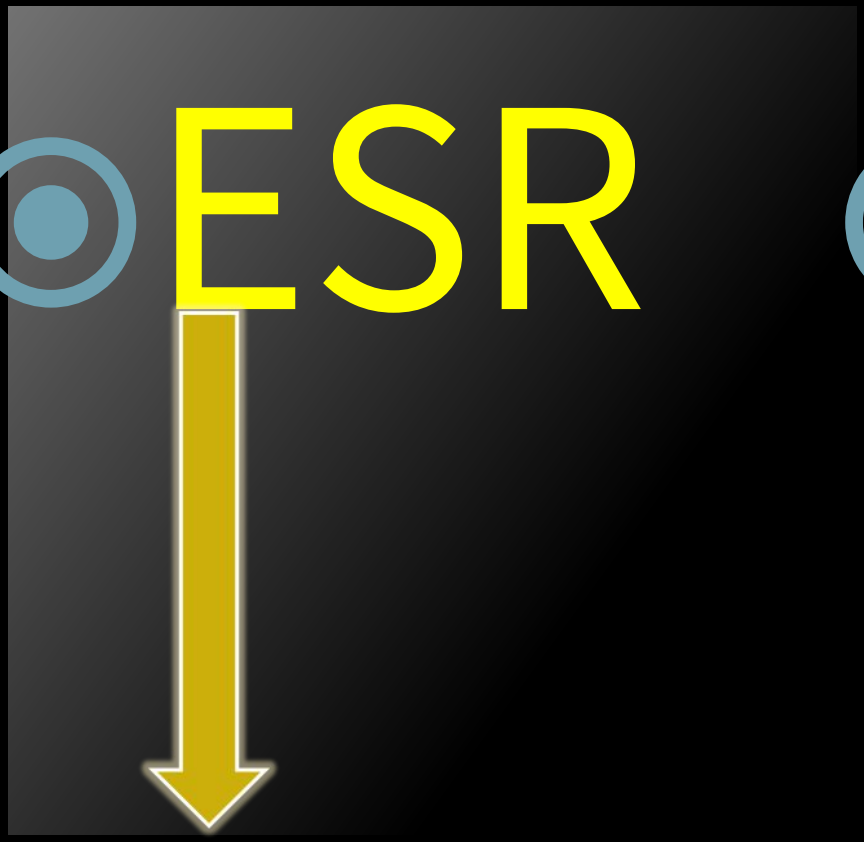
Define:-

- Rate of sedimentation is the rate at which red blood cells sediment in a period of one hour.

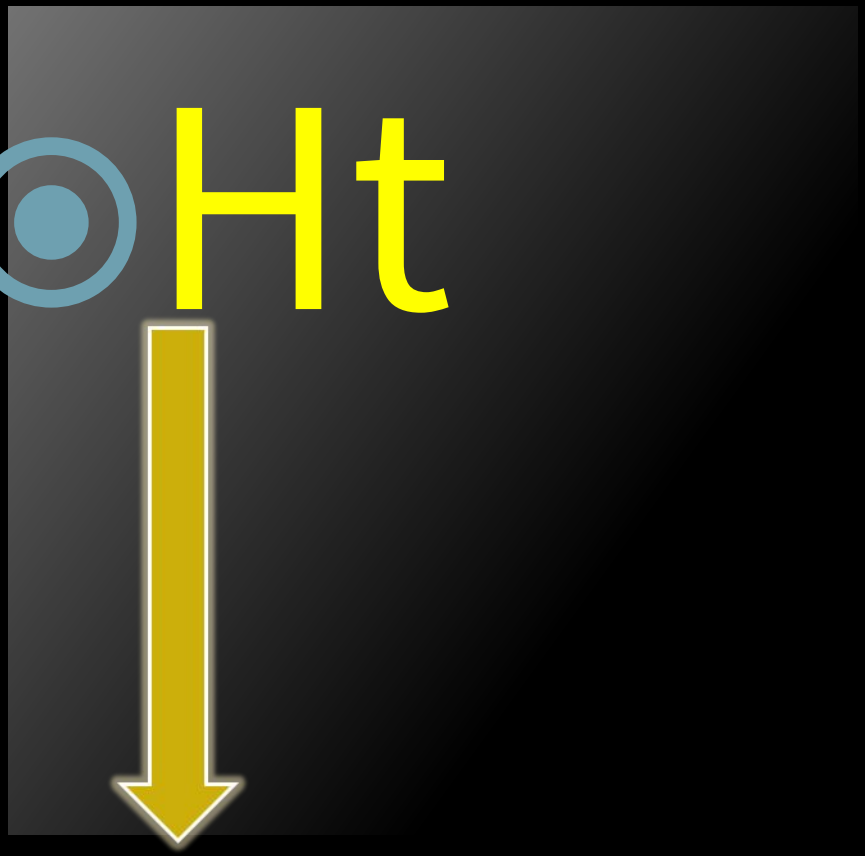
How To perform the test?

- Anticoagulated blood is placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall is measured and reported in mm/h





Depend on gravitational force



Depend on centrifugal force

Thank you…