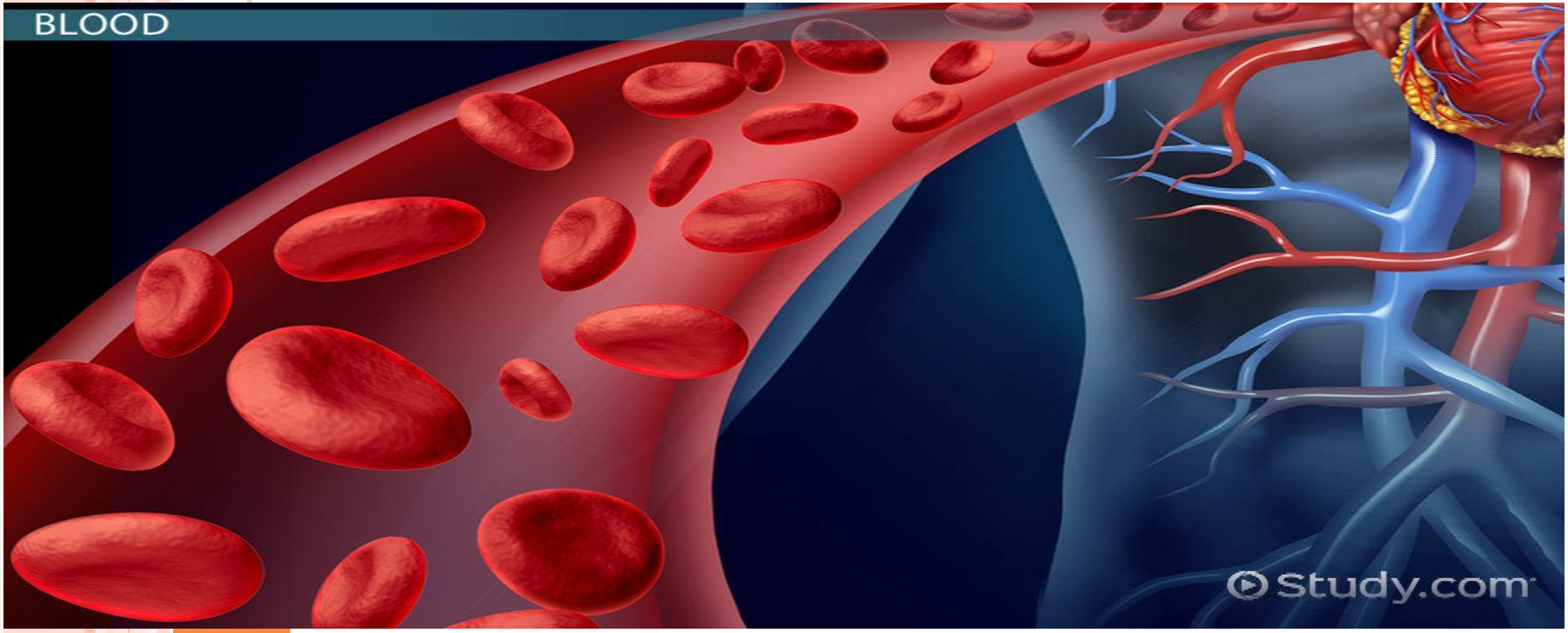
HEMATOLOGY



Dr. Amr Abd Elrhman
Medical Science Specialist
British University in Egypt

characterisics ..?

Function ..?

Composition ..?

Formation ..?

characteristics

pH of blood	• The normal pH range of blood is 7.35 to 7.45,which is slightly alkaline. The venous blood normally has a lower pH than the arterial blood because of presence of more Carbon dioxide.
Temperature	• The temperature of the blood is 38°C(100.4°F), about 1°Chogher than oral or rectal body temperature.
Viscosity	• 'Viscosity' means thickness or resistance to flow. Blood is about 3-5 times denser & more viscous(thicker) than water & feels slightly sticky. Viscosity is increased by the presences of blood cells & plasma proteins. This thickness contributes to normal blood pressure.
Colour	• The colour of blood varies with its oxygen content. Arterial blood is bright red due to it's high level of oxygen. Venous blood has given up much of it's oxygen in tissues & thus has a darker, dull red colour.
Amount	• Blood constitutes about 20% of extracellular fluid, amounting to 8% of total body mass. The blood volume is 5L to 6L(1.5gal) in average sized adult male & 4L to 5L(1.2gal) in an average sized adult female.

FUNCTIONS OF BLOOD

TRANSPORTATION

- Respiration
- Nutrient carrier from GIT
- Transportation of hormones from endocrine glands
- Transports metabolic wastes

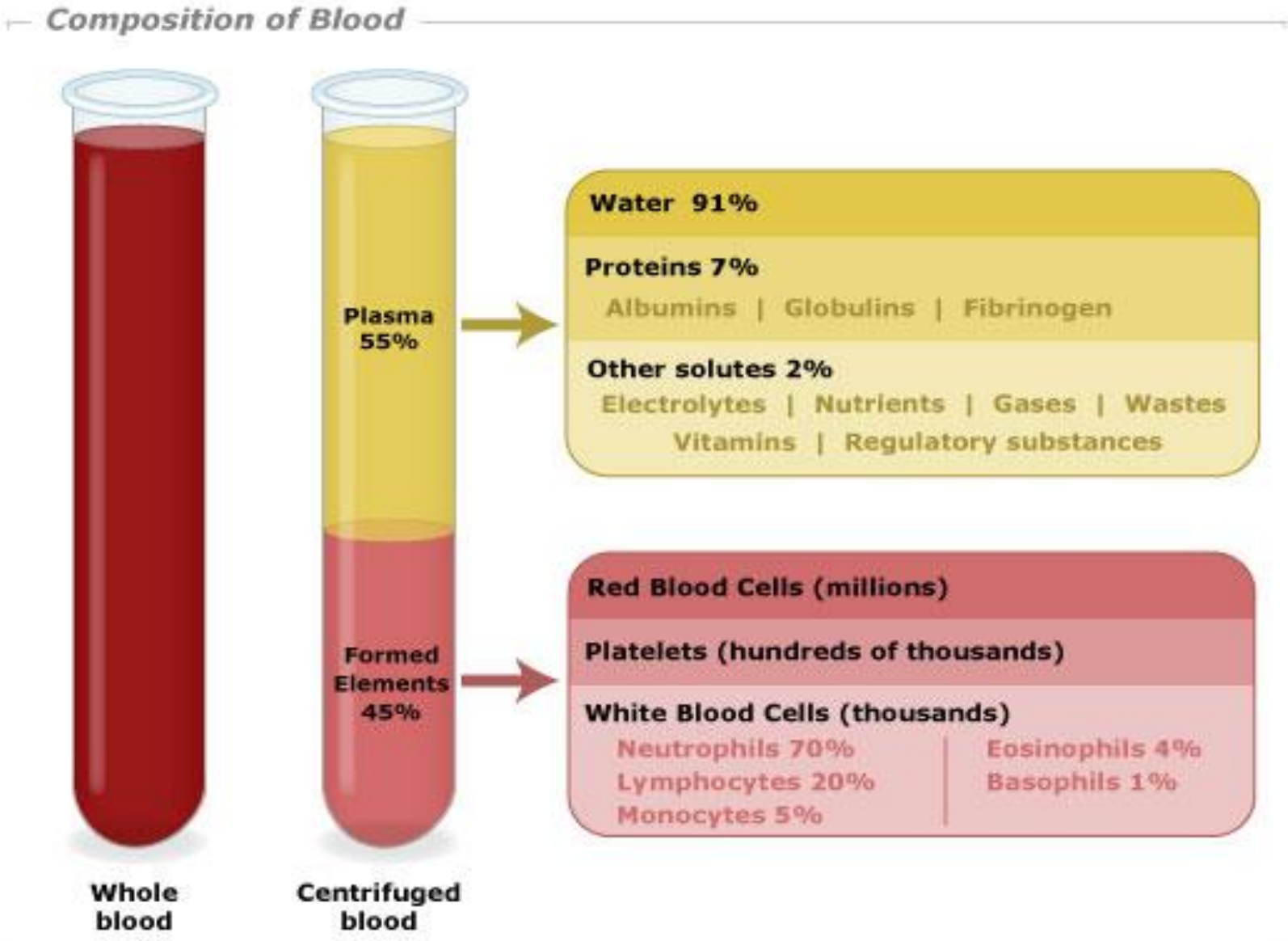
REGULATION

- Regulates pH
- Adjusts and maintains body temperature
- Maintains water content of cells

PROTECTION

- WBC protects against disease by phagocytosis
- Reservoir for substances like water, electrolyte etc.
- Performs haemostasis

