Physiology Lab

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The Experiments

- Red Blood Cell (RBC) Count
- White Blood Cell (WBC) Count
- Differential Leukocyte count (DLC)
- Reticulocyte count
- Packed cell volume (PCV)
- Hemoglobin concentration
- Erythrocyte Sedimentation rate (ESR)
- Blood Type
- Bleeding Time
- Clotting Time
- Osmotic Fragility Test

Red Blood Cells (RBCs)

- Normal RBCs are biconcave discs, they have few organelles and no nuclei.
- A major function of RBCs is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues.
- The average number of RBCs in healthy men is 5,200,000/mm^3 (±300,000) and in healthy women 4,700,000/mm^3 (±300,000)
- The number of RBCS is regulated within narrow limits, so that oxygen is transported adequately to the tissues and at the same time the cells do not become so numerous that they impede blood flow.

- RBC count is typically ordered as a part of complete blood count (CBC) and may be used as a part of health checkup to screen for variety of conditions.

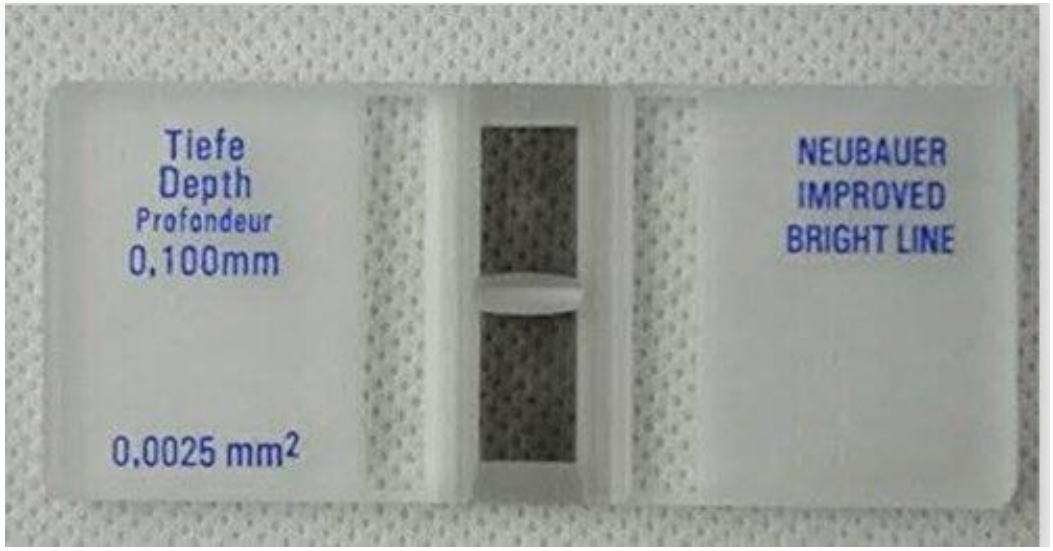
- **Causes of high RBC count (Polycythemia)**

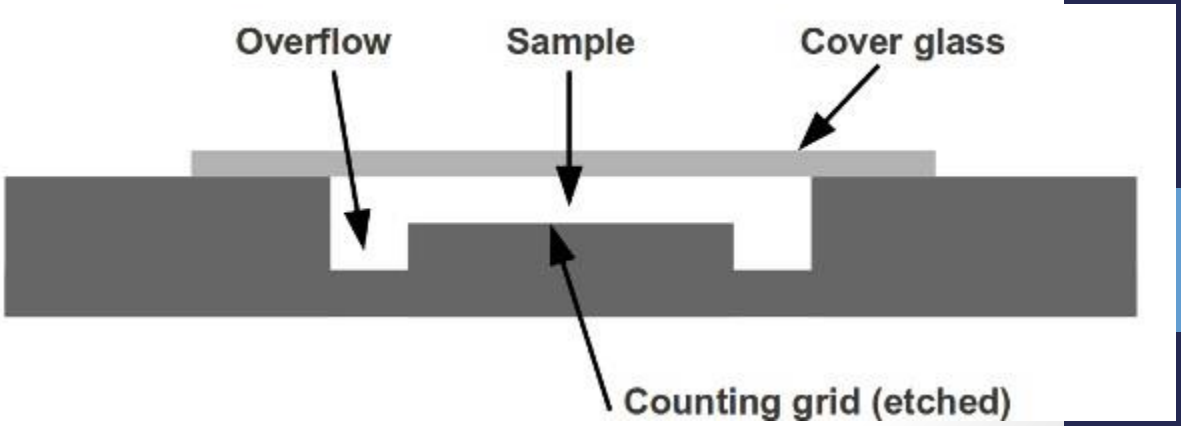
1. Living at high altitudes
2. Cardiac or pulmonary diseases
3. Erythropoietin secreting tumors
4. Smoking.
5. Polycythemia Vera
6. Dehydration

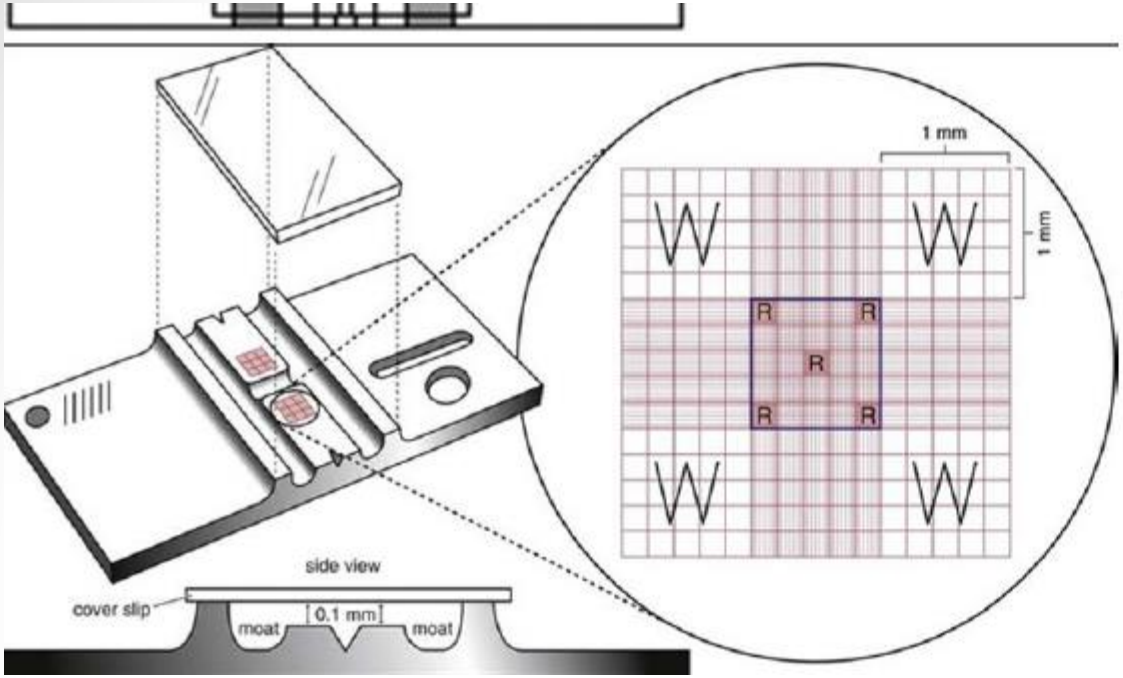
- **Causes of low RBC count (Anemia)**

1. Internal or external bleeding
2. Nutritional deficiencies
3. Bone marrow failure
4. Hemolysis of RBCs
5. Chronic renal failure

- Hemocytometer is a special microscopic slide that has specific grids engraved on it’s counting chamber and is designed to hold a specific volume of fluid.



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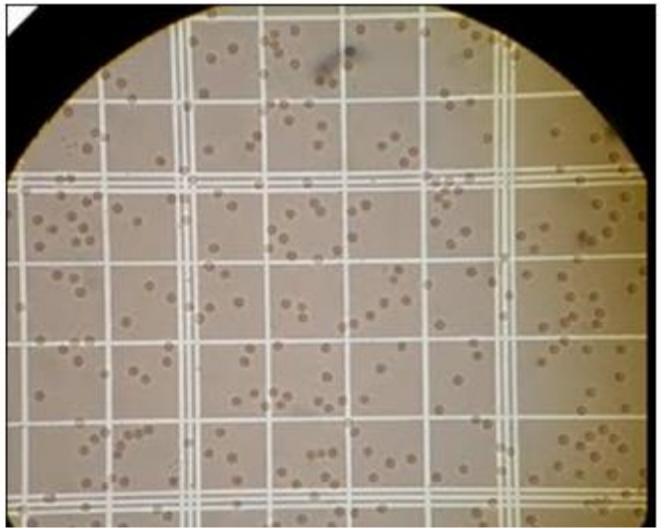
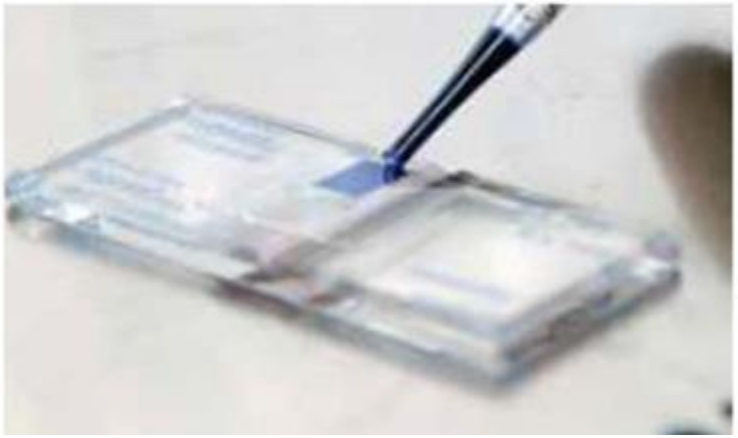
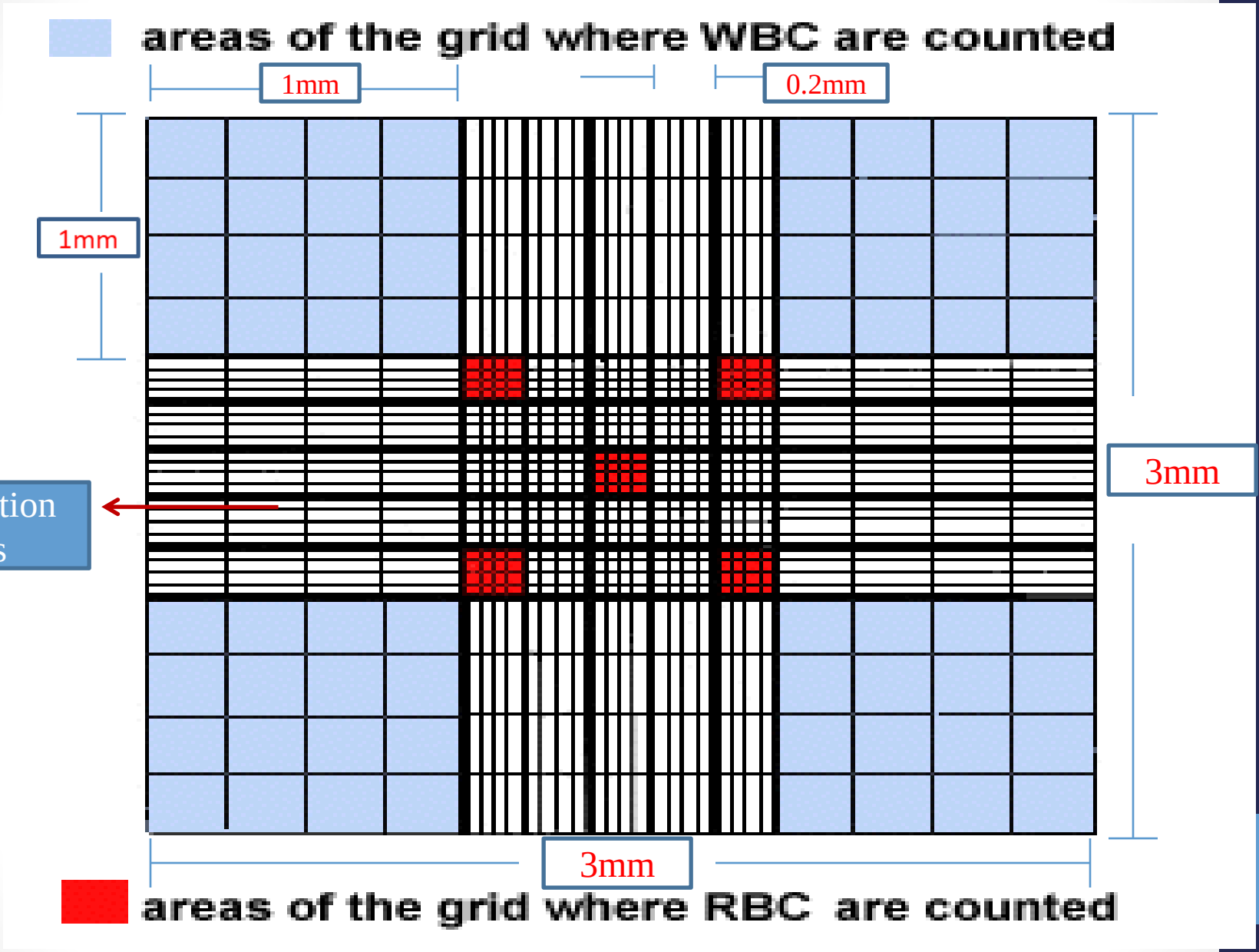
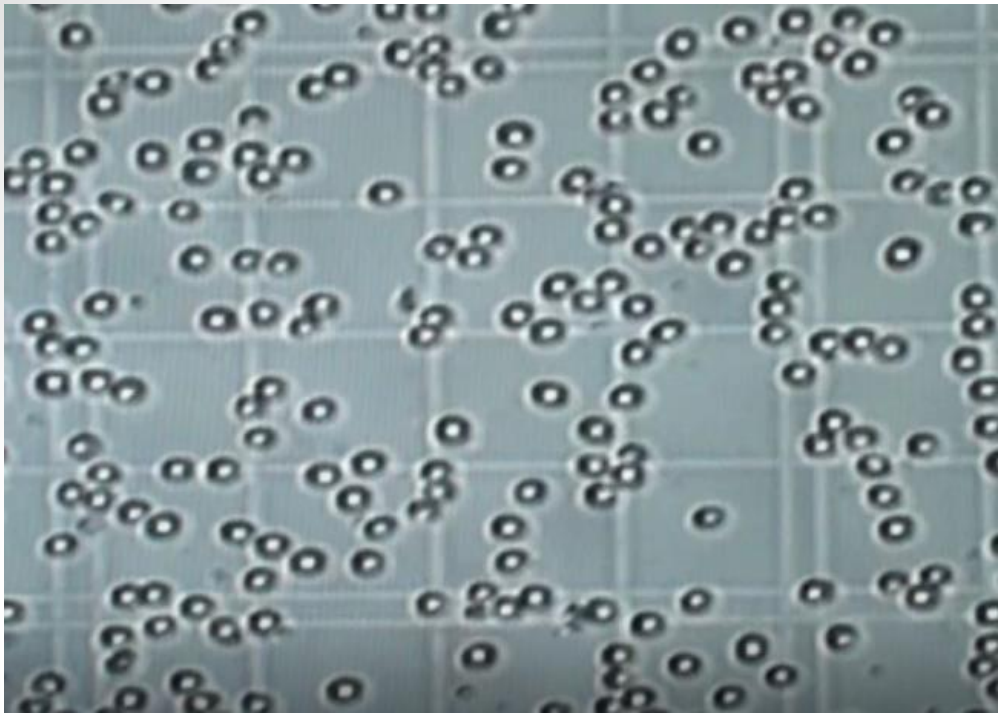