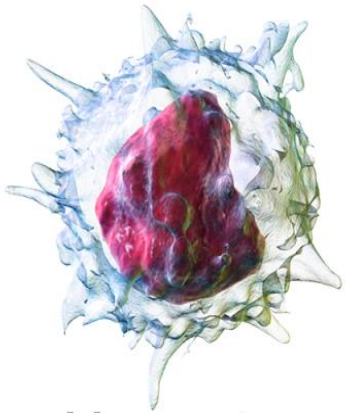
COMPOSITION OF WHOLE BLOOD



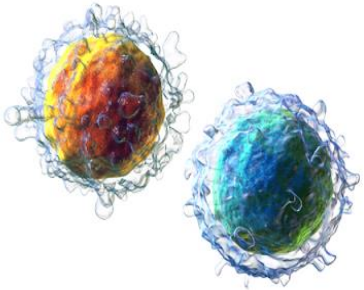
Formed Elements of Blood

Red Blood Cells

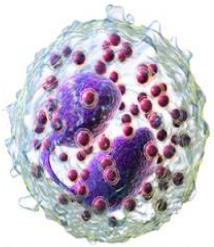
Platelets



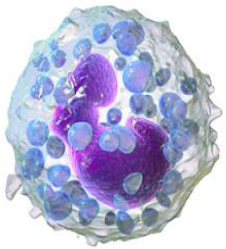
Monocyte



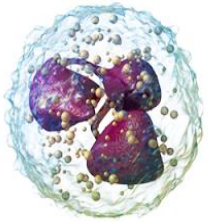
Lymphocytes



Eosinophil



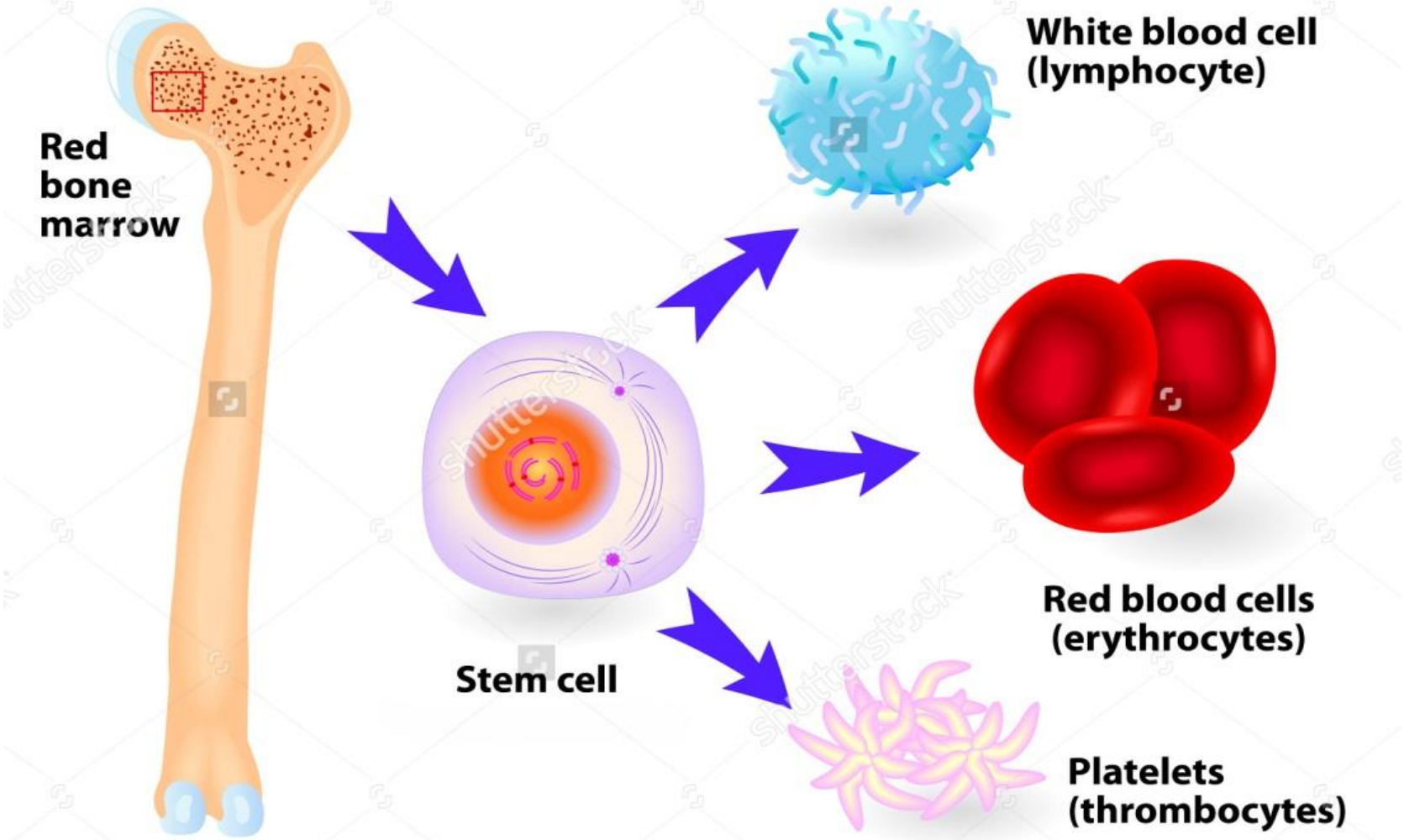
Basophil



Neutrophil

White Blood Cells

Haematopoiesis & Stem cell



⇨ It tests for disorders and **abnormalities of the blood** and gives an indication of disease present in other organs.

⇨ The components of CBC test are:

- **RED BLOOD CELLS**
- **WHITE BLOOD CELLS**
- **PLATELETS**

COMPLETE BLOOD COUNT

COMPONENT :