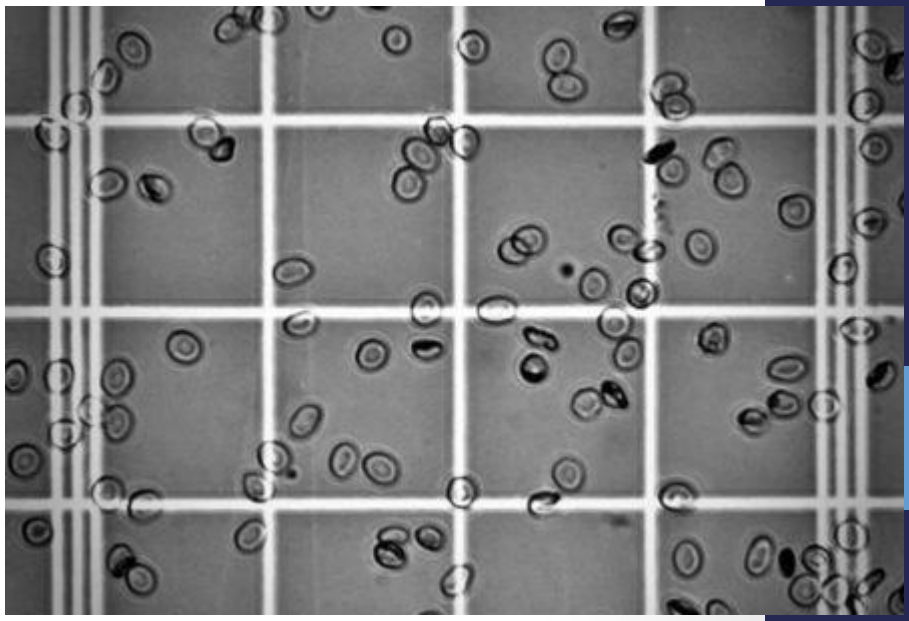

areas of the grid where WBC are counted
1mm
0.2mm
1mm
3mm
Orientation lines
3mm
areas of the grid where RBC are counted

The procedure

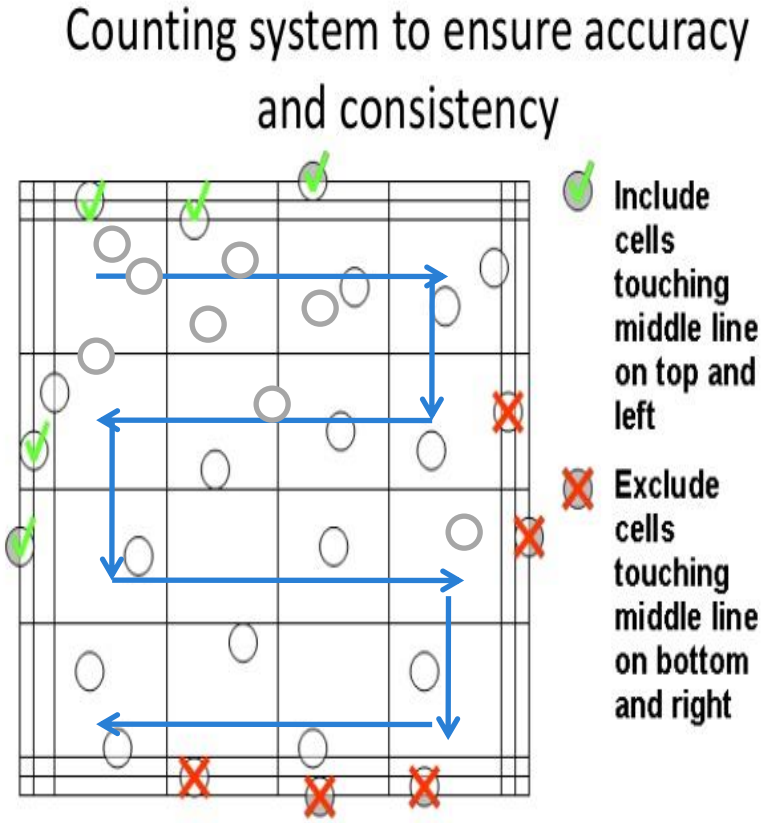
1. Clean the hemocytometer well
2. Place a coverslip over the counting area. Now the distance between the bottom of the coverslip and the surface of the counting area is **0.1 mm**
3. Dilute the blood sample by adding 1 unit of blood to 199 units of an isotonic solvent and thoroughly mix the mixture
4. Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer. The diluted blood will flow by capillary attraction to fill the chamber.
    - ✓ Let it stand for 3 min before you start counting the cells.
5. Use the 10X lens to identify the center square , then use 40X lens to focus on the smaller squares and count the RBCs (RBCs appear circular in shape with a light center)
6. Count the number of cells in the five small squares and obtain an average number.



Count all the cells contained within the square and those touching the upper and right outer lines. The cells that touch the left and bottom outer lines are not to be counted. In each of the four areas, conduct the count in a zig-zag line.



- Start counting from the left to the right and proceed in a zig-zag.
  - To avoid counting the same cells twice, cells that are touching the lines at the tops and left sides of the squares are counted, but cells that are touching the bottoms and right sides of the squares are not counted.



- If the RBC count was (100, 90, 94, 96, 95). What is the RBC count in the blood sample?

1. In this case the average RBC count is 95
2. Dilution factor (DF) = Final volume/ volume of blood
   - Blood is diluted at (1:199) so DF= 200
3. The volume of fluid contained in one small square = (0.2x 0.2 x 0.1)= 0.004 mm^3
   - Volume Correction Factor (VCF)= Desired Volume/ Actual volume
     - Desired volume = 1 mm^3
   - VCF = 1/0.004 = 250

- The number of RBCs in blood sample= The average number of RBCs X DF X VCF = 95 X 200 X 250 = 4,750,000 cells/mm^3

Important Points

- Before you obtain the average number of RBCS make sure the count in the five squares doesn't vary by more than 20 cells.
- If there is a big variation discard the sample from the slide and repeat the experiment.
- DF can change based on the dilution performed during the experiment
- VCF is always the same

WBC count

- White Blood Cells are part of the immune system
- Move to areas of severe infection or inflammation to provide a rapid and potent defense for the body
- Normal WBC count is 4000 - 11,000 cells/mm^3
- This test is often included in the complete blood count (CBC), it is done to get an impression about the immune system since the Leukocytes (WBC) play an integral role, to get more informative results it is often combined with the differential count

- **Causes of High WBC count (Leukocytosis)**

1. Active inflammation or infection.
2. Certain malignancies
3. Recent vigorous exercise, thermal burn, electric shock, surgery, or trauma.
4. Certain medications e.g. glucocorticoids (neutrophilia)
5. Dehydration.

- **Causes of Low WBC count (Leukopenia)**

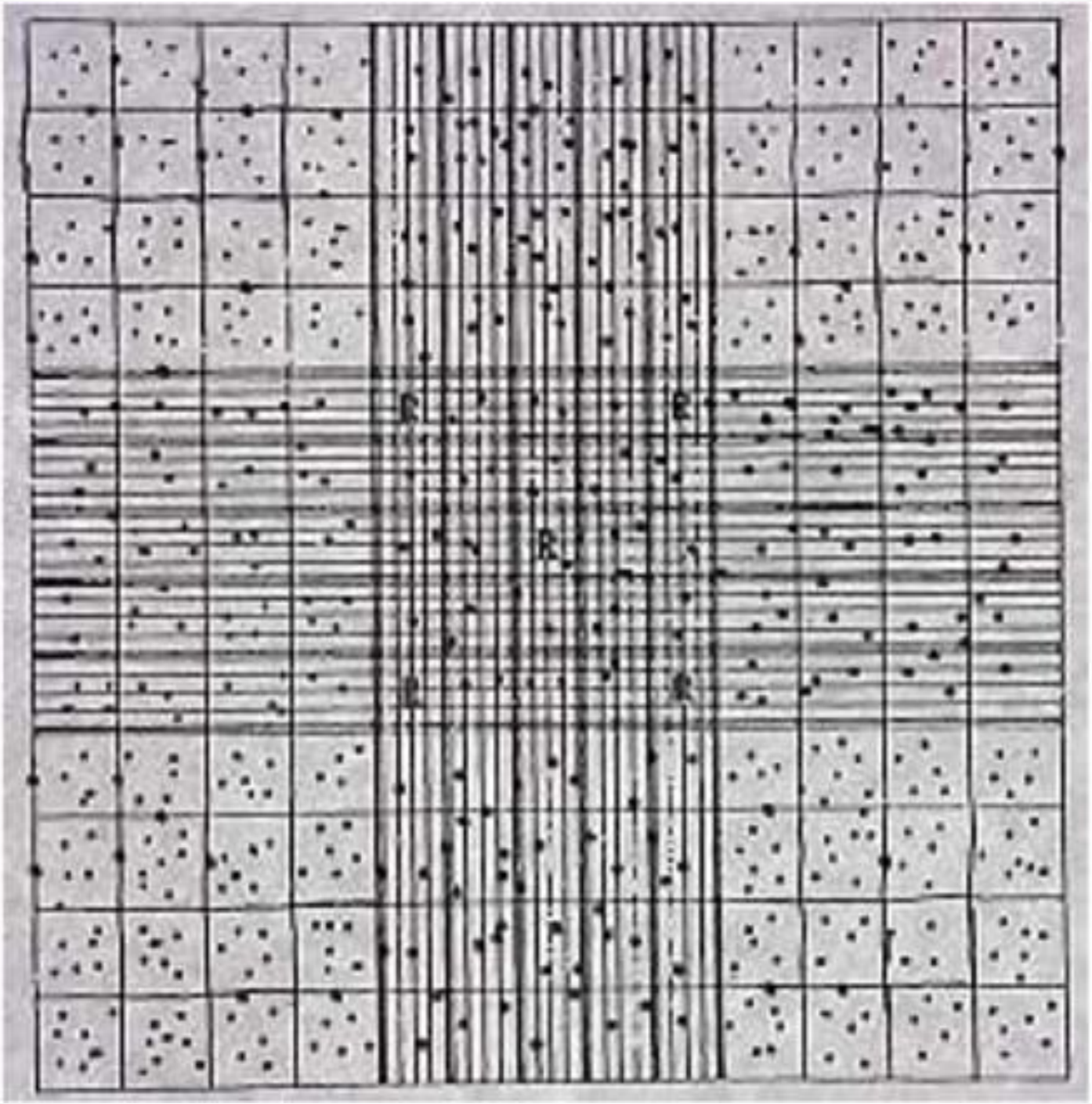
1. Bone marrow failure due to aplastic anemia, fibrosis, metastatic cancer, radiotherapy or chemotherapy
2. Autoimmune diseases.
3. Infections like HIV & tuberculosis.

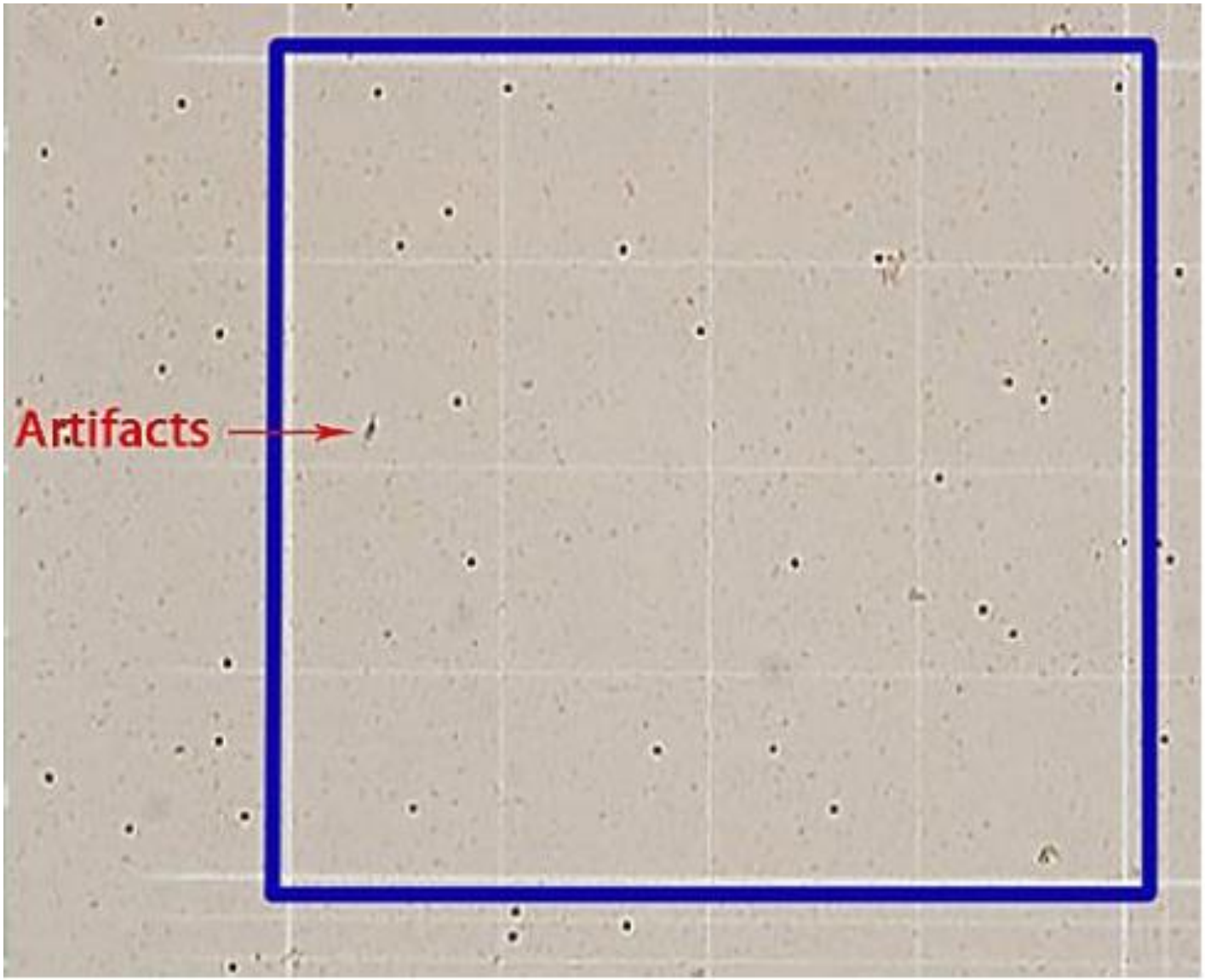
The procedure

1. Clean the hemocytometer well
2. Place a coverslip over the counting area.
3. Dilute the blood sample by adding 1 unit of blood to 19 units of solvent and thoroughly mix the mixture.
   - ✓ The dilution fluid contains an agent (glacial acetic acid) which lyses the red cells. It also contains a dye that stains the nuclei of WBCs. This allows a proper count of WBCs.
4. Draw a sample using a pipette and gently touch the junction of the coverslip and hemocytometer . The diluted blood will flow by capillary attraction to fill the chamber.