- RBC
- HB
- PCV OR HCT
- MCV
- MCH
- MCHC

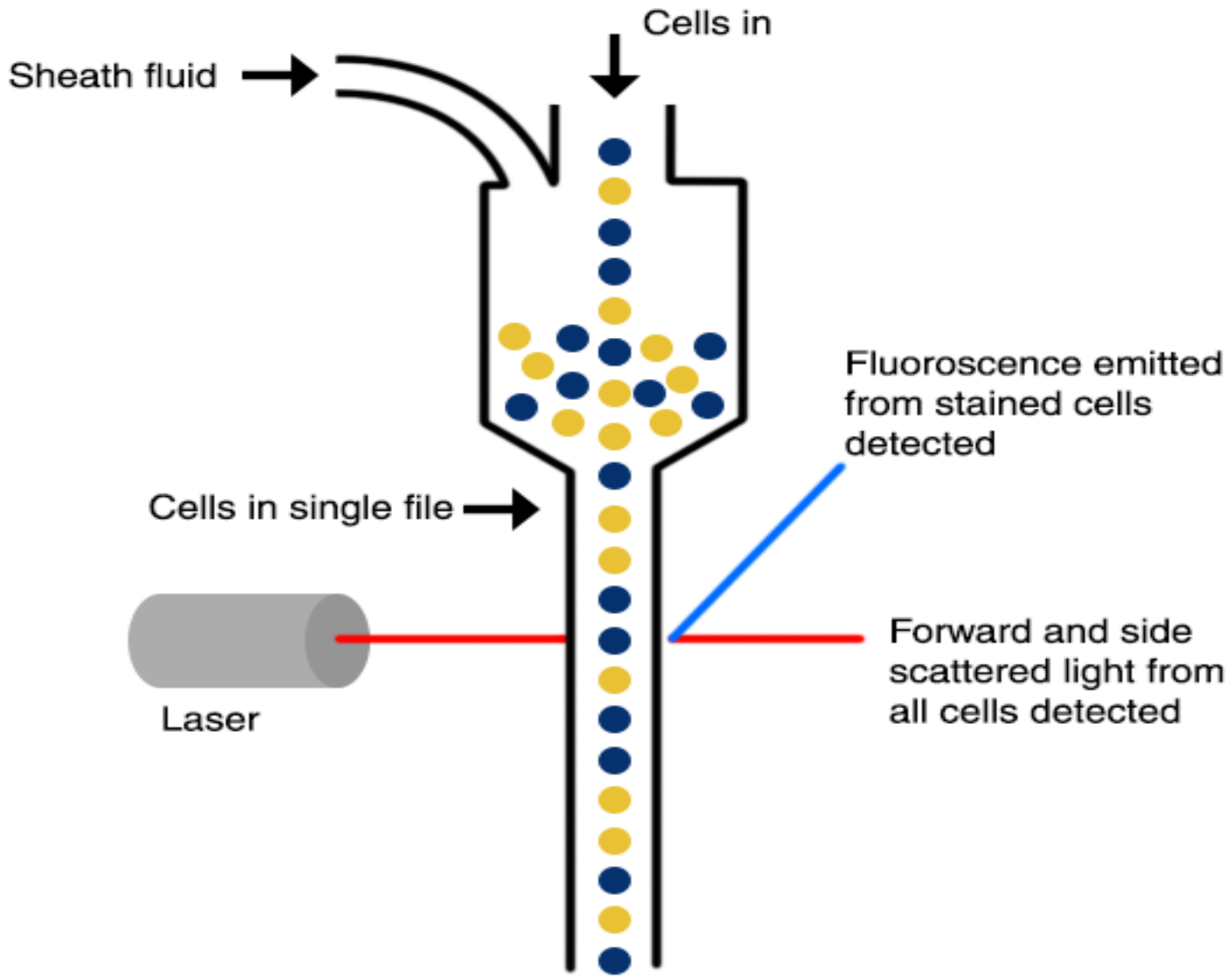
platelets

- WBC or TLC
- Neutrophiles
- Lymphocytes
- Monocytes
- Easinophils
- Basophiles

FLOW CYTOMETER

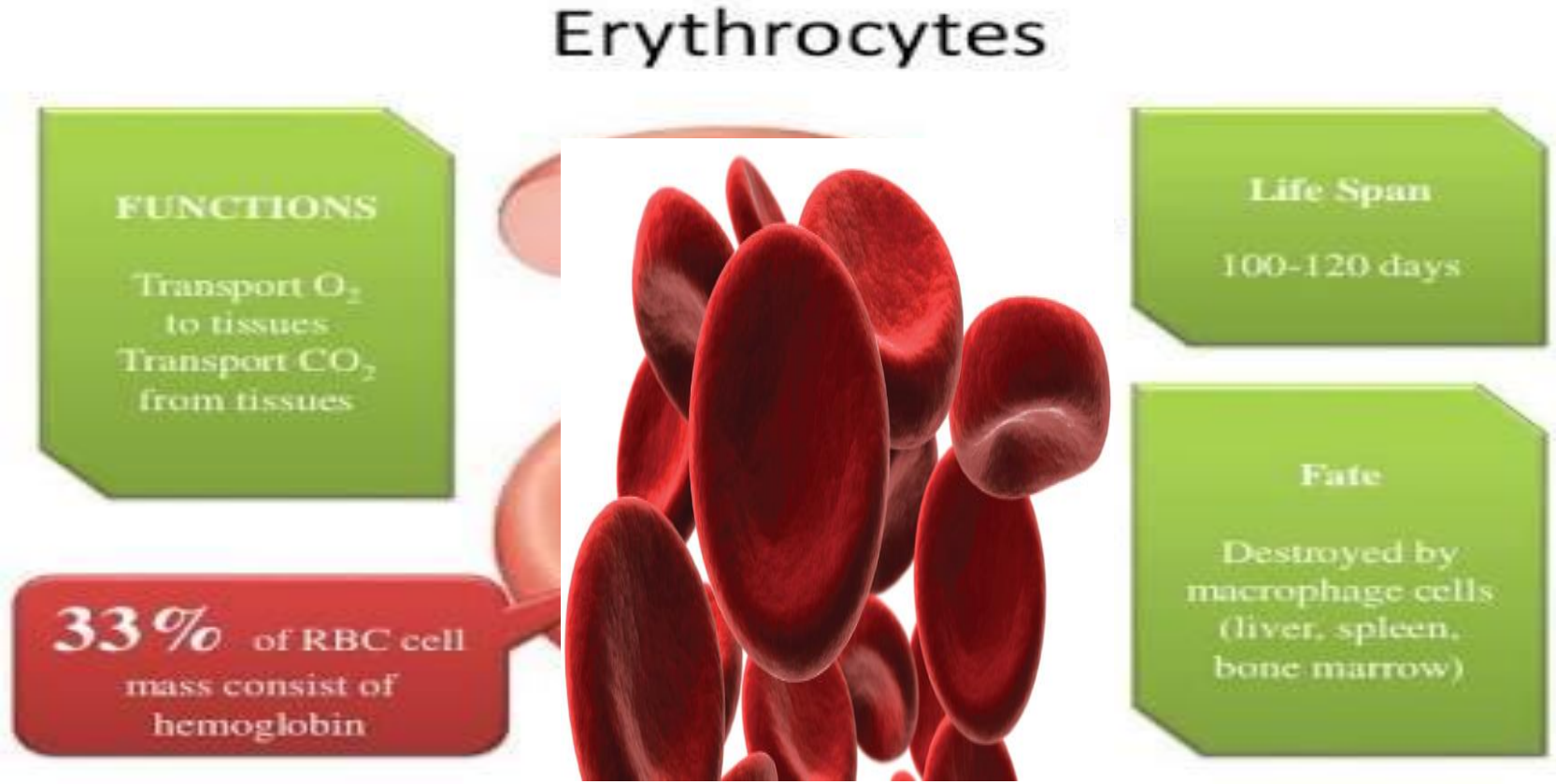
CELL COUNTER

Principle of Cell counter

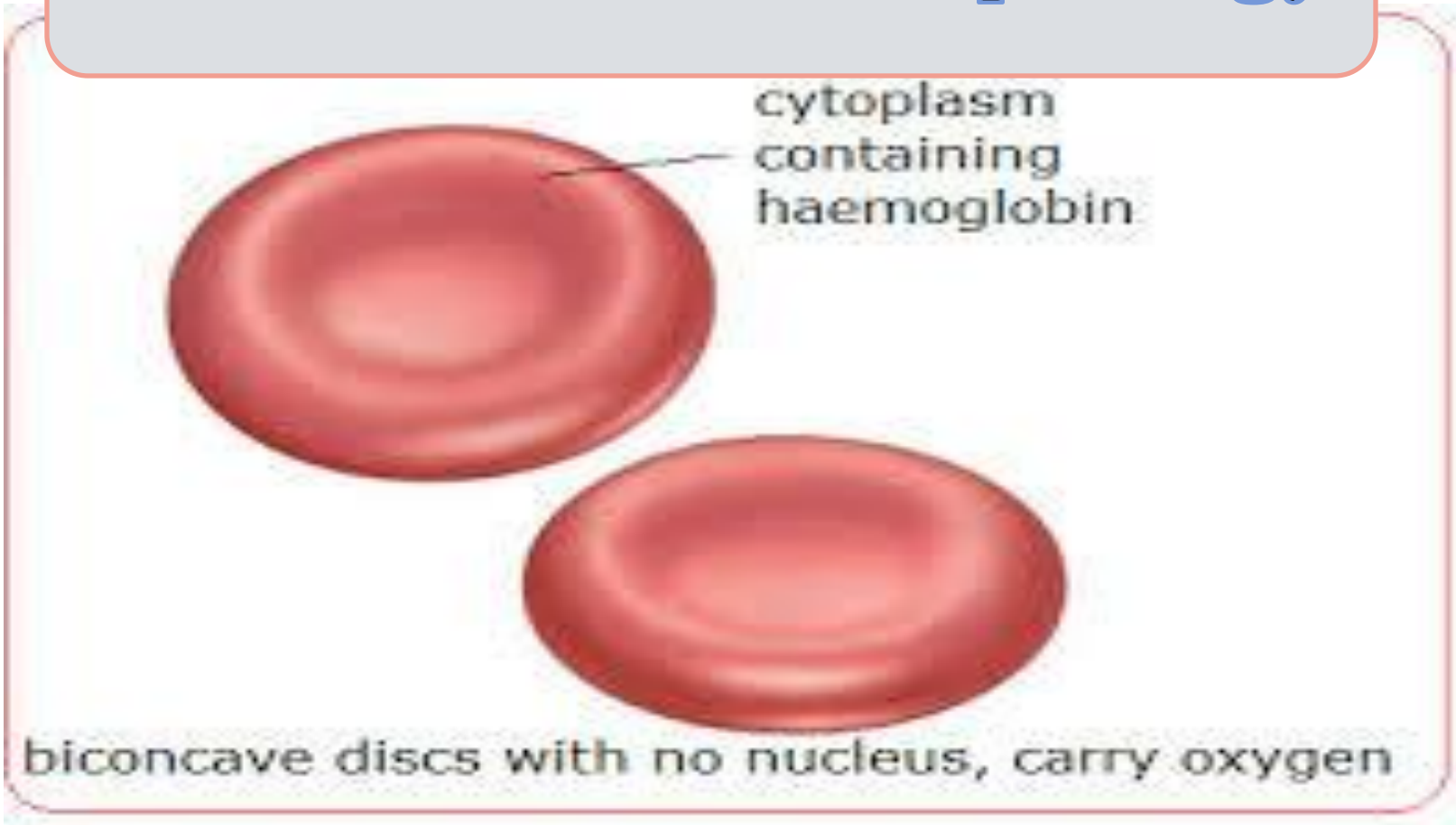


Red blood cells..

- Normal Values
- Males 4.7 to 6.0 million cells per microliter
- Females 4.2 to 5.4 million cells per microliter



Red blood cells morphology



Red Blood Cells count . (RBC)

By using Diluting Fluid (Hayem's fluid)

This should be isotonic so that RBCs are not hemolyzed.

Normal saline can be used but it may cause crenation of the RBCs and allow rouleaux formation.

.

Tools and Method :

1-Blood sample

- **Sample Required?**

  A blood sample drawn from a vein in your arm or a fingerstick or heelstick (newborns)

- **Test Preparation Needed?**

  None.