   ✓ Let it stand for 3 min before you count the cells.

5. Use the 10X lens to count the WBC in the four large corner squares .(WBCs appear as dark dots)




Artifacts

The calculation

1. Blood is diluted at (1:19) so DF = 20
2. The volume of fluid in the corner square is (1 **X** 1 **X** 0.1= 0.1 mm^3) SO the VCF is 10

✓ If we counted an average of 40 cells in the 4 squares the count of WBCs is….

40 **X** 20 **X** 10 = 8000 cells/mm^3 which is a normal value

•Before you obtain the average number of WBCS make sure the count in the four squares doesn't vary by more than 10 cells

Differential Leukocyte Count (DLC)

- The blood contains 5 different types of white blood cells which are classified into:

1. Granulocytes: have cytoplasmic granules which contain enzymes or chemicals, and have a single multi lobed nucleus (segmented)
2. Agranulocytes: have a single non lobulated nucleus, their cytoplasmic granules are too small to be seen under the light microscope.

1. Neutrophils: 40-80 %
2. Eosinophils: 1-4 %
3. Basophils: < 1%

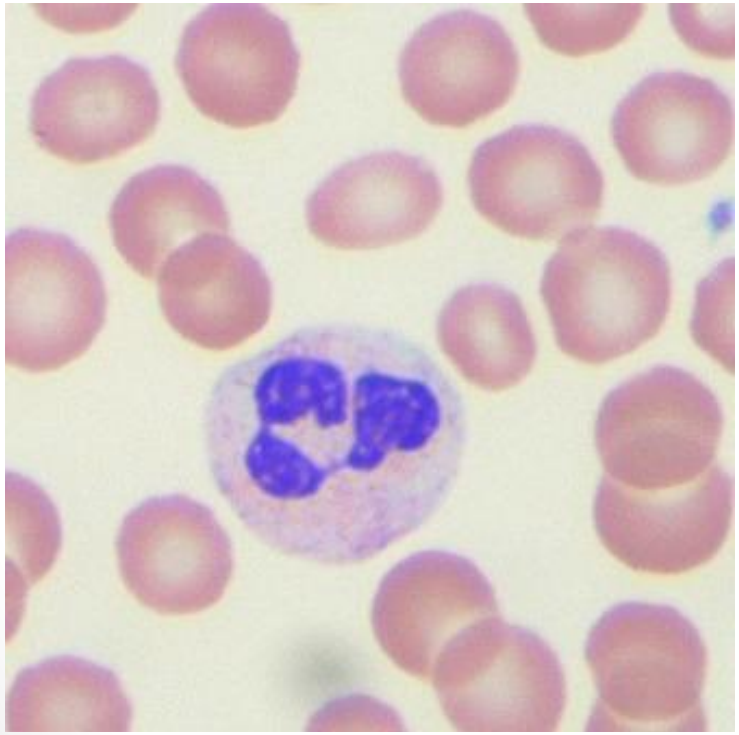
Granulocytes

4. Lymphocytes: 20-40%
5. Monocytes: 2-8%

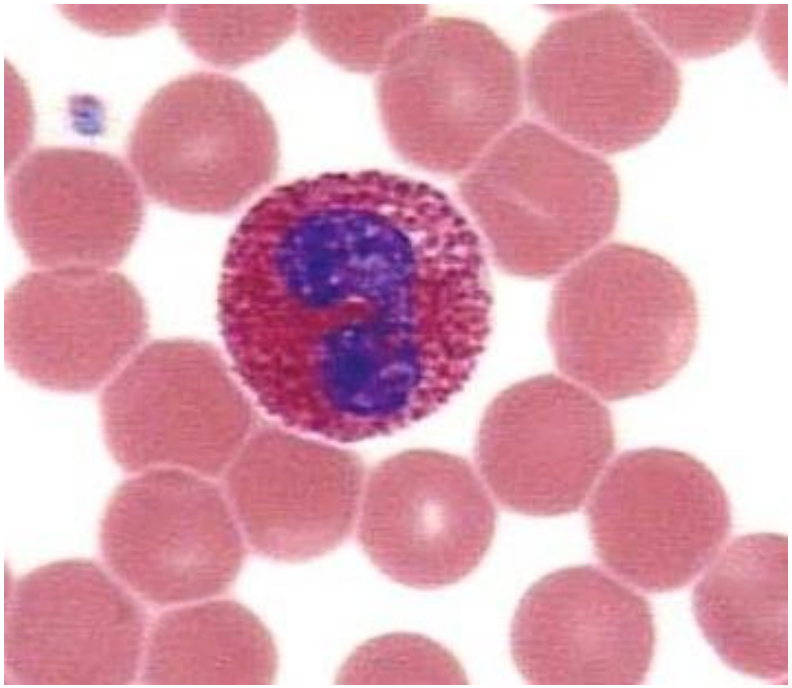
Agranulocytes

Neutrophils have nuclei with several lobes and fine pink granules in their cytoplasm.

They are called neutrophils, because their granules are not very amenable to staining with either acidic or basic dyes

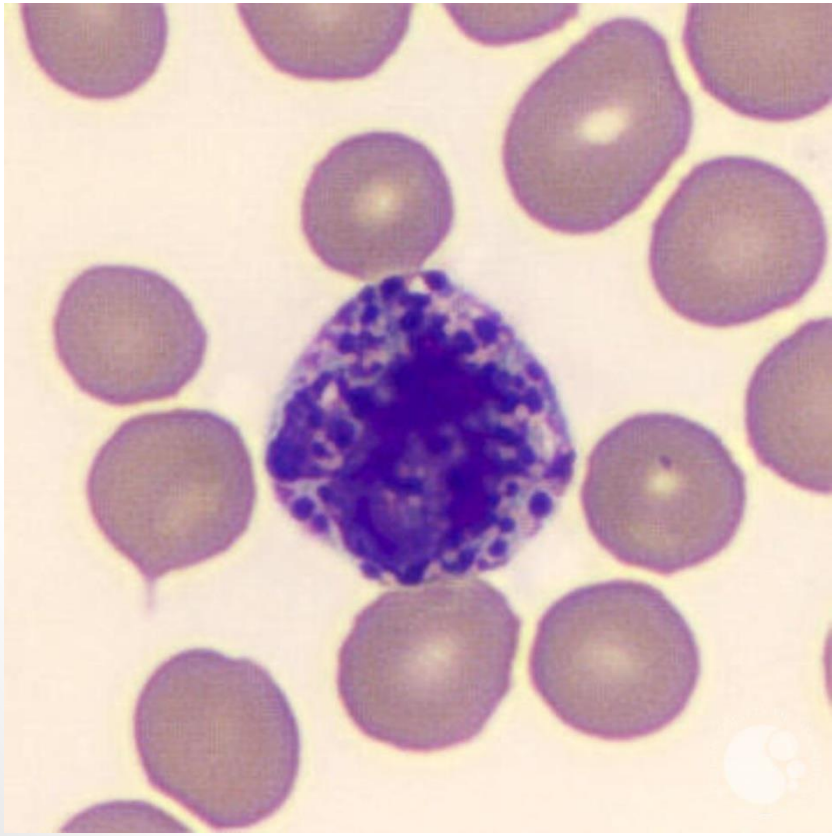


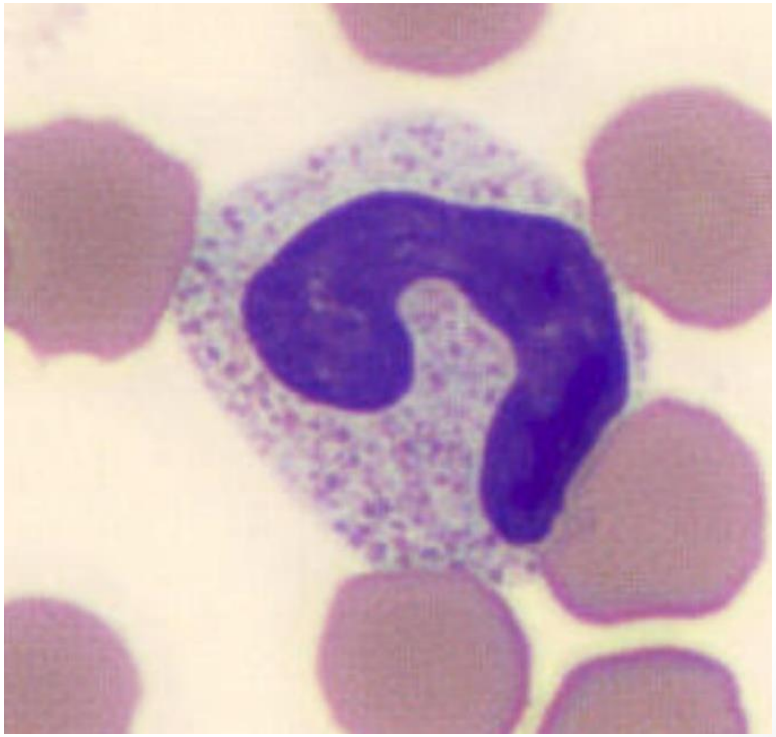
Eosinophils have bi-lobed nuclei and medium-sized granules that can be stained bright red with an acidic dye.



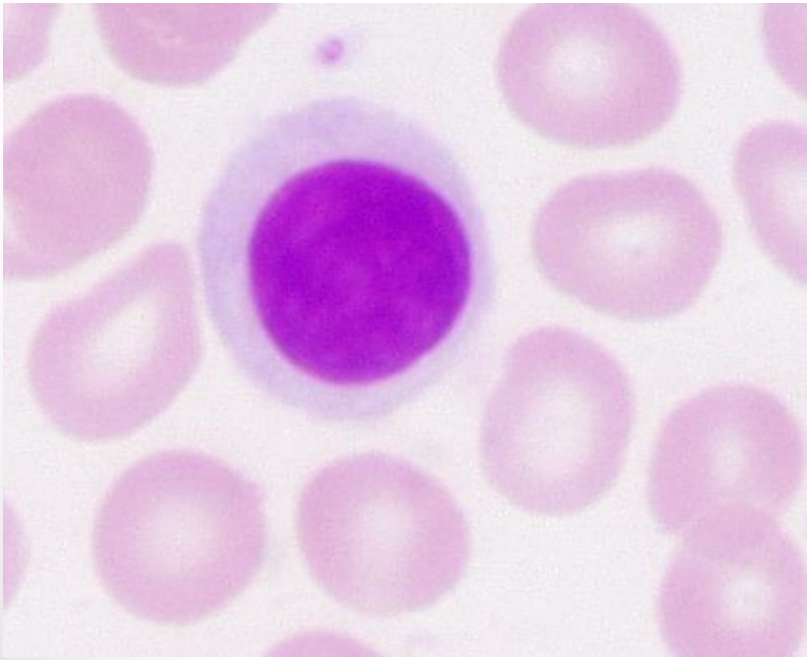
Basophils have bi-lobed or S shaped nuclei and large granules which stain dark blue with basic dyes and completely obscure the nucleus

Neutrophilic Band cells are immature neutrophils, usually make less than 5% of the total WBC count, their nucleus isn't segmented

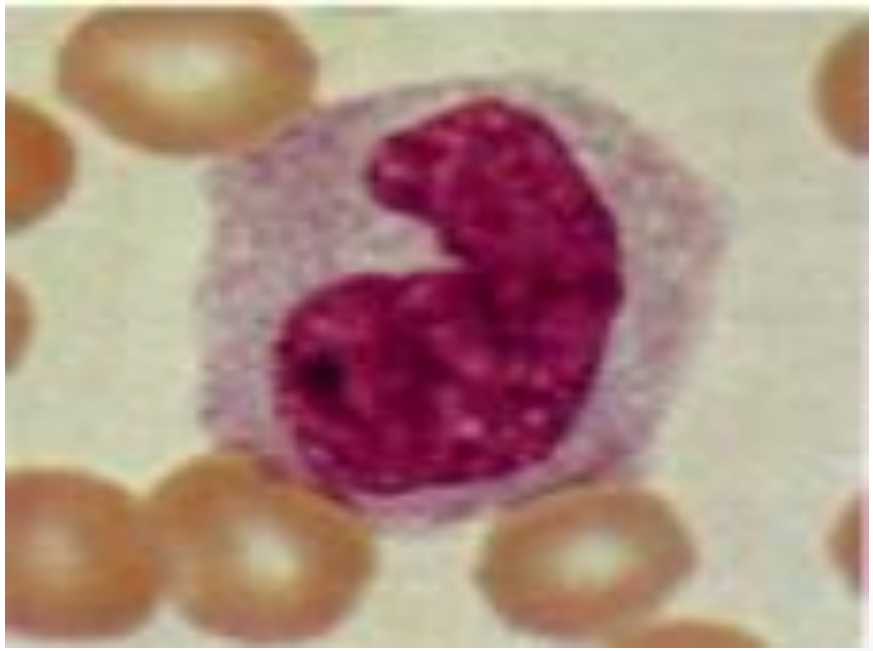


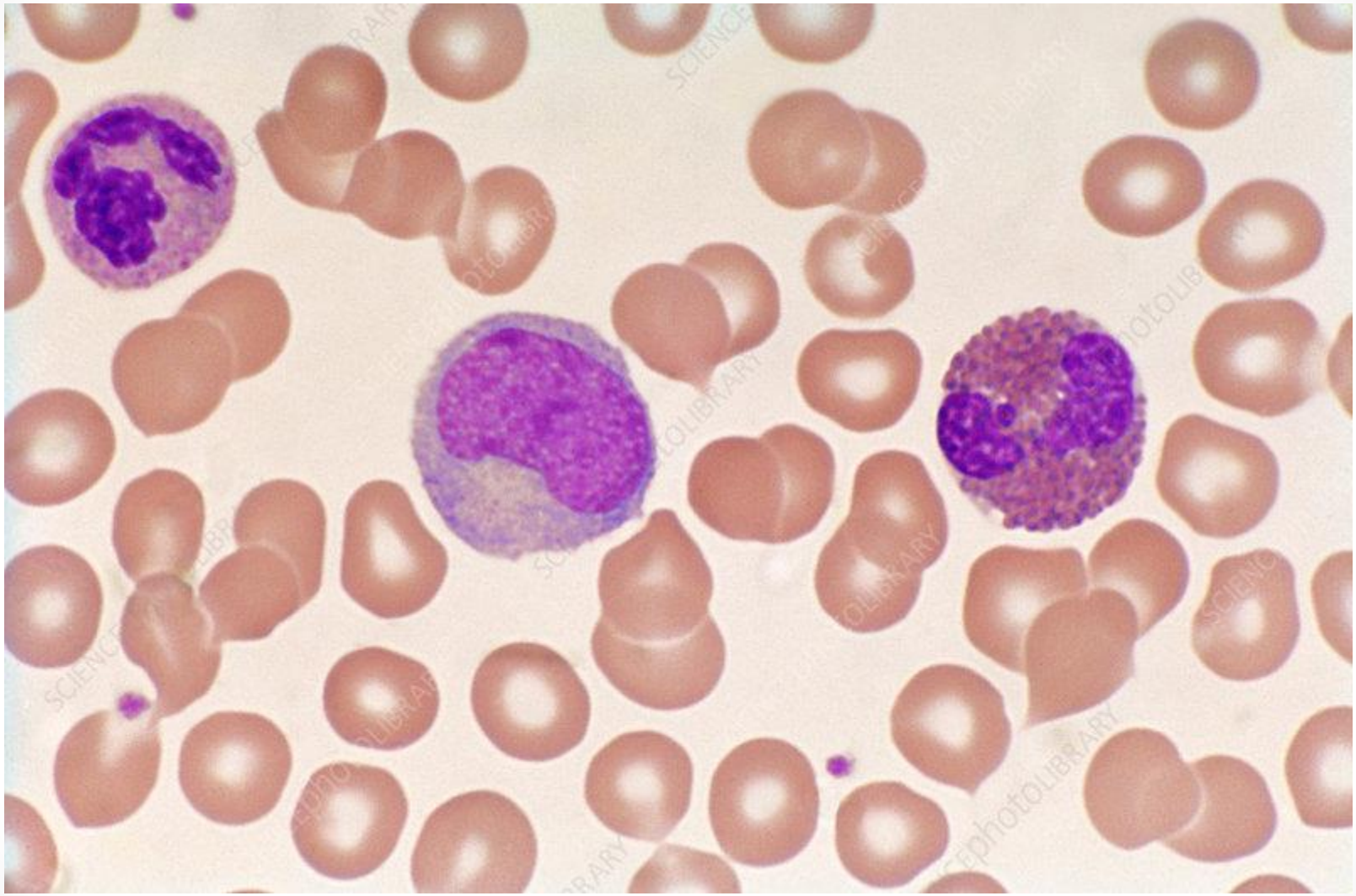


- **Lymphocytes** have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules. Most cells are small in size.
- We can’t differentiate between the B and T lymphocytes under the light microscope.



- **Monocytes** are large cells. They have large indented nuclei, often kidney-shaped. Their cytoplasm has fine purple granules which give it a "ground glass" appearance.

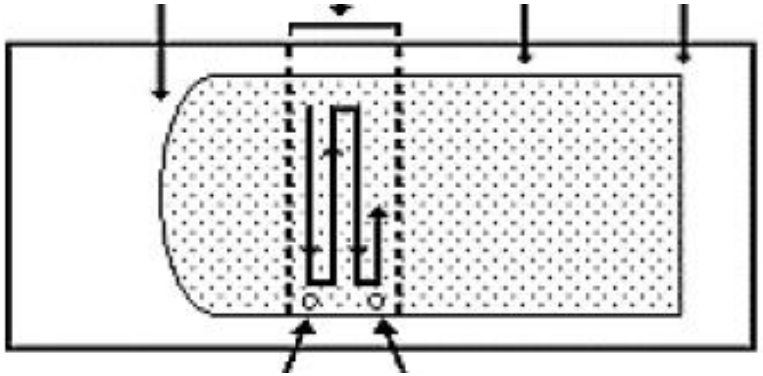




The procedure

1. A drop of blood is thinly spread over a glass slide, air dried, and stained with an acidic dye (red) and a basic dye (blue-purple).
2. The slide is examined under a microscope using an oil immersion lens.
3. Two hundred white cells are then counted and classified.
4. The number of each type of cells is expressed as a percentage.

- To do this one must be able to distinguish between the 5 types of WBCs



Importance of DLC

- Gives relative percentage of each type of WBC
- Helps reveal the presence of abnormal WBCs like blasts or lymphoma cells.
- Used along with WBC count to generate an **absolute value** for each type of WBCs.
  - Relative percentages can be misleading
  - Absolute values are also useful for monitoring certain conditions.
  - Absolute count =WBC (cells/ μL) x percent of the specific WBC type ÷100

Absolute count calculation

- If the WBC count is 6000 cells/mm^3 and the lymphocytes make 30% of the DLC, the Absolute lymphocyte count (ALC) will be:

WBC count **x** (Lymphocyte%)/100 =

(6000 X 30)/100 = 1800 cells/mm^3

• Absolute neutrophil count (ANC)=WBC (cells/ μL) x percent (neutrophils + neutrophilic band cells) ÷100

1. Neutrophilic leukocytosis: is defined as a total WBC above 11,000/μL along with an absolute neutrophil count (ANC) greater than 7700/μL
   - Bacterial infections, inflammatory conditions, stress.

2. Lymphocytic leukocytosis : is defined as a total WBC above 11,000/μL along with an absolute lymphocyte count greater than 4500/μL
   - Viral infections as infectious mononucleosis, mumps, rubella and pertussis or in acute and chronic lymphocytic leukemias.

3. Monocytic leukocytosis:
   - Acute or chronic bacterial infection and chronic inflammation

4. Eosinophilic leukocytosis :
   - Parasitic infections & allergic conditions

6. Basophilic leukocytosis:
   - Allergic conditions

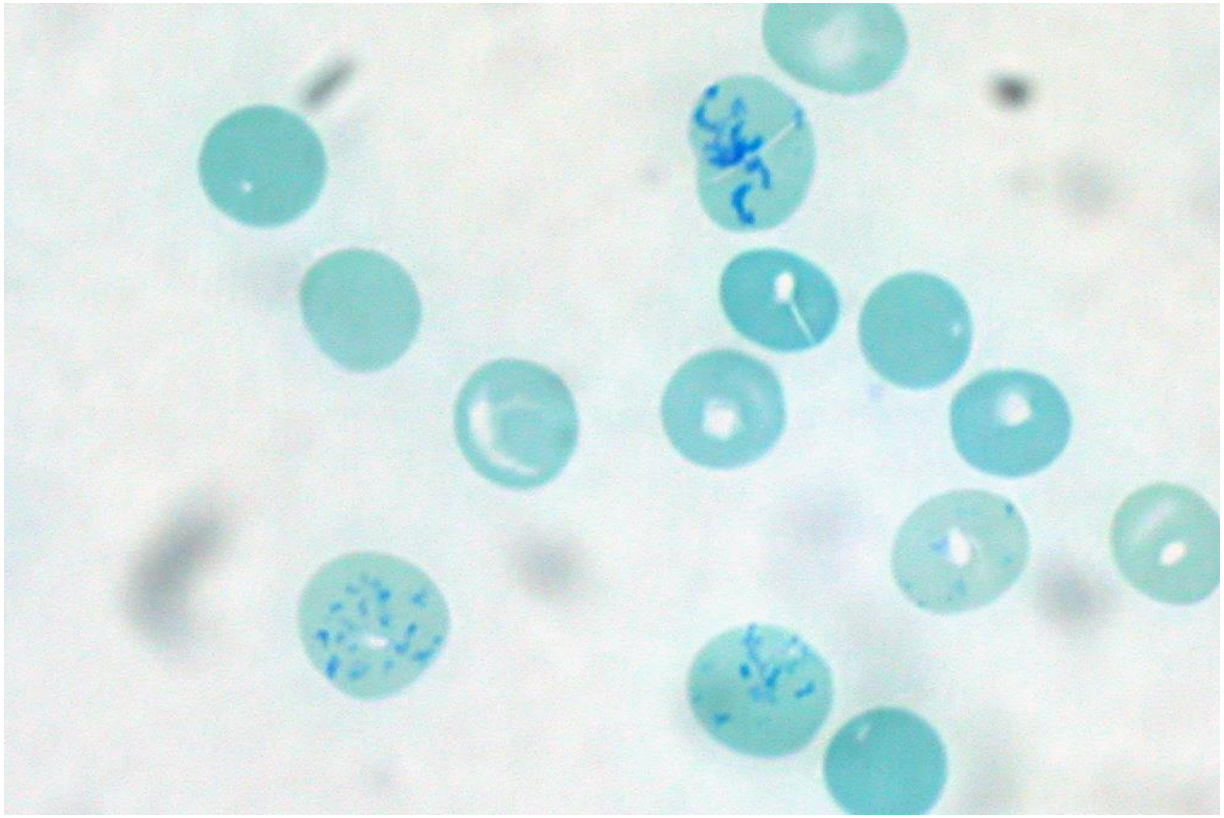
7. Neutropenia : absolute neutrophil count is less than 1,500 cells/ mm^3
   - Certain infections like typhoid fever, HIV & CMV, chemotherapy, radiotherapy, and autoimmune diseases.

8. Lymphocytopenia:
   - May occur in the normal elderly or be associated with chronic infection or malignancy.

Reticulocyte Count

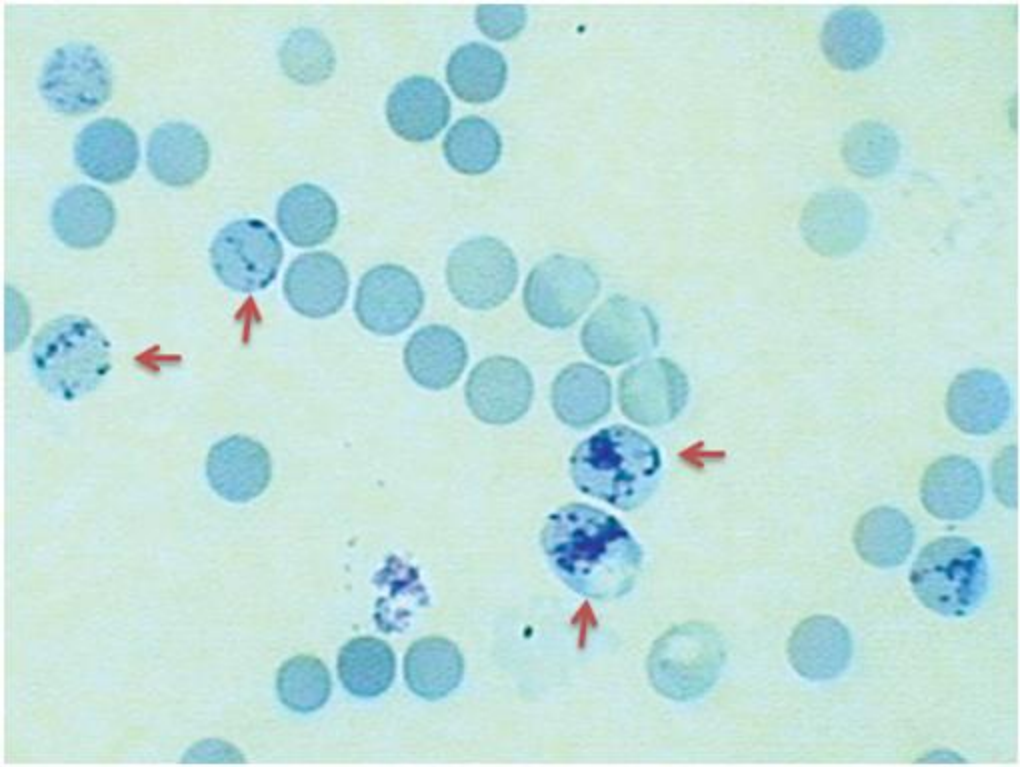
- Reticulocytes are the immediate precursor of RBCs, following their release to the blood stream they mature within 1-2 days into RBCs.
- Contain a small amount of basophilic material, mainly remnants of the Golgi apparatus & mitochondria
- They normally make less than1-2% of all RBCs
- Used to estimate the degree of effective erythropoiesis
- Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure

If supravital staining (new methelene blue) is performed on a blood smear, the reticulocytes appear larger than RBCs and contain dark blue dots and curved linear structures in their cytoplasm (remnants of ribosomes).



The procedure

- 500-1000 RBCs should be counted and the number of reticulocytes noted. The count is expressed as a percentage which can be used to calculate the absolute reticulocyte count (ARC) .