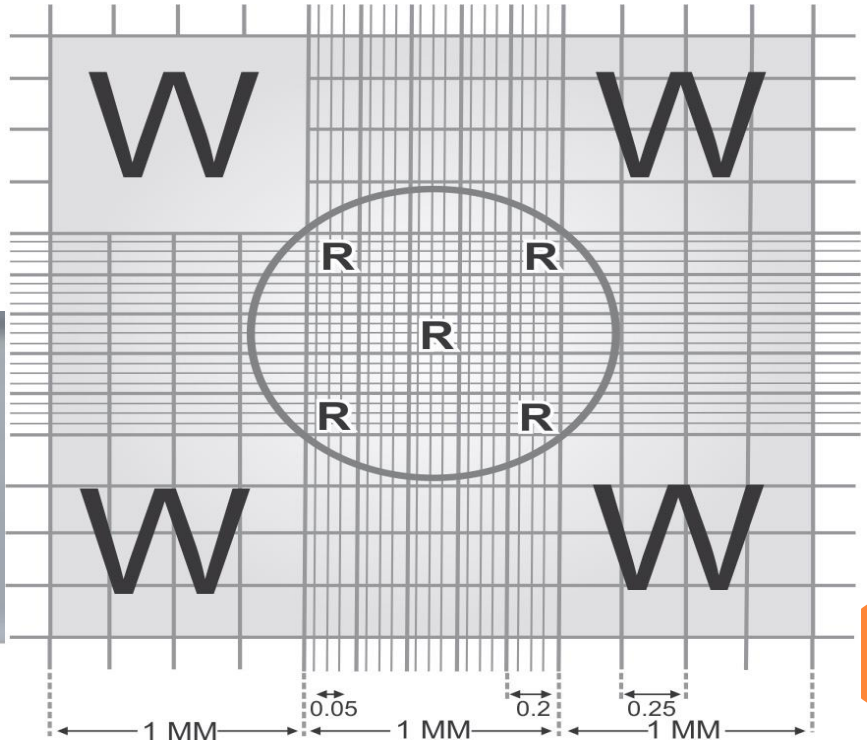
3- HEMOCYTOMETER

Hemo: blood
Cyto: cell
Meter: measurement/counter

Thus, it is an instrument used to count the blood cells.

It includes:

a) **Neubauer's slide**
b) **Cover slip**



4-Microscope

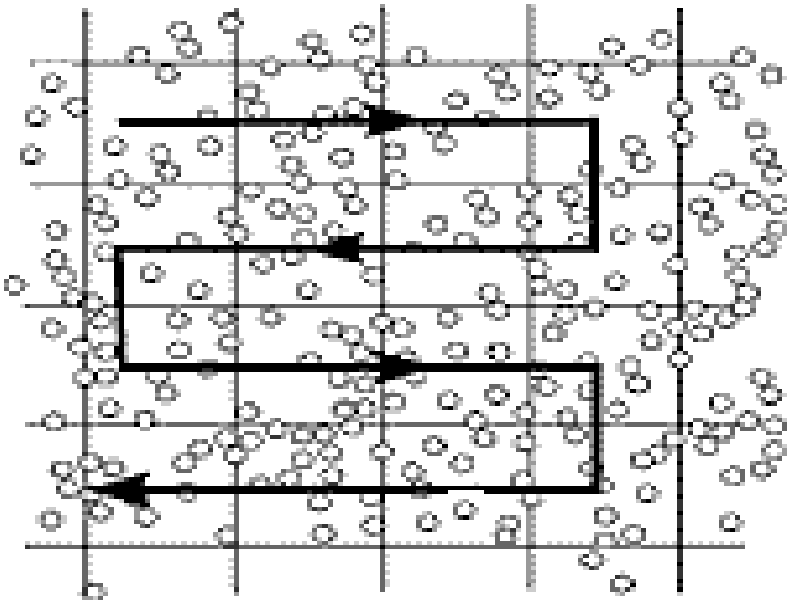
Count the RBCs using 40X objective in the 80 smallest squares as indicated in the diagram of the chamber

Counting rules :-

Do not count cells touching

- **Bottom line**
- **Right line**

This is to avoid double counting.



Calculation :-

Cell count = N x dilution factor x depth factor / area counted

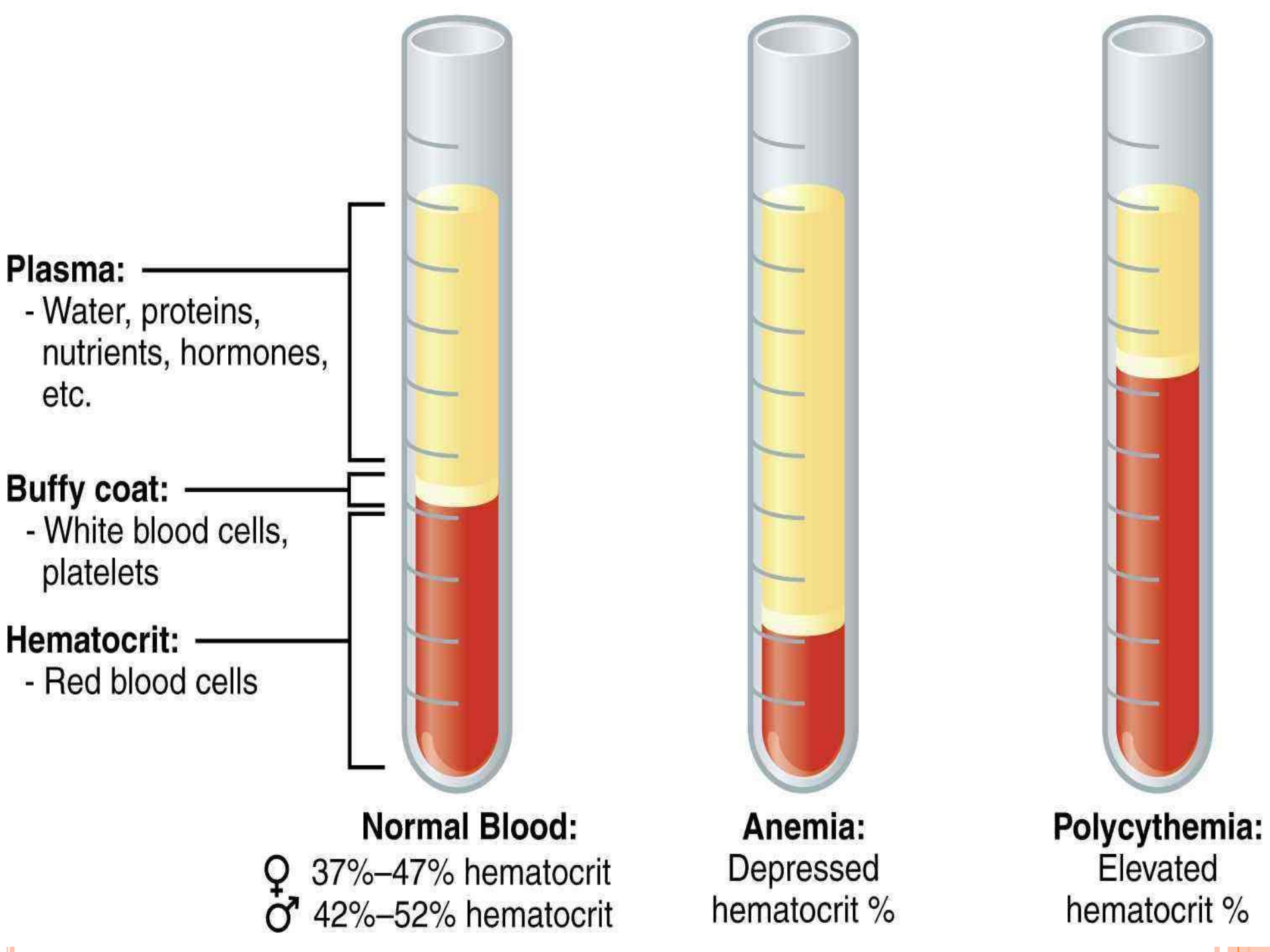
Cell count = N x 10.000

Low RBC Count

- Known as **anemia**
- Acute or chronic bleeding
- RBC destruction (e.g., hemolytic anemia, etc.)
- Nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency)
- Bone marrow disorders or damage
- Chronic inflammatory disease
- Kidney failure

High RBC Count

- Known as polycythemia
- Dehydration
- Pulmonary disease
- Kidney or other tumor that produces excess erythropoietin
- Smoking


Plasma:
- Water, proteins, nutrients, hormones, etc.
Buffy coat:
- White blood cells, platelets
Hematocrit:
- Red blood cells
Normal Blood:
♀ 37%–47% hematocrit
♂ 42%–52% hematocrit
Anemia:
Depressed hematocrit %
Polycythemia:
Elevated hematocrit %

HEMOGLOBIN

- Hemoglobin (Hb) is the main constituent of the RBCs and carries out the important function of transportation oxygen from lungs to various parts of the body it transports back carbon dioxide from the body to the lungs.

- The red cell mass of an adult contains approximately 600 g of hemoglobin , capable of carrying 800 mL of oxygen

  13.0-17.0 g/dl in males

  11.5-15.5 g/dl in females

Hemoglobin estimation methods

1- Sahli method or (acid hematin)

2- Cyanomethemoglobin method

Procedures

2- Cyanomethemoglobin method

- Accurate.
- Commonly used.
- Recommended by ICSH (international committee for standardization in haematology).

a) **Principle of cyanmethaemoglobin method:**

Blood + diluent (Drabkin's solution)

" potassium ferricyanide + potassium cynaide"

Converts: Haemoglobin (**Hb**) and Methaemoglobin (**Hi**)

↓

*Cyanmethaemoglobin (**HiCN**)*

b) Reagent and equipment for Cyanmethaemoglobin method:

- Diluent (Drabkin's solution)
- 5 ml pipette.
- Cuvettes.
- Test tube.
- 20 micro liter pipettes.

c) **Procedure of Cyanmethaemoglobin method:**

20ul blood + 4ml diluent **mix, 5-10min** ⟶ HiCN.