- ARC accurately reflects the degree of reticulocytosis regardless of the degree of anemia. The normal absolute reticulocyte count is between 25,000 to 75,000/mm^3

- ARC = (RBC count X reticulocyte%)/100

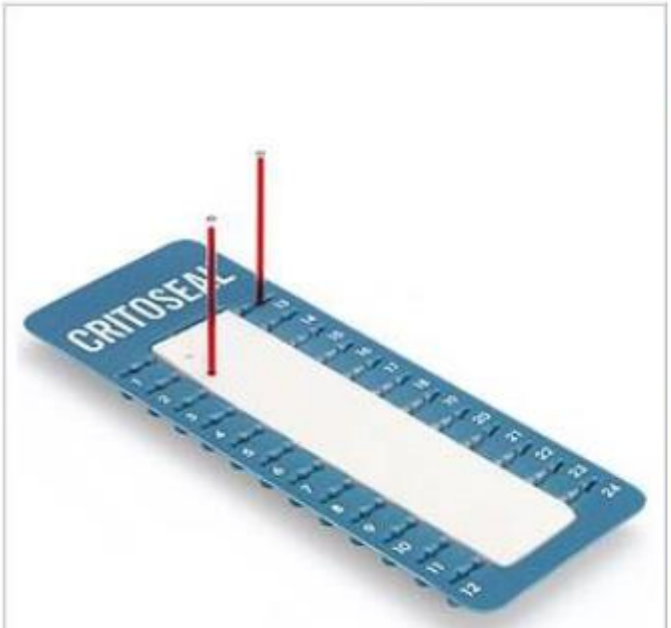
Reticulocytosis and Reticulocytopenia

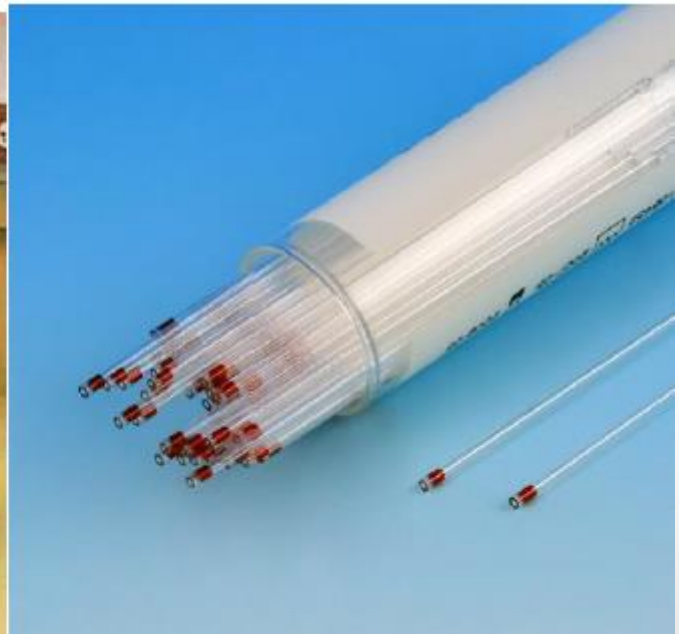
- Condition associated with an increase in reticulocytes:
  - Hemolytic anemias: Immune hemolytic anemia, RBC membrane defects, Sickle cell diseases,
  - Following hemorrhage
  - Following treatment of anemias

- Condition associated with a decrease in reticulocytes:
  - Iron deficiency anemia
  - Aplastic anemia
  - Radiation therapy
  - Tumor in bone marrow

Packed Cell Volume (PCV) Hematocrit (HCT)

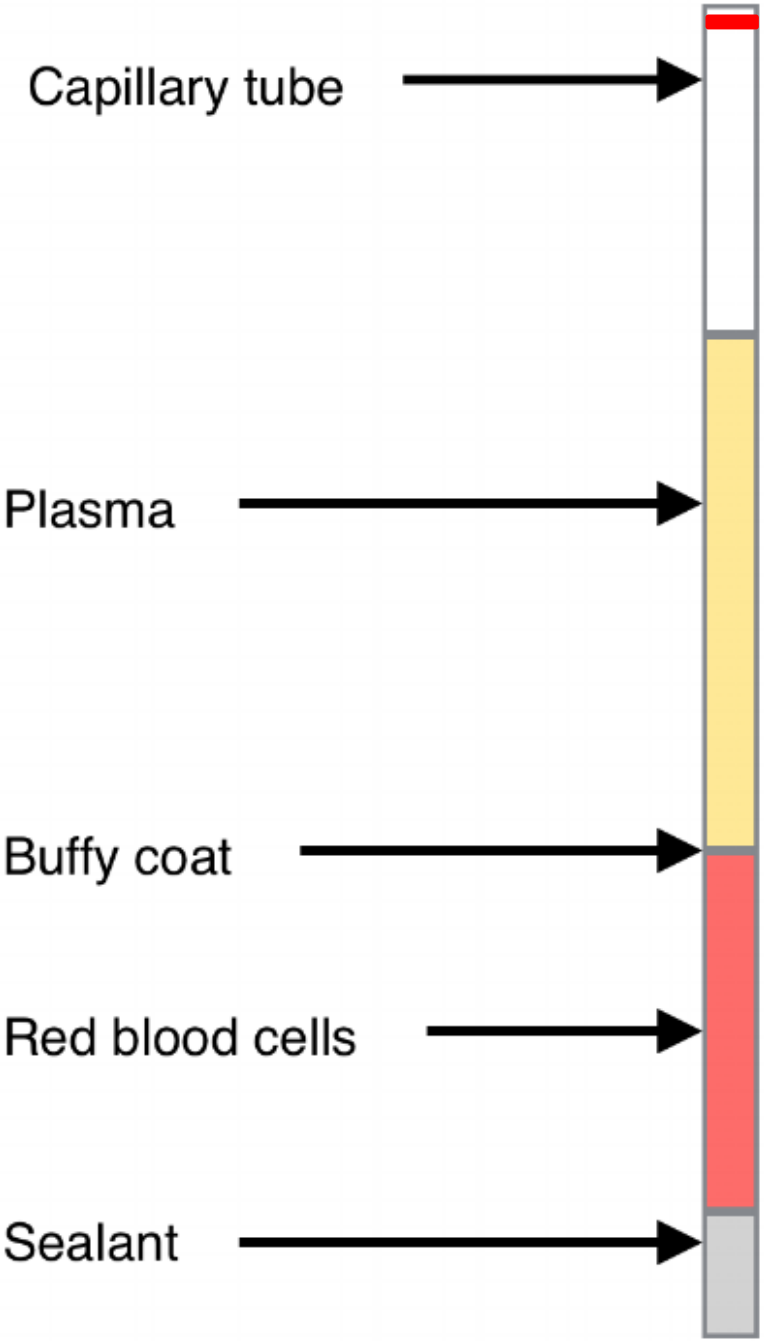
- PCV is the ratio of the volume of packed red cells to the total blood volume.
  - Males: 40%- 54%
  - Females: 36% - 46%
- It decreases in cases of anemia and increases in polycythemia and dehydration.





The procedure

- A blood sample is centrifuged in a heparinized capillary tube (red tip),
- The RBCs become packed at the bottom of the tube.
- The PCV is then calculated according to the following formula:
- PCV= $\frac{\text{RBC height}}{\text{Total height}}$ x100
- Beware not to include the buffy coat



Hemoglobin Concentration

- Hemoglobin is a globular protein made up of four subunits. Each subunit contains a **heme** group conjugated to a polypeptide. Heme is an iron-containing porphyrin derivative.
- Heme has the ability to bind oxygen reversibly and carry it to tissues.

➢ Normal values of hemoglobin

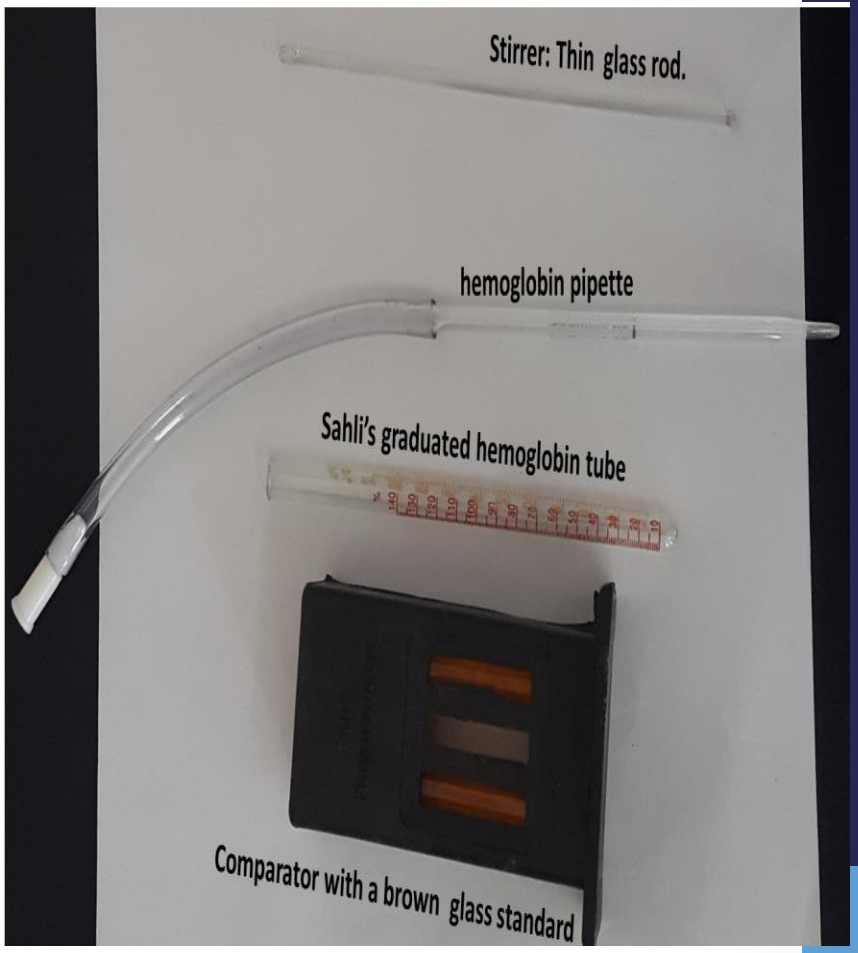
- 14-17.5 g/ 100 ml in males
- 12-15 g/ 100 ml of in females

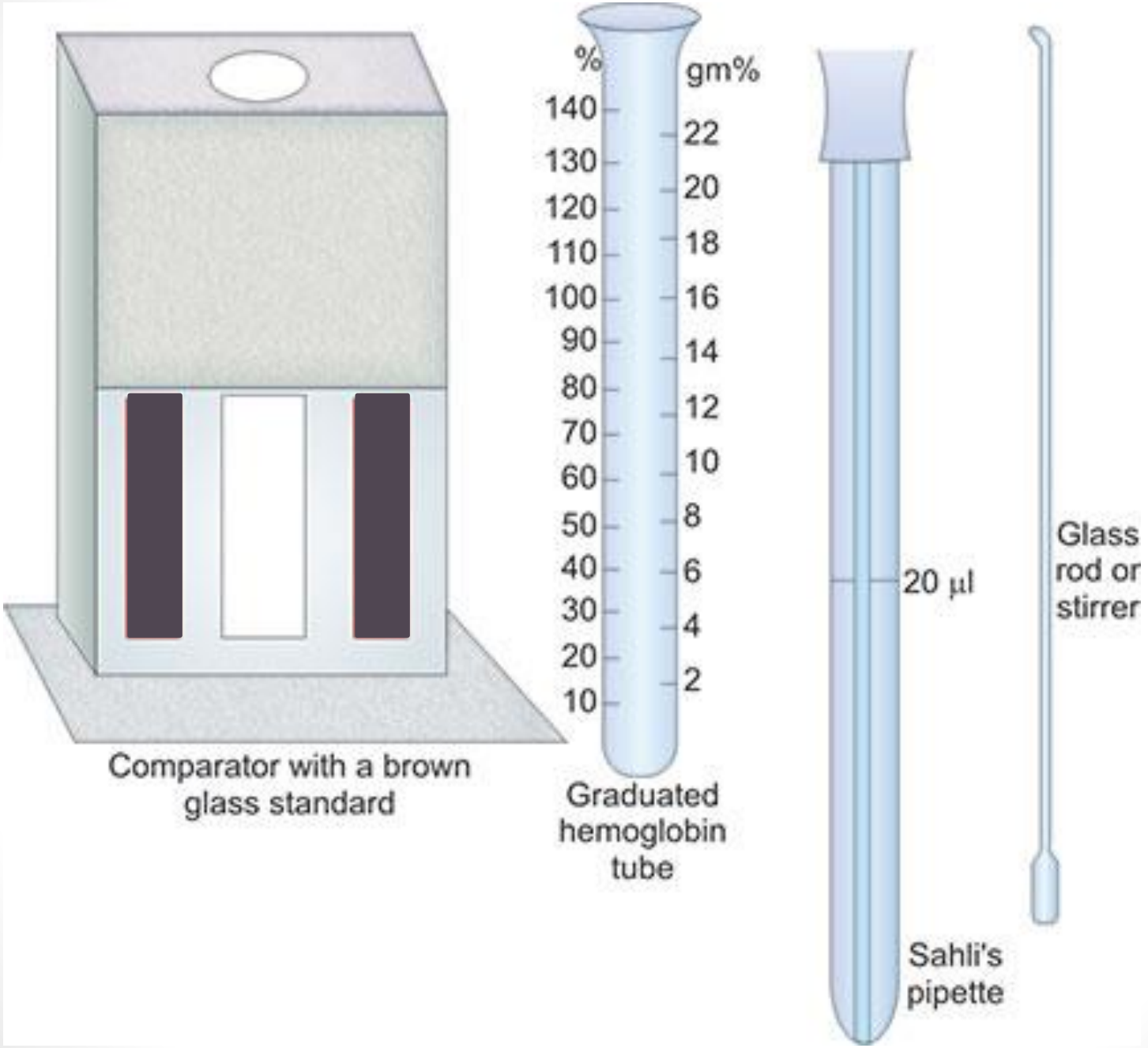
➢ Different methods can be used to find the hemoglobin concentration one of them is Sahli's method.

➢ Based on the fact that when blood is mixed with HCl, hemoglobin is converted to acid hematin which is brown in color

Sahli's apparatus

Sahli's haemoglobinometer

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18
16
14
12
10
8
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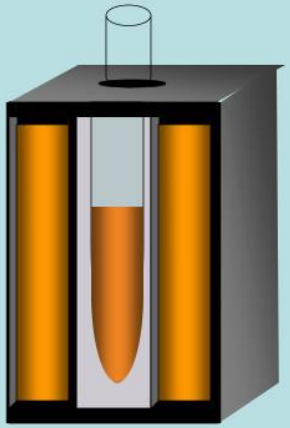
4
2
20 µl
Glass rod or stirrer
Sahli's pipette
Comparator with a brown glass standard
Graduated hemoglobin tube

The procedure

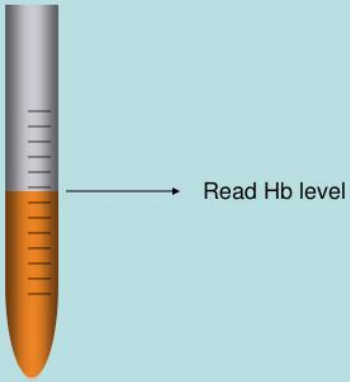
1. Add HCl into the tube up to 2g% mark
2. Mix the EDTA sample gently and fill the pipette with 20 Ul blood.
3. Wipe the external surface of the pipette to remove any excess blood.
4. Add the blood into the tube containing HCl. Wash out the contents of the hemoglobin pipette by drawing in and blowing out the acid few times so that the blood is mixed with the acid thoroughly.
5. Allow to stand undisturbed for 10 min. (This is because, maximum conversion of hemoglobin to acid hematin, occurs in the first ten minutes)
6. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod until it's colour matches that of the comparator glass.
7. Remove the stirrer and take the reading directly

- Hemoglobin concentration is read directly from the graduated scale on the dilution tube.

Continue adding, stirring until colour matches with standard



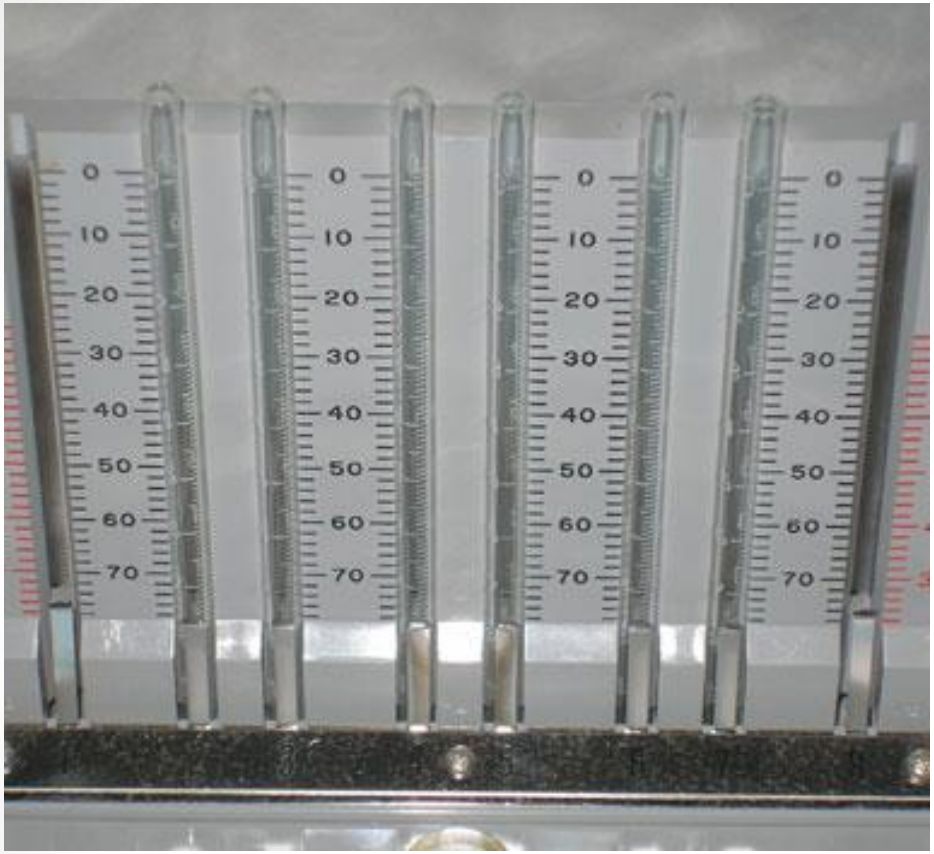
Read Hb from lower meniscus, express as g/dl



Erythrocyte Sedimentation Rate (ESR)

- The rate at which RBCs sediment in a period of one hour.
- The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body.
- It reflects the tendency of red blood cells to settle more rapidly in the presence of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins.
- Changes in red cell shape or numbers may also affect the ESR.

The procedure



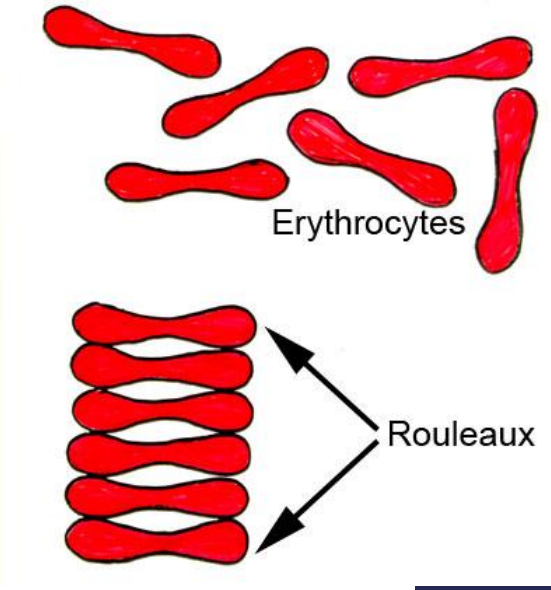
- In our lab we use the Wintrobe tube which is 100 mm long.
- EDTA anticoagulated blood is drawn into the Wintrobe tube till the zero mark
- The tube is placed in its rack in a strictly vertical position for 1 hour at room temperature
- the RBCs – under the influence of gravity - settle out from the plasma.
- The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

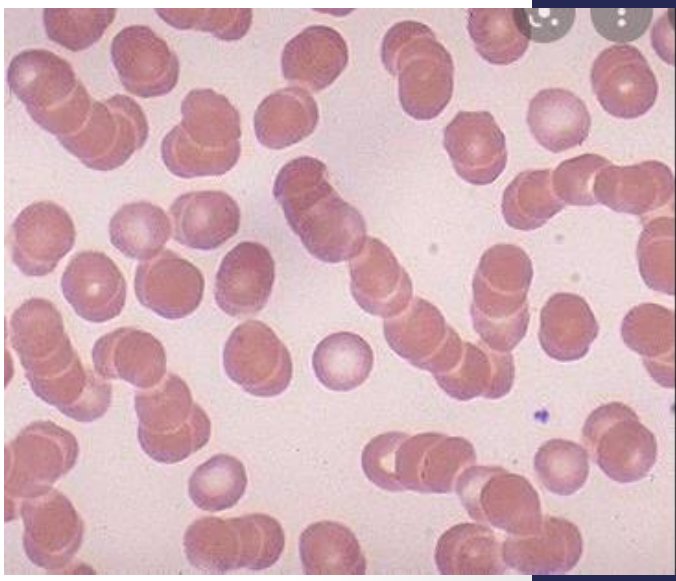
At the beginning of the experiment

After one hour

RBCs sedimentation

- The RBCs sediment because their density is greater than that of plasma. The sedimentation increases with stacking of RBCs (rouleaux formation)
- Rouleaux formation is possible because of the discoid shape of RBCs
- Normally, RBCs have negative charges on the outside of the cells, which cause them to repel each other.
- Many plasma proteins have positive charges and can neutralize the negative charges of the RBCs, which allows for the formation of the rouleaux.
- Therefore, an increase in plasma proteins (present in inflammatory conditions) will increase the rouleaux formations, which settle more readily than single red blood cells





- Normal ESR values
  - Men < 15mm/hr
  - Women < 20mm/hr

- High ESR
  - Inflammation
  - Anemia
  - Old age
  - Pregnancy
  - Technical factors: tilted ESR tube, high room temperature.

- **Some interferences which decrease ESR:**
- Abnormally shaped RBC (sickle cells and spherocytosis)
- Polycythemia
- Technical factors: low room temperature, delay in test performance (>2 hours), clotted blood sample

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CAP ACCREDITED
COLLEGE of AMERICAN PATH

Pa [redacted]

Patient No. : **19/899500**

Sample No. : **BA0120/1983778**

[redacted]

[redacted]

Age : **38 Year(s)**

Sex : **Female**

Sample Date / Time : **15-Aug-2020** **1:14 PM**

Routine Haematology

				Reference limit
Haemoglobin	: 127	g/L		120 - 160
Haematocrit	: 36.8	%		37.0 - 48.0
RBC	: 4.8	x10^12/L		4.2 - 5.2
MCV	: 76.8	fL		80.0 - 99.0
MCH	: 27.0	pg		27.0 - 32.0
MCHC	: 34.5	g/dL		32.0 - 36.0
RDW	: 14.3	%	RDW (Red Cell Distribution Width)	11.7 - 15.2
Platelets	: 292	x10^9/L		150 - 450
MPV	: 9.2	fL	MPV Mean platelet volume	7.2 - 11.7
WBC	: 6.080	x10^9/L		4.0 - 11.0

Differential					Reference limit
Neutrophils	: 49	%	2.979	x10^9/L	1.800 - 7.500
Lymphocytes	: 43	%	2.614	x10^9/L	1.200 - 4.000
Monocytes	: 7	%	0.426	x10^9/L	0.200 - 1.000
Eosinophils	: 1	%	0.061	x10^9/L	0.040 - 0.500
Basophils	:	%		x10^9/L	0.015 - 0.100

Blood Film : The red blood cells are mainly normochromic normocytic.
The white blood cells are normal in total count and differential.
The platelets are adequate with normal size.

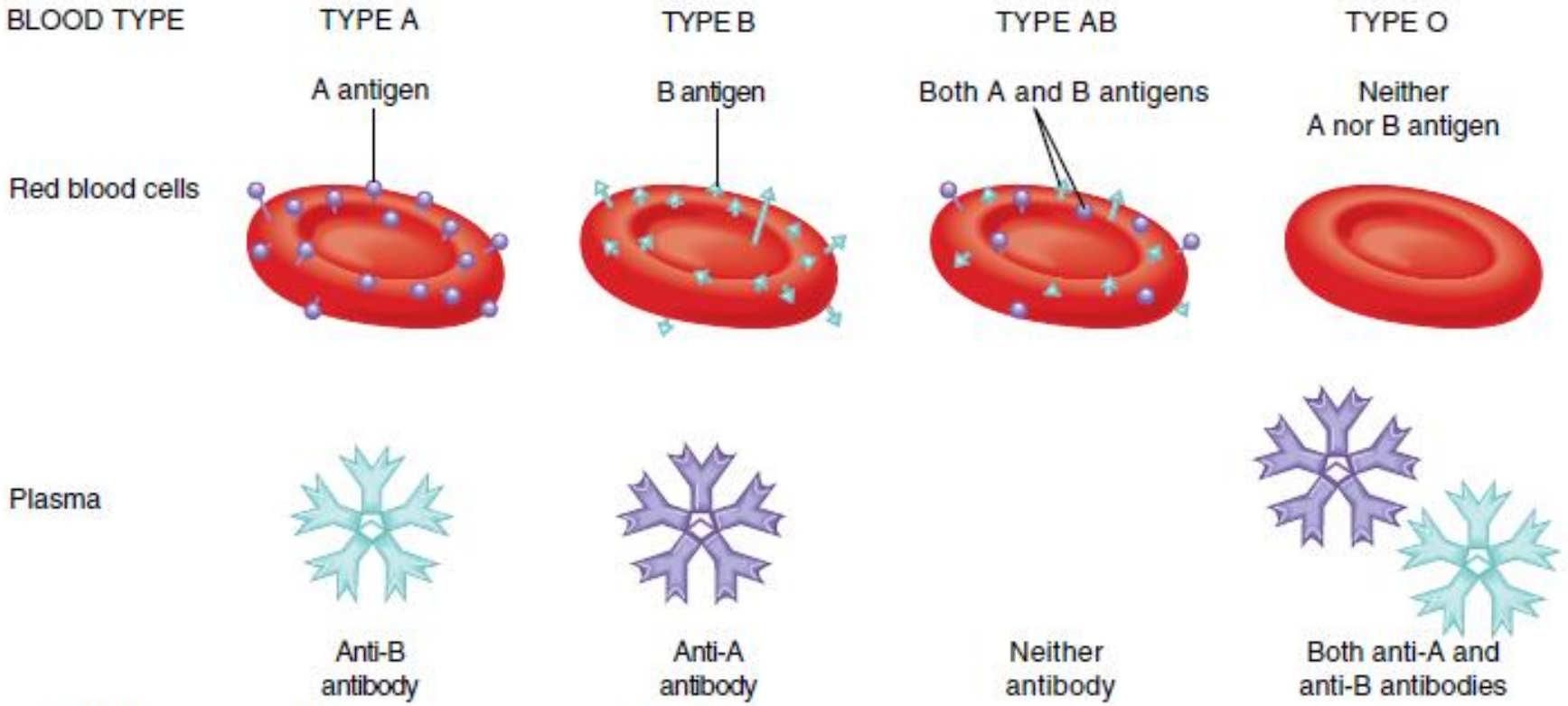
- Serum iron profile is recommended.

Blood Groups

- At least 30 commonly occurring antigens and hundreds of other rare antigens composed of glycoproteins and glycolipids are found on the surface of RBCs.
- Each of which can at times cause antigen- antibody reactions leading to immediate or delayed agglutination and hemolysis of RBCs.
- Most of the antigens are weak.
- Two particular types of antigens (agglutinogens) are likely to cause blood transfusion reactions: the *ABO* system of antigens and the *Rh* system.
- Based on these two systems we have 8 blood groups:
- A +ve, A –ve, B +ve, B –ve, AB +ve, AB –ve, O +ve & O -ve

ABO Blood Group

- The ABO blood group is based on two glycolipid antigens called A and B.
- Blood plasma usually contains antibodies called agglutinins that react with the A or B antigens. These are the anti-A antibody, which reacts with antigen A, and the anti-B antibody, which reacts with antigen B.
- Agglutinins start to appear in the blood within a few months after birth.
- They are formed naturally. Their production is thought to be stimulated when the immune system encounters the "missing" ABO blood group antigens in food or in micro-organisms.


BLOOD TYPE
TYPE A
TYPE B
TYPE AB
TYPE O
A antigen
B antigen
Both A and B antigens
Neither
A nor B antigen
Red blood cells
Plasma
Anti-B
antibody
Anti-A
antibody
Neither
antibody
Both anti-A and
anti-B antibodies

Rh blood group

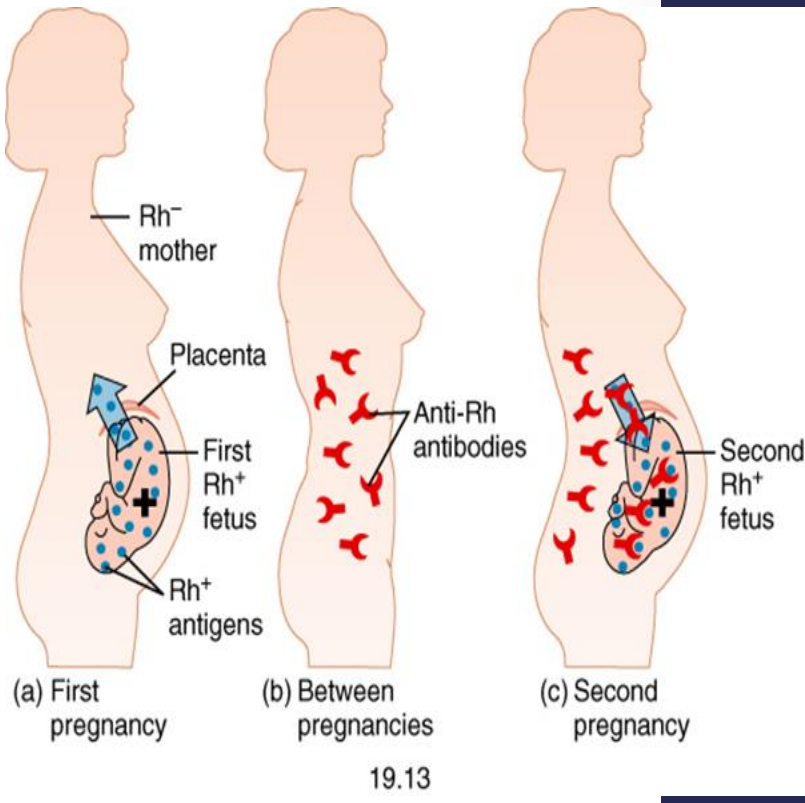
- There are six common types of Rh antigens, each of which is called an Rh factor. These types are designated C, D, E, c, d, and e.

- The type D antigen is widely prevalent in the population and considerably more antigenic than the other Rh antigens.
- Anyone who has this type of antigen is said to be Rh positive (85% of population), whereas a person who doesn’t have type D antigen is said to be Rh negative.