Measured by spectrophotometer at 540nm ↗ sample

↘ standard

Use the calculator:

$$\text{Hb (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of standard}$$

Measure the absorbance of the solution by using a calorimeter at a wavelength = 540nm. Then compare it with the standard solution of HiCN.

Spectrophotometer

HEMATOCRIT (HCT)
PACKED CELL VOLUME (PCV)

HEMATOCRIT IS THE VOLUME OF RED CELLS EXPRESSED AS A PERCENTAGE OF THE VOLUME OF WHOLE BLOOD IN THE SAMPLE .

METHODS

1. USING WINTROBE'S TUBE.

2. USING MICROHEMATOCRIT CAPILLARIES

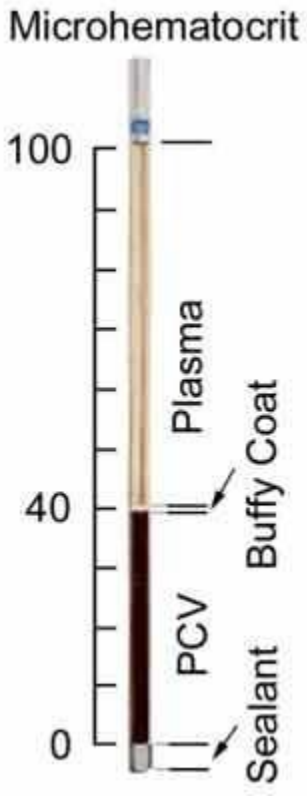
- **Males normal 45%**
- **Females normal 40%**

Microhematocrit

Capillary tubes coated with anticoagulant can be filled with blood obtained from finger puncture or from a venipuncture or with blood already anticoagulated.
One end of the filled capillary tube is sealed with sealing wax (Plasticine) or the empty end is sealed with heat.
The sealed tube is centrifuged for 3 minutes in a special high-speed centrifuge

Capillary tubes

Centrifuge



RBC Index

- **MCV**
  - Mean corpuscular volume
- **MCH**
  - Mean corpuscular hemoglobin
- **MCHC**
  - Mean corpuscular concertration

1 Mean Curpuscular Volume (MCV)

$$\text{MCV (femtoliter)} = \frac{\text{Hematocrit (\%)} \times 10}{\text{RBC count (millions/mm}^3\text{ blood)}}$$

- Adults 76–96 fl
- MCV is reduced in microcytic anemia
- MCV is raised in macrocytic anemia

2 Mean Corpuscular Hemoglobin (MCH)

- The MCH indicates the average weight of hemoglobin in the red blood cells.

- MCH = $\frac{\text{Weight of hemoglobin in 1 µl of blood}}{\text{Number of red blood cells in 1 µl of blood}}$

$$\frac{\text{Hemoglobin} * 10}{\text{Red blood cell count in millions}} \quad \text{(pg)}$$

- Normal value for the MCH : 27~31 pg

$1\ g = 10^{12}\ pg$

$1\ ml = 10^{3}\ \mu l$

- If MCH is lower than 27 pg the condition is called **Hypochromic**
- If MCH is higher than 31 pg the condition is called **Hyperchromic**
- If MCH is within the rang of 27~31 pg condition is called **Normochromic**

3 Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC is an expression of the average concentration of hemoglobin in the red blood cells. It gives the ratio of the weight of hemoglobin to the volume of the red blood cells.

- $$\text{MCHC} = \frac{\text{Hemoglobin in g/dl}}{\text{Hematocrit /dl}} * 100$$ (to convert to %)

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{Hct (\%)}} = \frac{15 \times 10}{45} * 100 = \frac{150}{45}$$

Normal value for the MCHC : 32~36 %

An MCHC below 32% indicates hypochromia, an MCHC above 36% indicates hyperchromia, and red blood cells with a normal MCHC are termed normochromic.

- **Red cell distribution width (RDW)** – is a measurement of the variation in RBC cell size
  - Standard deviation/mean MCV x 100
  - The range for normal values is 11.5-14.5 %
  - A value > 14.5 means that there is increased variation in cell size above the normal amount (**anisocytosis**)
  - A value < 11.5 means that the RBC population is more uniform in size than normal.

White cell count (WBC)

- White cell count (WBC) is the total number of leukocytes in a volume of blood, expressed as thousands/µl.

- WBC can be done by manual methods or by automated cell counters.

- **Normal Values:**
  - Newborn 9.0 - 30.0 x 10^3/µl
  - 2 years 6.0 - 17.0 x 103/µl
  - adult 4.0 – 11.0 x 103/µl

Requirement & Method

- **1 - Sample Required?**

A blood sample drawn from a vein in your arm or by a fingerstick (children and adults) or heelstick (infants)

- **Test Preparation Needed?**

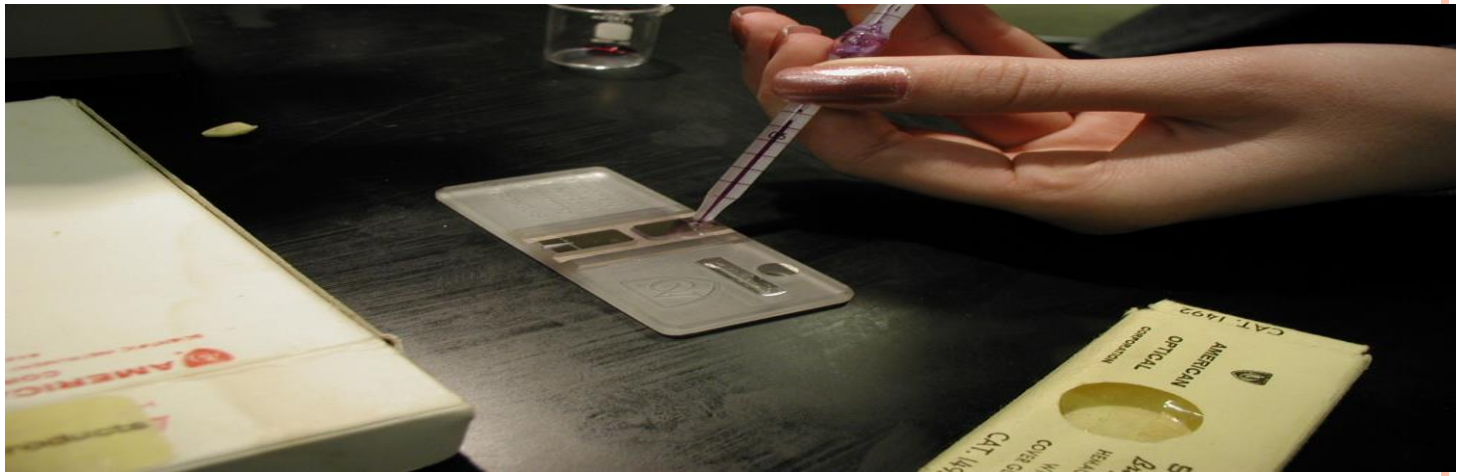
None

EDTA blood

The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes.
The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid.

Mix the contents in the pipette and after five minutes by discarding few drops

fill the counting chamber ON Hemocytometer and allow the cells to settle for two to three minutes.



3- HEMOCYTOMETER

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Cyto: cell
Meter: measurement/counter

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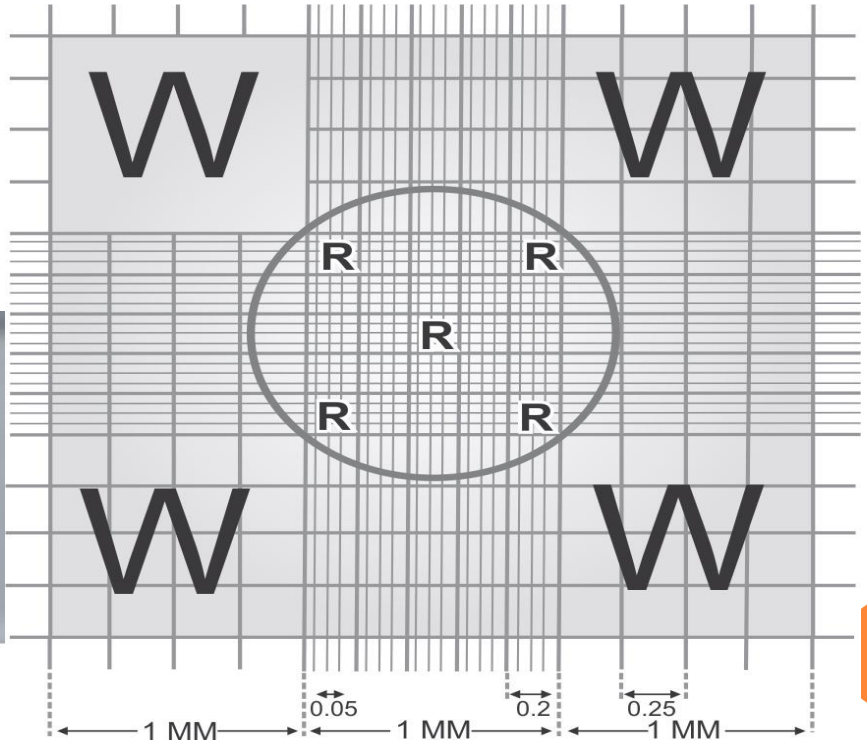


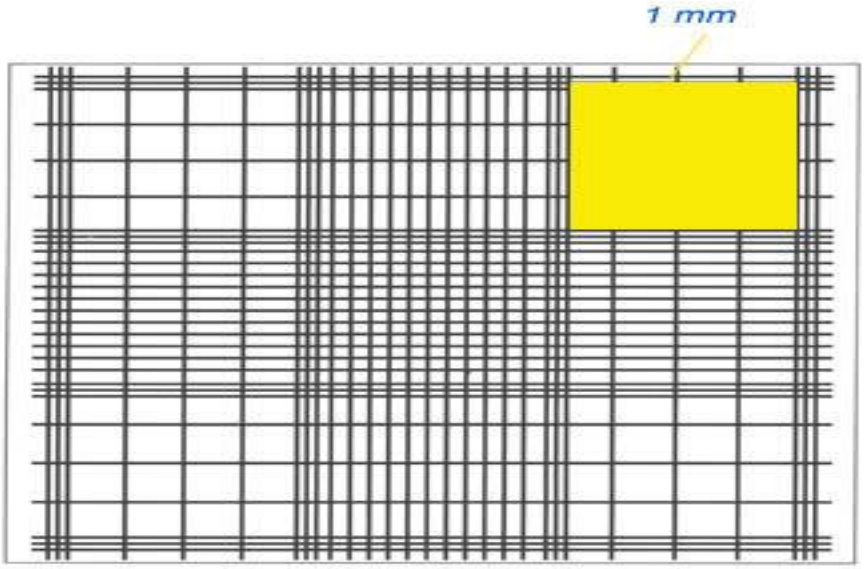
4-Microscope

Count the WBCs using 40X objective in the 64 smallest squares as indicated in the diagram of the chamber

FOCUSING

4X to see the general formation of slide.

10X for WBC counting



Counting Rule

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  - Bottom line
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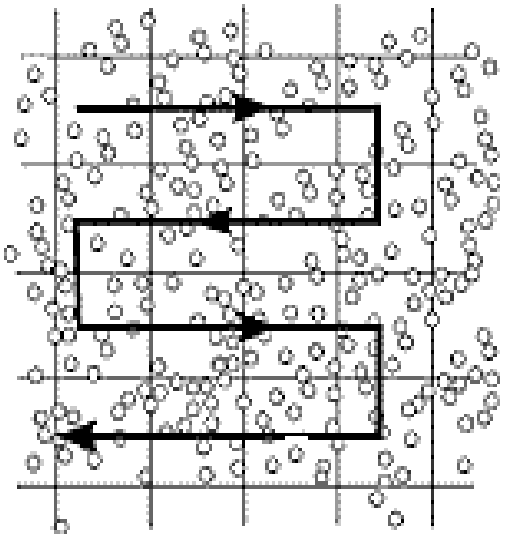
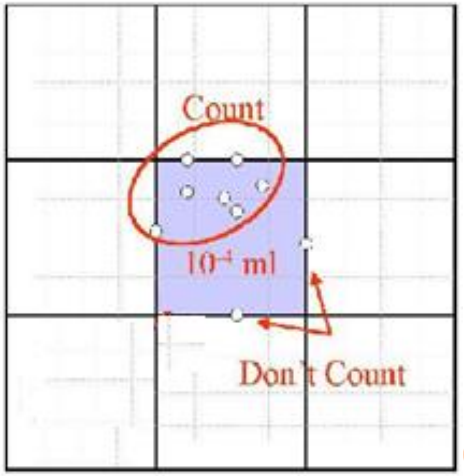
  - This is to avoid double counting.





Calculation :-

No. *of* WBCs in undiluted blood = X / 64 x 160 x 20/mm³

= X x 50 / mm³

- ***Total WBC Count : 4000 upto 11000 / cu.mm.***

WBC differential count

1. Neutrophils: 50-74 (62%, 3000-7000/mm^3
2. Lymphocytes: 20-40% (30%), 1500-3500/mm^3
3. Monocytes: 2-8% (5.3%), 100-700/mm^3
4. Eosinophils: 1-4% (2.3%), 100-440/mm^3
5. Basophils: 0.3-0.5% (0.4%), 20-50/mm^3

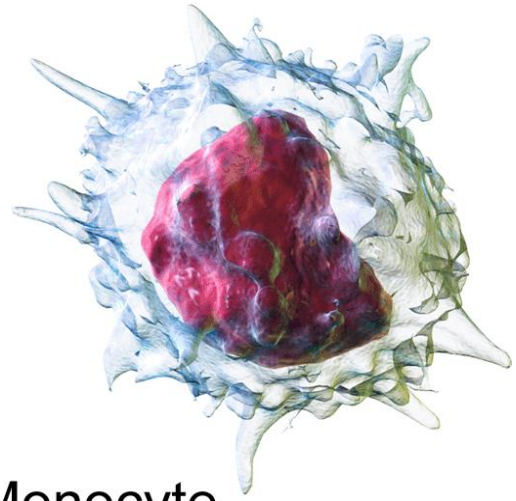
Relative Quantities- mnemonics

– "Never Let Monkeys Eat Bananas!"(descending order in abundance)

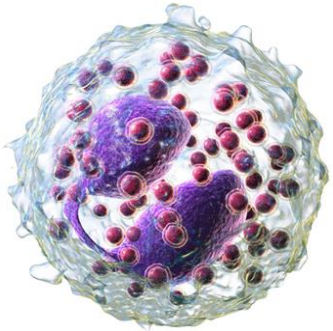
Types of White Blood cells

AGRANULOCYTES

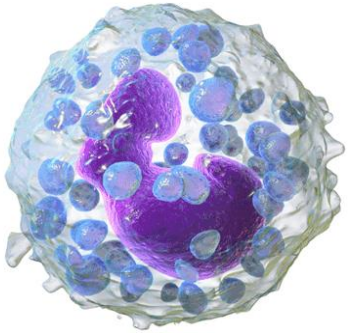
GRANULOCYTES



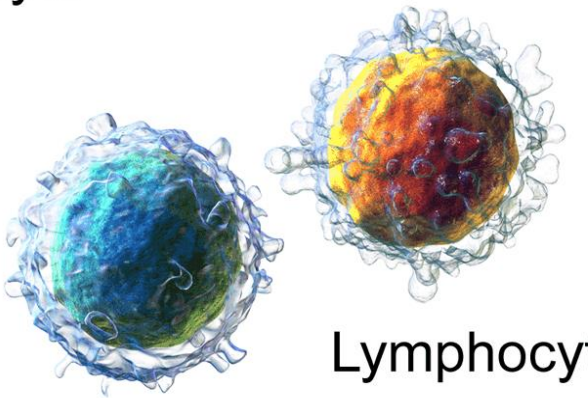
Monocyte



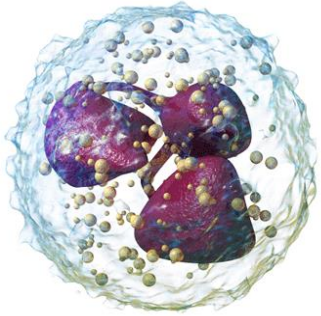
Eosinophil



Basophil



Lymphocytes

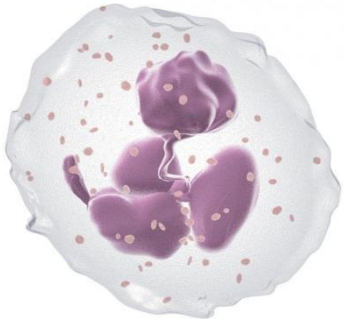


Neutrophil

MORPHOLOGY (GRANULOCYTES)

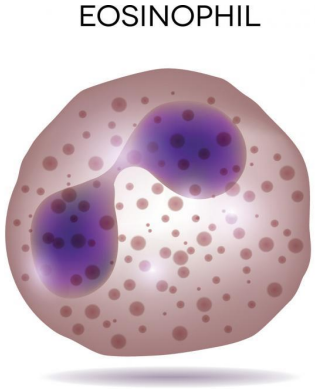
Neutrophils

have fine or small granules in the cytoplasm
granules appear violet in colour.
The nucleus is multilobed . nucleus has 4-5 lobes
diameter – 10-12 microns