- In contrast to ABO system there is no preformed Anti-D in the Rh–ve individual

Hemolytic disease of the newborn (HDN)

- Rh^{-ve} mother is exposed to Rh^{+ve} blood from the fetus through the placenta during birth, abortion , or miscarriage.
- The mother will start to make anti-Rh antibodies.
- The firstborn baby usually is not affected.
- If the mother becomes pregnant again anti-Rh antibodies can cross the placenta and enter the bloodstream of the fetus. If the fetus is Rh^{+ve} agglutination and hemolysis occur in the fetal blood leading to anemia and jaundice. The disease - erythroblastosis fetalis or hemolytic disease of the newborn- may result in fetal death.
- An injection of anti-Rh antibodies called anti-Rh gamma globulin can be given to prevent HDN.
- Rh^{-ve} women should receive it before delivery, and soon after every delivery, miscarriage, or abortion.



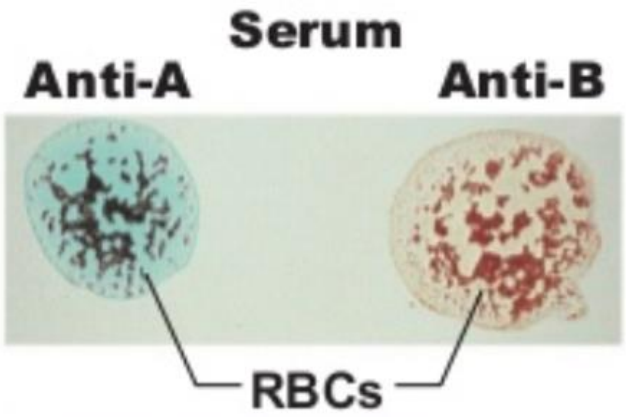
Determination of blood type

1. Prick the tip of a finger with a lancet and put three separate drops of blood on a clean microscopic slide.
2. Add one drop of Anti-A to the first drop, Anti-B to the second drop, and Anti-D to the third drop.
3. Mix well, using separate wooden sticks.
4. The results are read directly from the slide.

- If agglutination occurs in the first drop the blood type is A , if agglutination occur in the second drop the blood type is B, if it occurs in both it is AB and if it doesn't occur in any drop it is type O.
- If agglutination occurs in the Rh drop the blood is considered as Rh+ve. (This reaction might take some time to develop)

- The strength of agglutination reaction is not the same in all people, so in some cases it may be necessary to examine the slide under the microscope to look for agglutination.


Anti-A
MONOCLONAL ANTIBODIES
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522
Anti-B
MONOCLONAL ANTIBODIES
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522
Anti-D
Store at 2-8°C
DO NOT FREEZE
M L No. KD-522

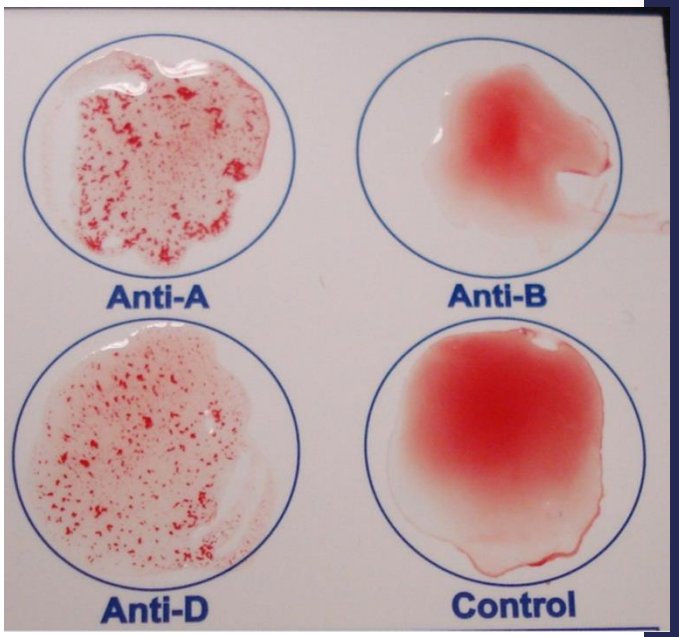


Type AB

Type A

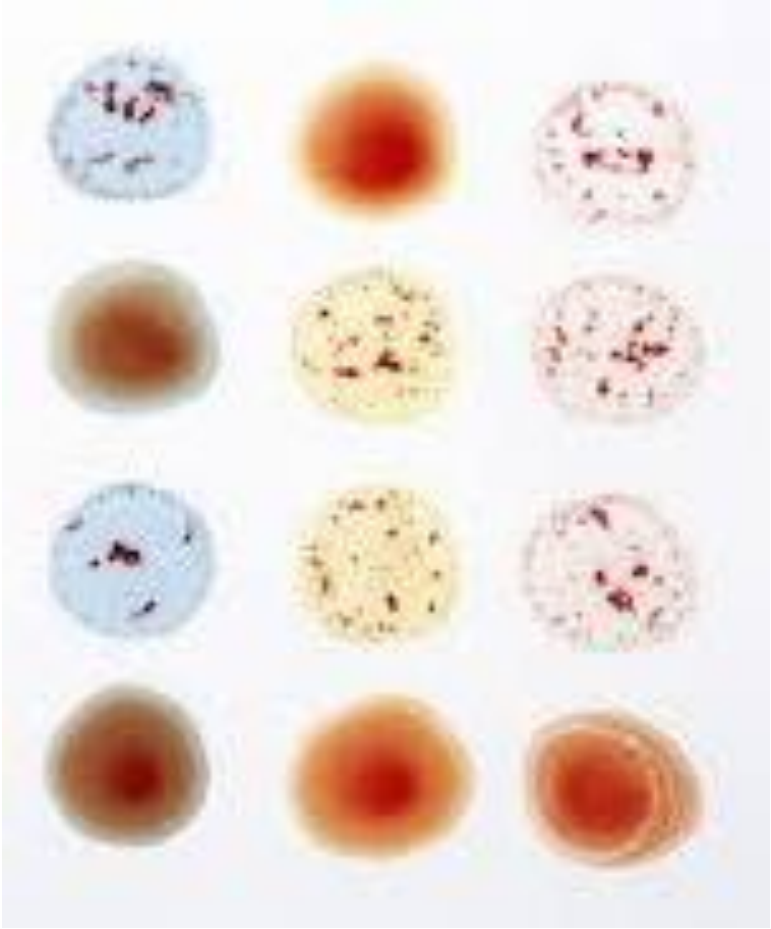
Type B

Type O



Type A +ve

What is the type of blood in each test presented below?



1.

2.

3.

4.

Hemostasis

- Hemostasis is prevention of blood loss from circulatory system.
- Depends on the integrity of blood vessels, platelets and clotting factors.

The hemostatic response to vascular injury is achieved by several mechanisms:

1. Vasoconstriction
2. Formation of a platelet plug
3. Formation of a blood clot

Bleeding time

- A **bleeding time** is used to evaluate the second phase of hemostasis, which involves adherence of the platelets to the injured vessel, platelet activation and aggregation (formation of a plug).
- ✓ The time measures how long it takes for a platelet plug to form.
  - ✓ Normal range: 3-5 minutes
- ✓ It increases when the platelets count is low (thrombocytopenia), platelet function is abnormal or with the use of aspirin .
- Disadvantages: Insensitive, Invasive & operator dependent.

- Duke method

1. Clean the tip of the finger or the ear lobe with alcohol.
2. Puncture the skin with a special lancet. The wound should be 3–4 mm deep.
3. Wipe the blood drop by a filter paper every 30 seconds
4. Repeat until no more blood is absorbed by the filter paper.
5. Multiply the number of blood drops by 30 seconds
    - Or divide the number of spots of blood by 2 and that will give you the bleeding time in minutes.

Clotting time

- It measures the time required for a blood sample to coagulate in vitro. Clotting time depends on the availability of coagulation factors.

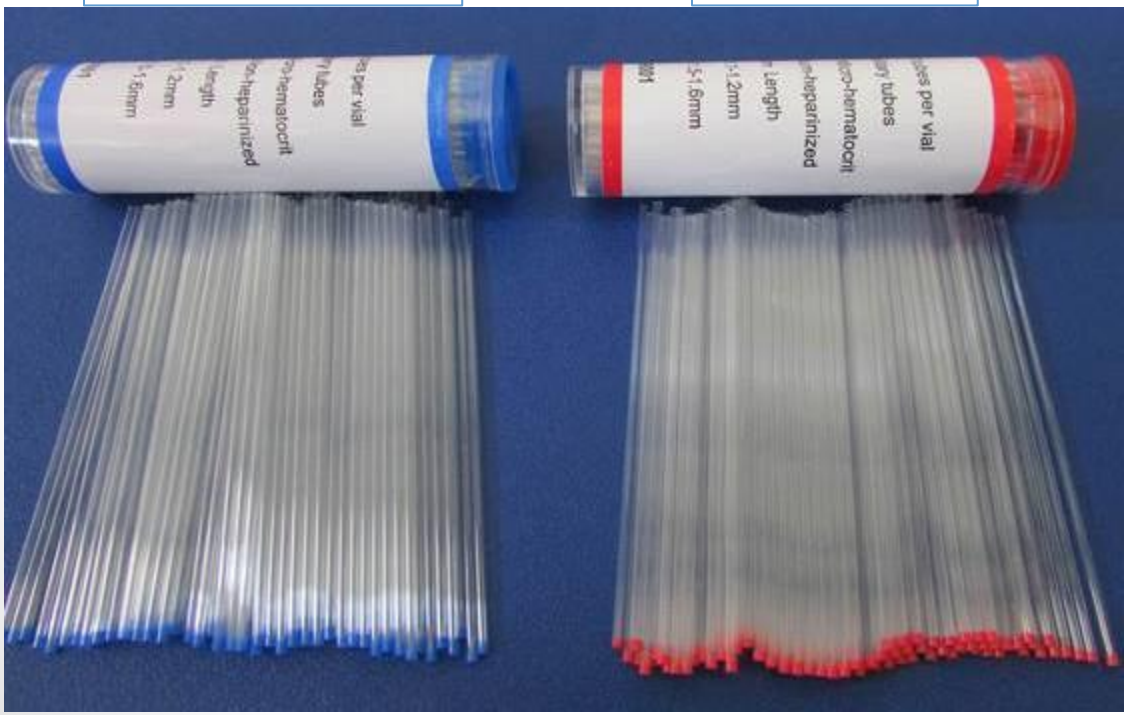
- Normal value is 6-10 minutes.

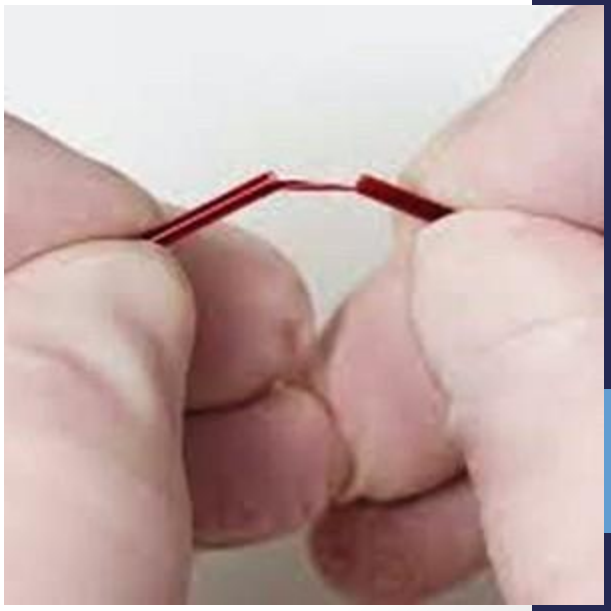
- It is prolonged in conditions like hemophilia, vitamin K deficiency, liver diseases, and warfarin overdose.

1. Clean the tip of the finger with alcohol then prick it with a lancet.
2. Draw blood into <u>non-heparinized</u> capillary tubes.
3. After 2 minutes, start breaking the capillary tubes to see whether a thread of coagulated blood is formed between the two broken ends.
4. It is preferred to calculate the clotting time from the average of two capillary tubes.

Non-heparinized

Heparinized





Osmotic fragility

- when RBCs reside in an isotonic medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water.

- When RBCs reside in a hypotonic medium, a net influx of water occurs so the cells swell and the integrity of their membranes is disrupted resulting in **hemolysis**

- When RBCs reside in a hypertonic media , a net efflux of water occurs so the cells lose their normal biconcave shape, undergoing collapse.

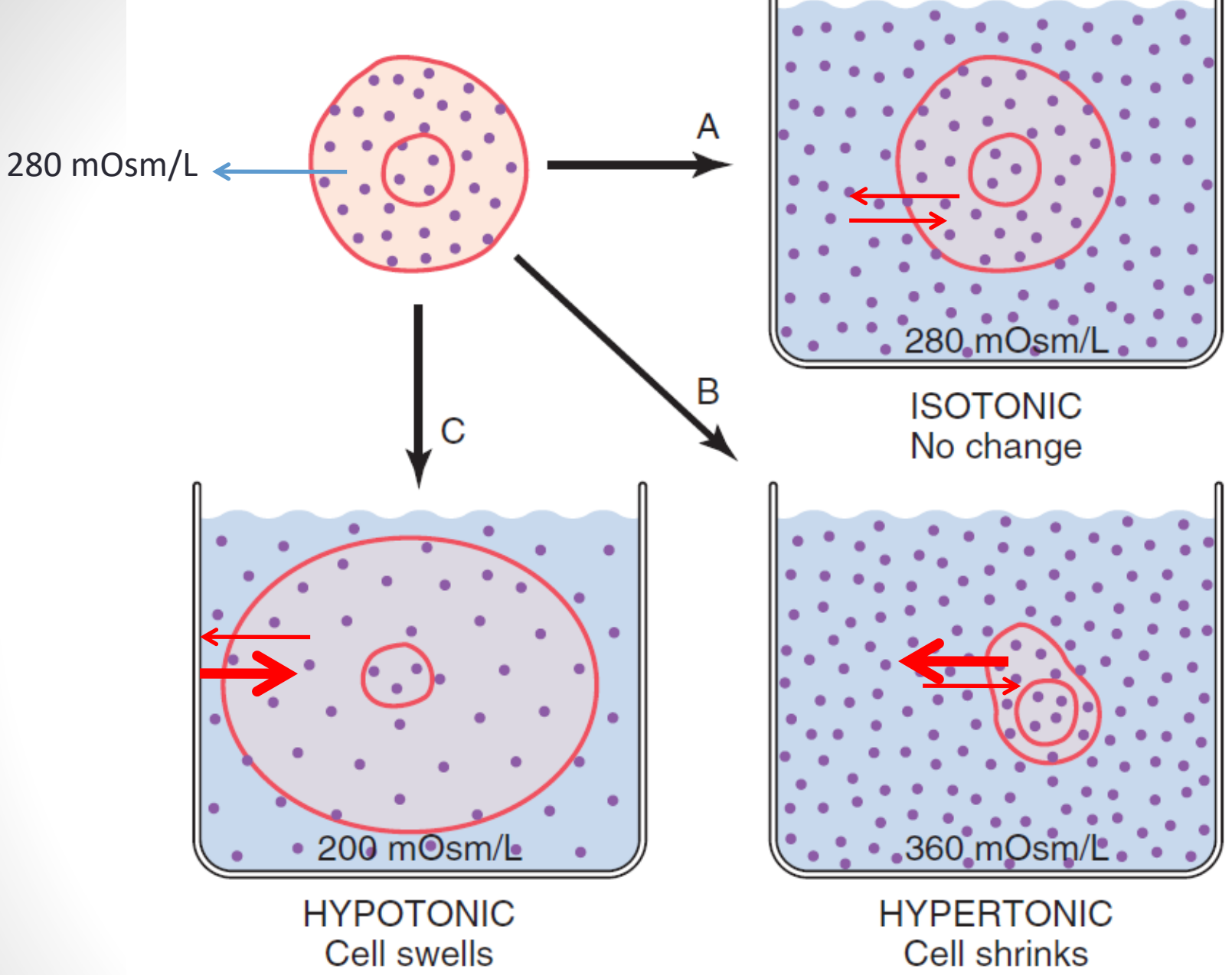


Figure 25-5. Effects of isotonic (*A*), hypertonic (*B*), and hypotonic (*C*) solutions on cell volume.