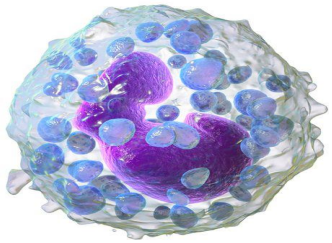
Eosinophils

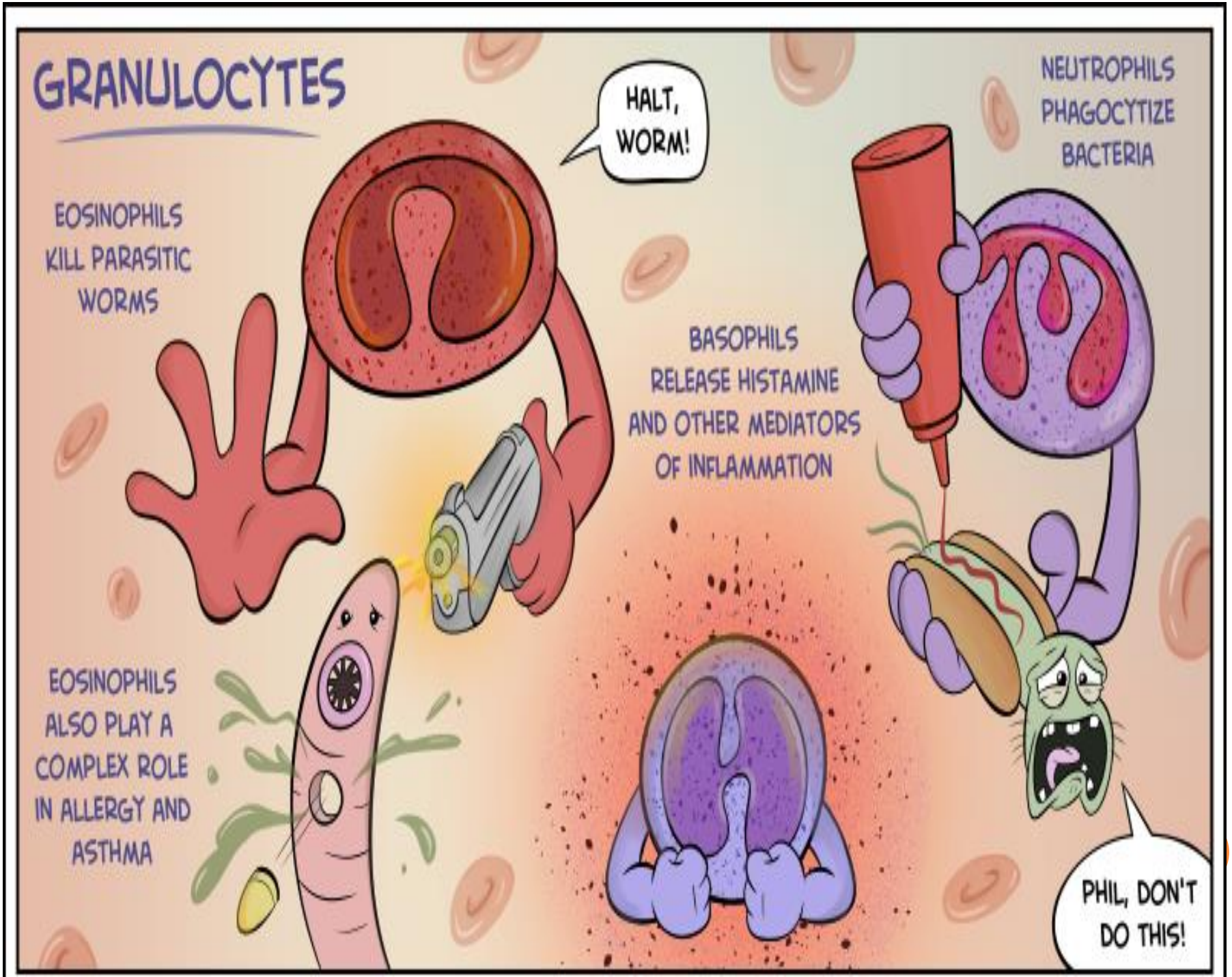
have larger granules in the cytoplasm
stain bright red colour.the nucleus is bilobed.
diameter-10 and 14 microns



Basophils

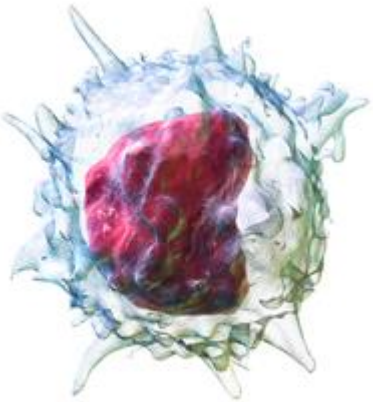
have coarse granules in the cytoplasm.
the granules stain purple blue
with basic dyes like methylene blue.
diameter-8 to 10 microns




GRANULOCYTES
EOSINOPHILS KILL PARASITIC WORMS
HALT, WORM!
NEUTROPHILS PHAGOCYTIZE BACTERIA
BASOPHILS RELEASE HISTAMINE AND OTHER MEDIATORS OF INFLAMMATION
EOSINOPHILS ALSO PLAY A COMPLEX ROLE IN ALLERGY AND ASTHMA
PHIL, DON'T DO THIS!

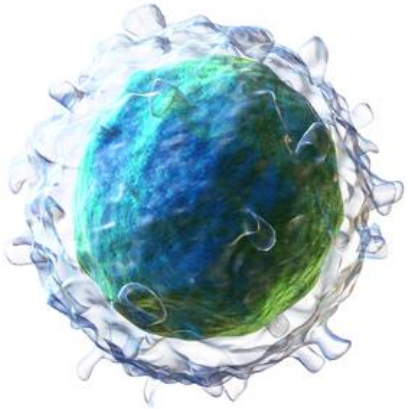
Agranulocytes

Monocytes



are largest leucocytes
with diameter of 14 to 18 microns.
the cytoplasm is clear without granules.
the nucleus is kidney,round, oval,horseshoe shaped.
- Play an important role in phagocytosis

Lymphocytes



have clear cytoplasm without granules.
the nucleus is ovel shaped
occupying the whole of the cytoplasm.
- Play an important role in Immunity

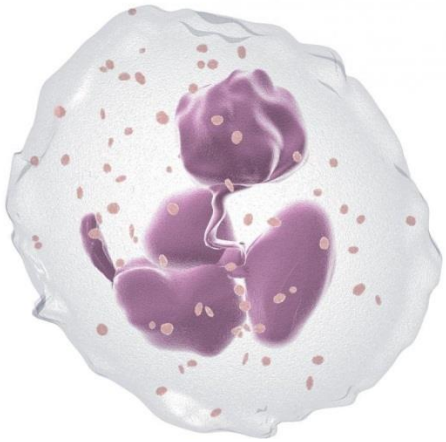
CONDITION IN WHICH ALTERATION IN DLC

Neutrophilia-

increased in neutrophil count called neutrophilic leukocytosis.

this occurs in the following condition:

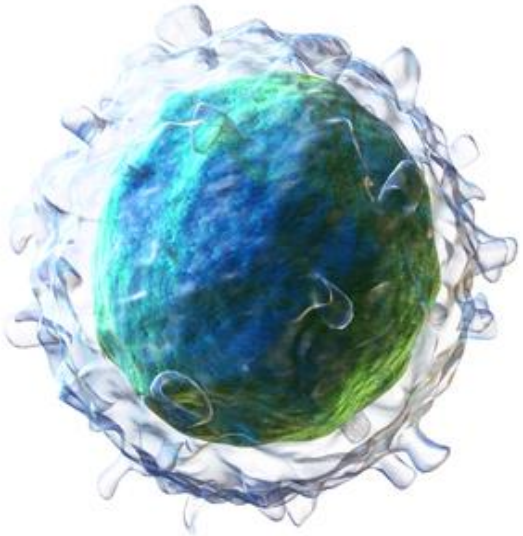
1) **Acute infections**
2) **Metabolic disorders**
3) **Injection of vaccines**
4) **After acute hemorrhage**



Lymphocytosis

- increasd in lymphocyte count is called lymphocytosis and this occurs in

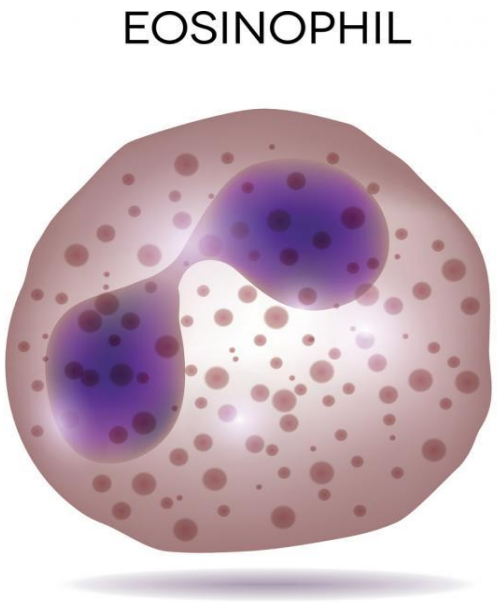
1) Diptheria
2) Infectious hepatitis
3) Malnutrition
4) Rickets



Eosinophilia

-increasd in eosinophil count is called eosinophilia and this occurs in

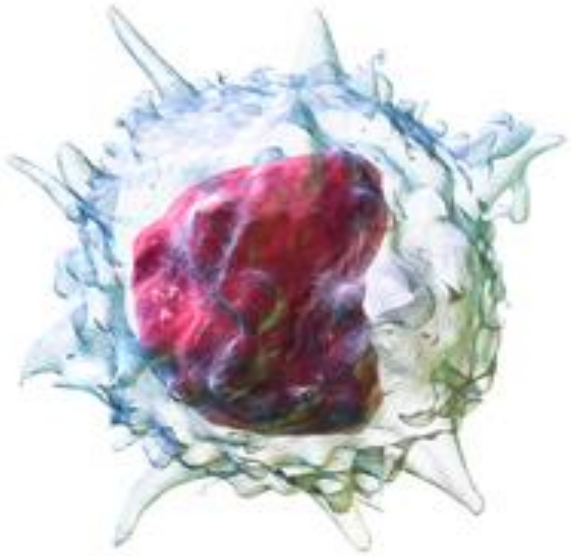
1) Allergic condition
2) Asthma
3) Blood parasitism
4) Scarlet fever