Osmotic fragility test

- A test designed to measures red blood cell's resistance to hemolysis when exposed to a series of increasingly dilute saline solutions.

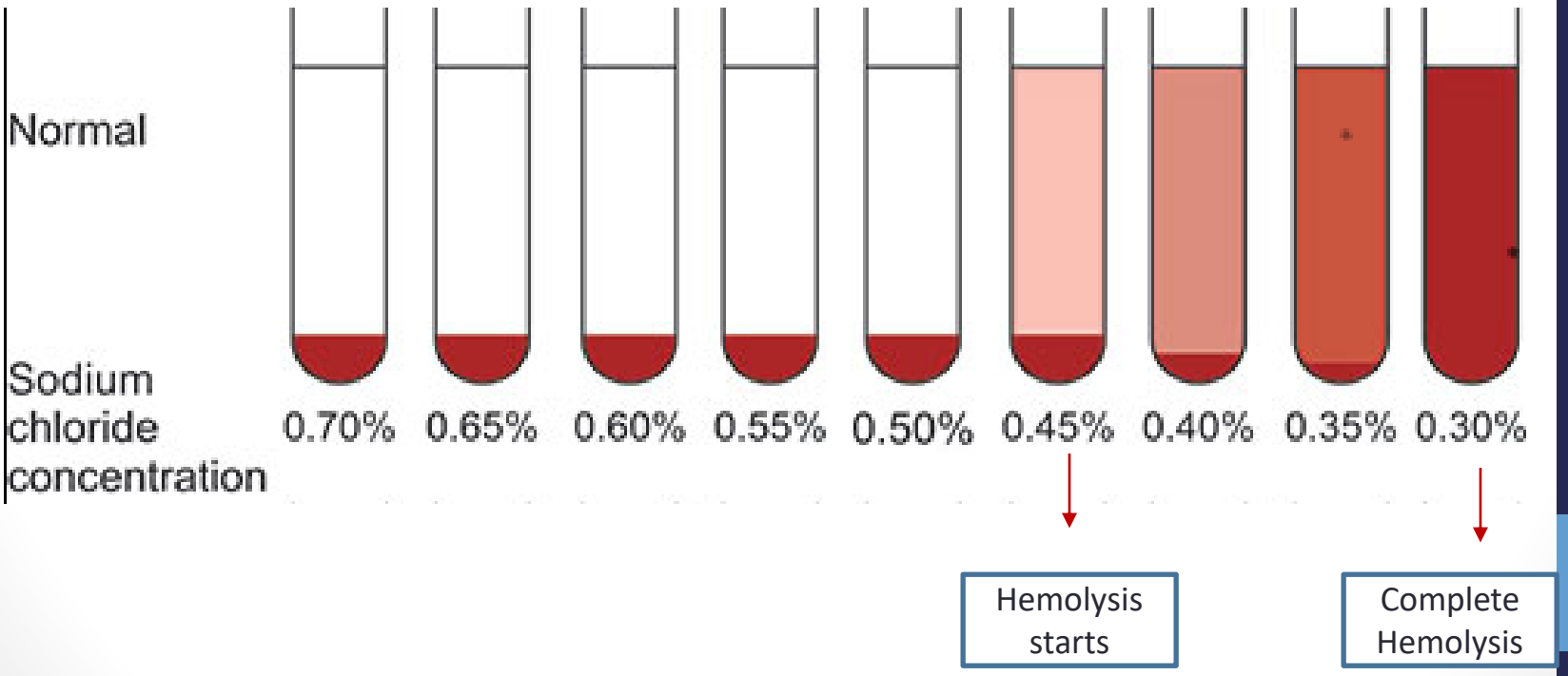
- The susceptibility of RBCs to hemolysis is a function of:
  - Surface area to volume ratio.
  - Cell membrane composition and integrity

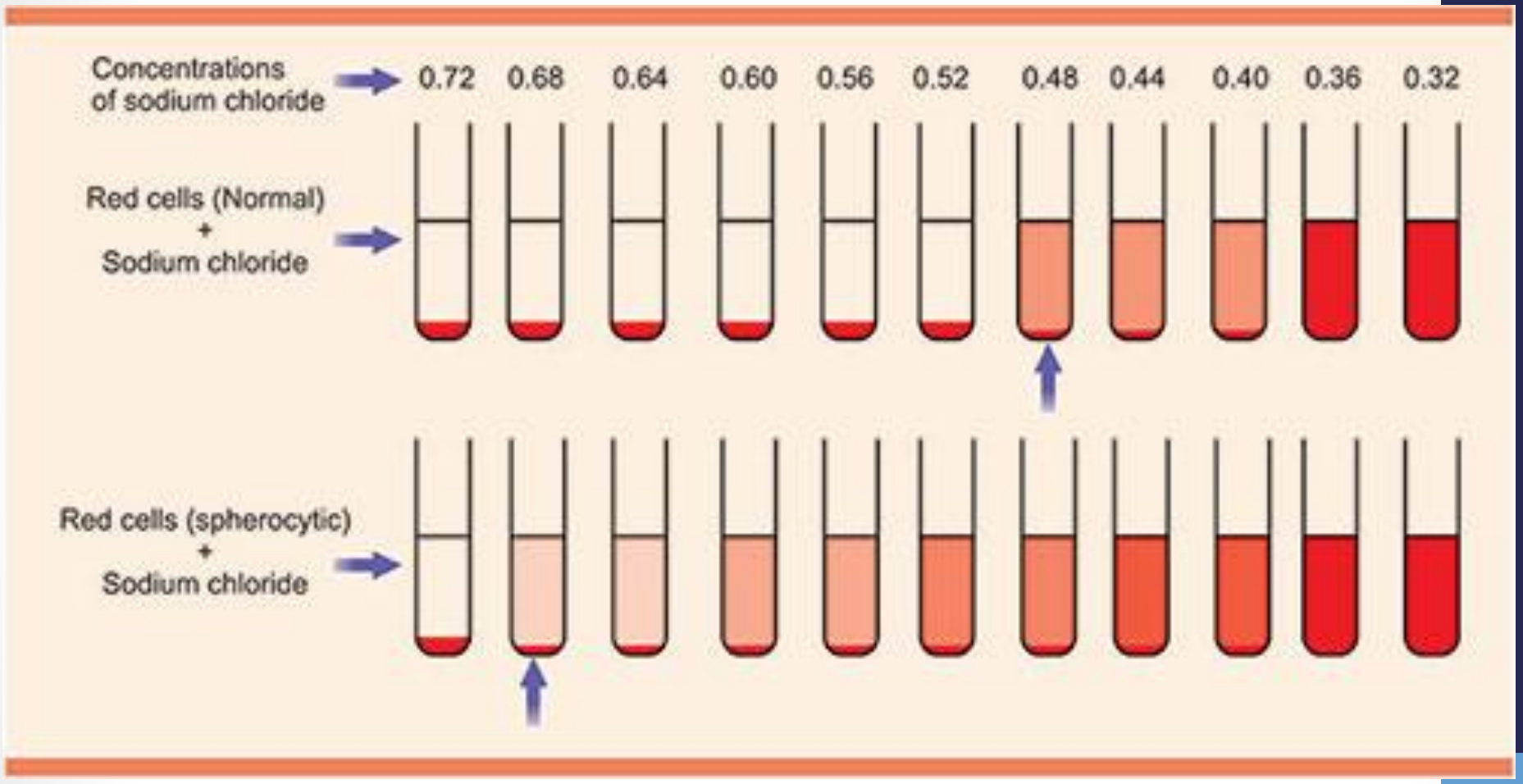
- This test is mainly used to diagnose hereditary spherocytosis but it is also used in some countries to screen for thalassemia.

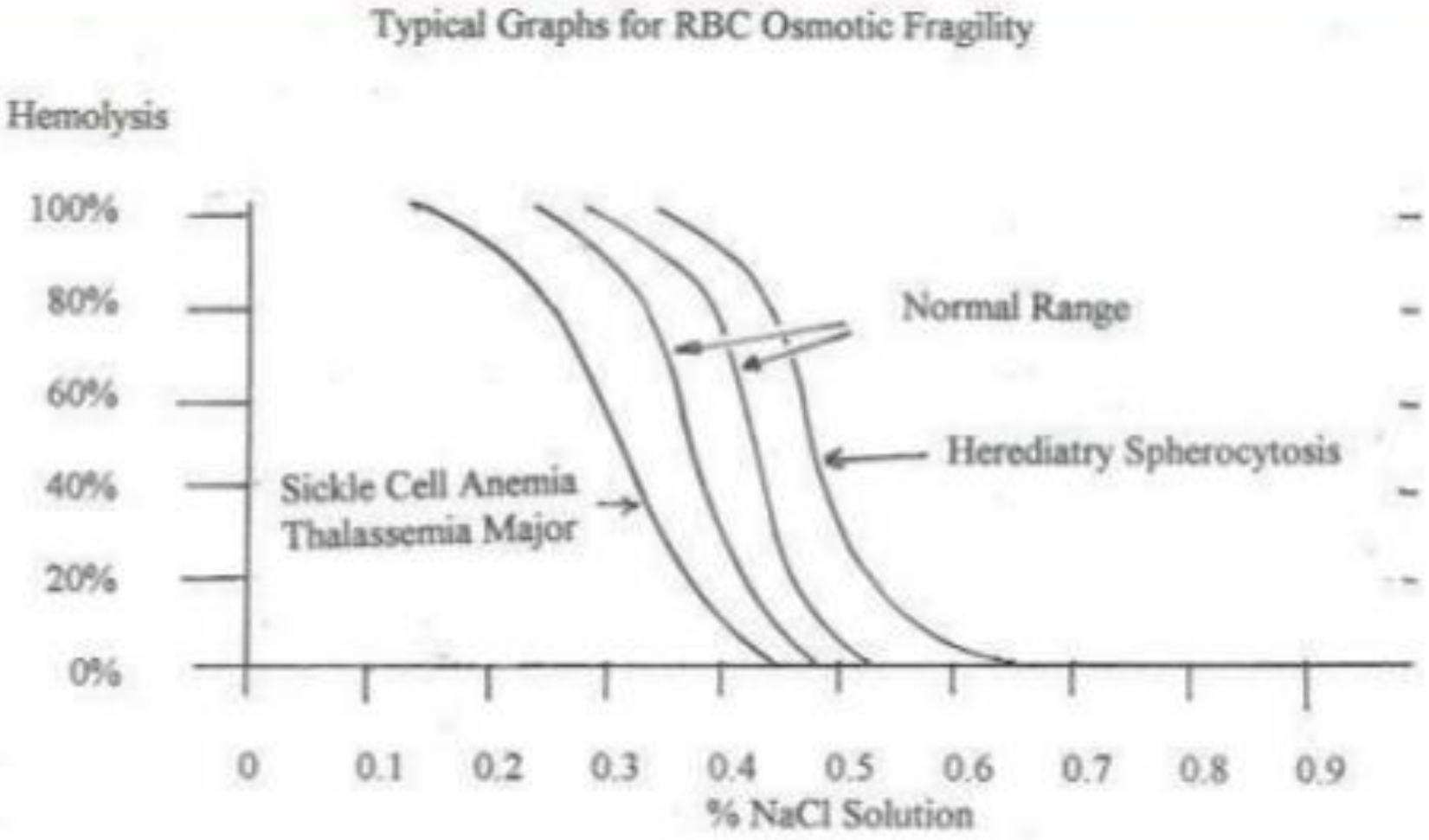
- The procedure:

1. Put labeled centrifuge tubes in a rack.
2. Prepare NaCl solutions of different concentrations starting from 0.9% NaCl till 0.2% NaCl.
3. Add 10 ml of each solution to a different tube then add one drop of blood to each tube.
4. Shake each tube well and allow them to stand for 20 minutes. After 20 minutes, the tubes are centrifuged for 10 minutes
5. Transfer supernatant fluid from each tube into spectrophotometer cuvettes
6. The absorbance is then measured at 540 nm and used to calculate the percentage of hemolysis for each solution.
7. The results are plotted against the NaCl concentrations, this yields an osmotic fragility curve which is then compared to a standard curve.

- In this example
- From 0.7% to 0.5% there is no hemolysis.
- At the concentration of 0.45% hemolysis starts and the solution becomes red in color, but there are some settled RBCs in the tube.
- At the concentration of 0.30%, the solution is bright red and there are no settled RBCs (complete hemolysis).




Concentrations of sodium chloride
0.72
0.68
0.64
0.60
0.56
0.52
0.48
0.44
0.40
0.36
0.32
Red cells (Normal) + Sodium chloride
Red cells (spherocytic) + Sodium chloride


Typical Graphs for RBC Osmotic Fragility
Hemolysis
100%
80%
60%
40%
20%
0%
Normal Range
Herediatry Spherocytosis
Sickle Cell Anemia
Thalassemia Major
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
% NaCl Solution

- Decreased red cell fragility (increased resistance to hemolysis) is seen with the following conditions:
  - Thalassemia.
  - Iron deficiency anemia.
  - Sickle cell anemia

✓ These cells have a high surface area: volume ratio

- Increased red cell fragility (increased susceptibility to hemolysis) is seen in the following conditions:
  - Hereditary spherocytosis
  - Autoimmune hemolytic anemia
  - Toxic chemicals, poisons, infections, and some drugs.
  - Severe burns.

✓ These cells have a low surface area: volume ratio