Monocytosis

-increasd in monocytes count is known as monocytosis and occurs in

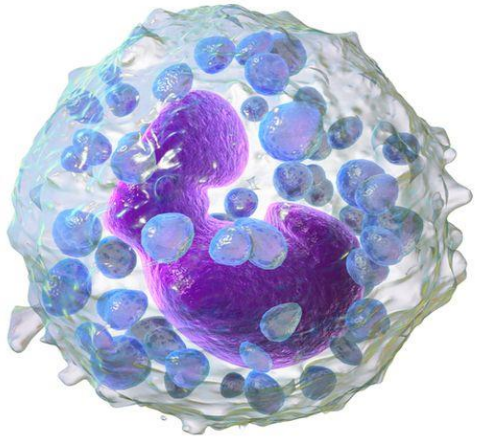
1) Tuberculosis
2) Syphilis
3) Malaria



Basophilia

-increased in basophill count is called basophilia and it occur in

1) Small pox
2) Chicken pox
3) Polycythemia vera

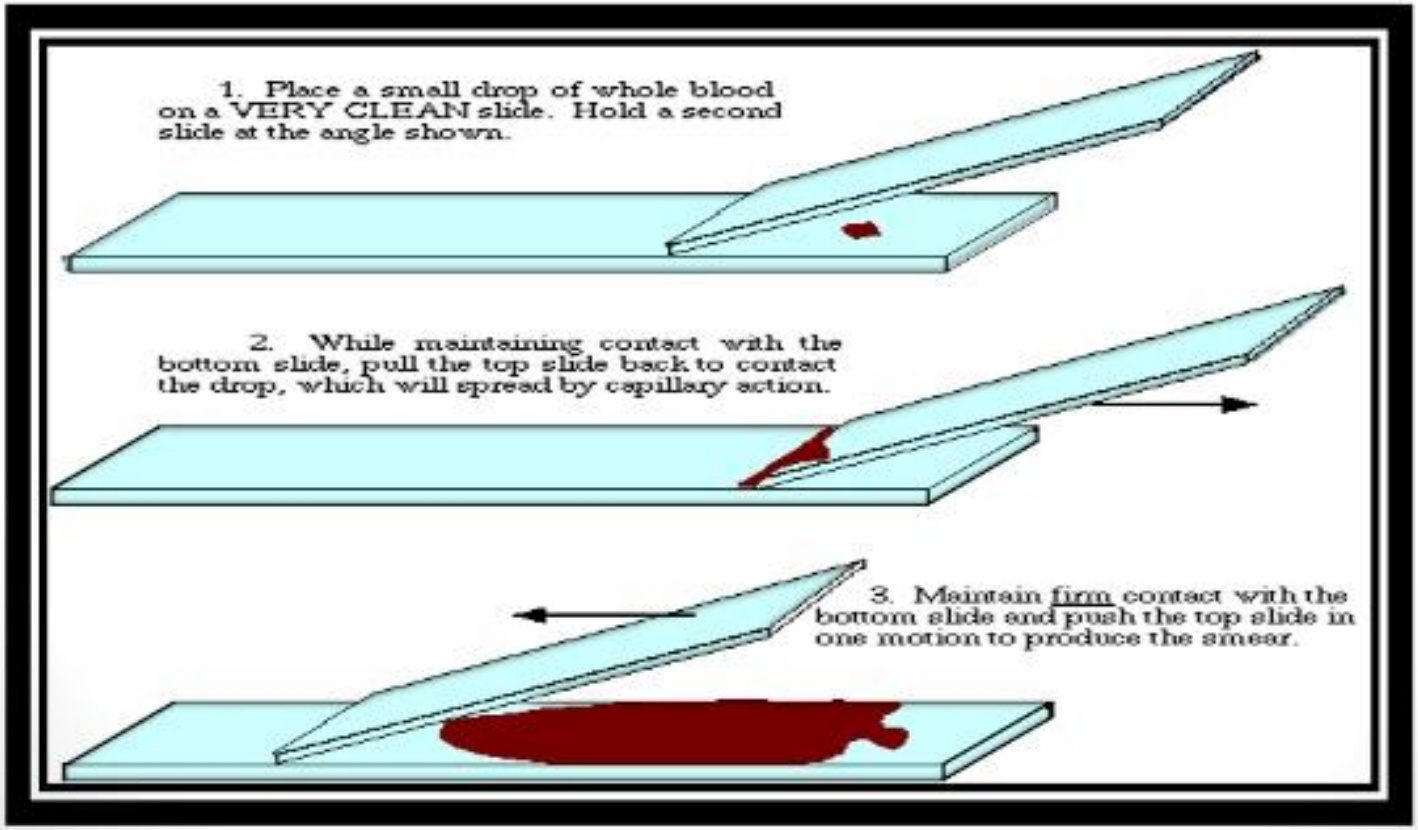


Basophil

Procedure

1

Preparation of blood smear



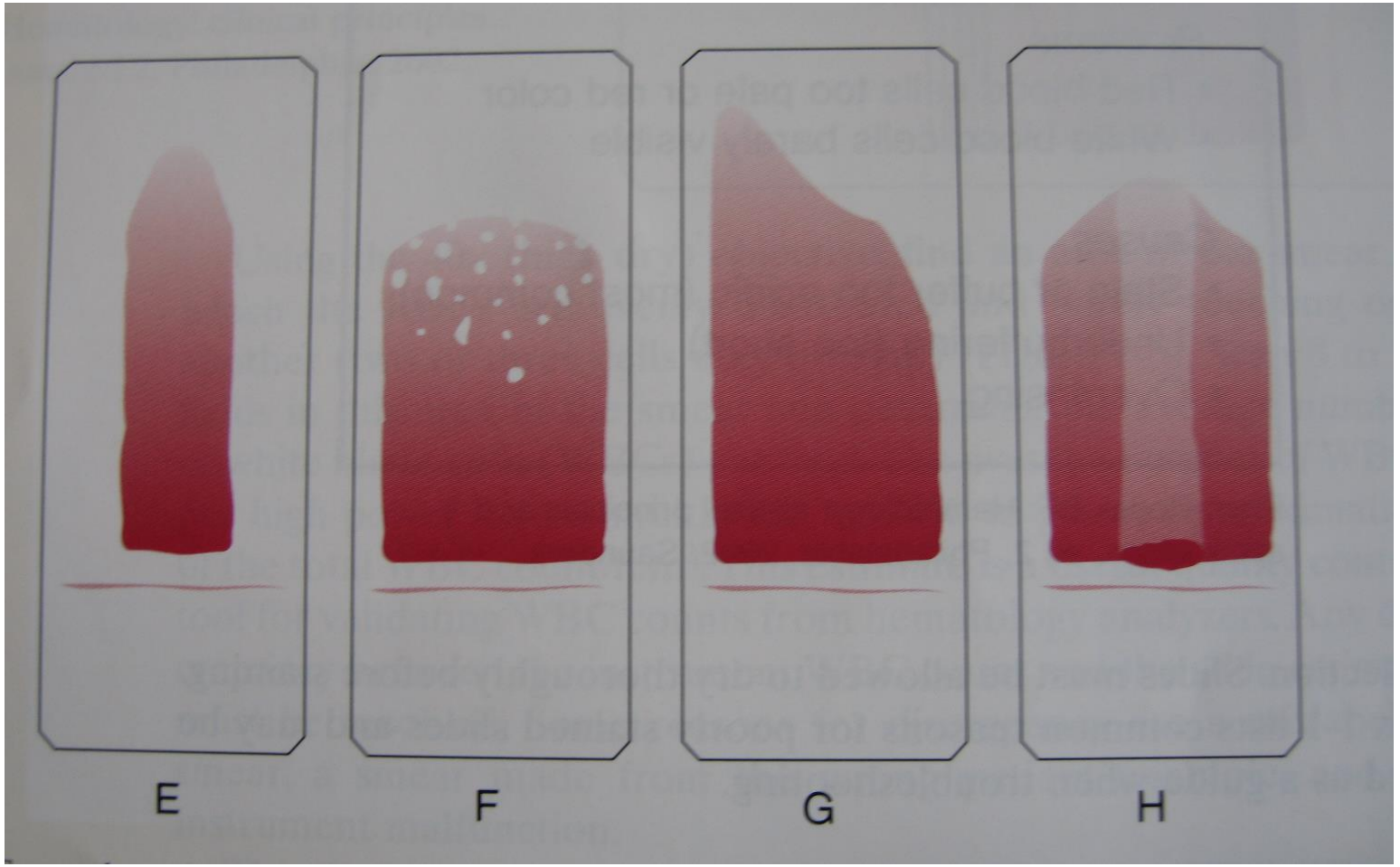
PERIPHERAL BLOOD SMEAR

- Characteristics:
  - It is smooth without irregularities, holes, or streaks
  - When the slide is held up to light, the featheredge of the smear should have a “rainbow” appearance
  - The whole drop is picked up and spread

Well-made PBS

PERIPHERAL BLOOD SMEAR

Examples of unacceptable smears



Method of staining :

1-The blood film is dried for 2 minutes .

2-Pour Giemsa stain over the smear for 8-10 minutes.

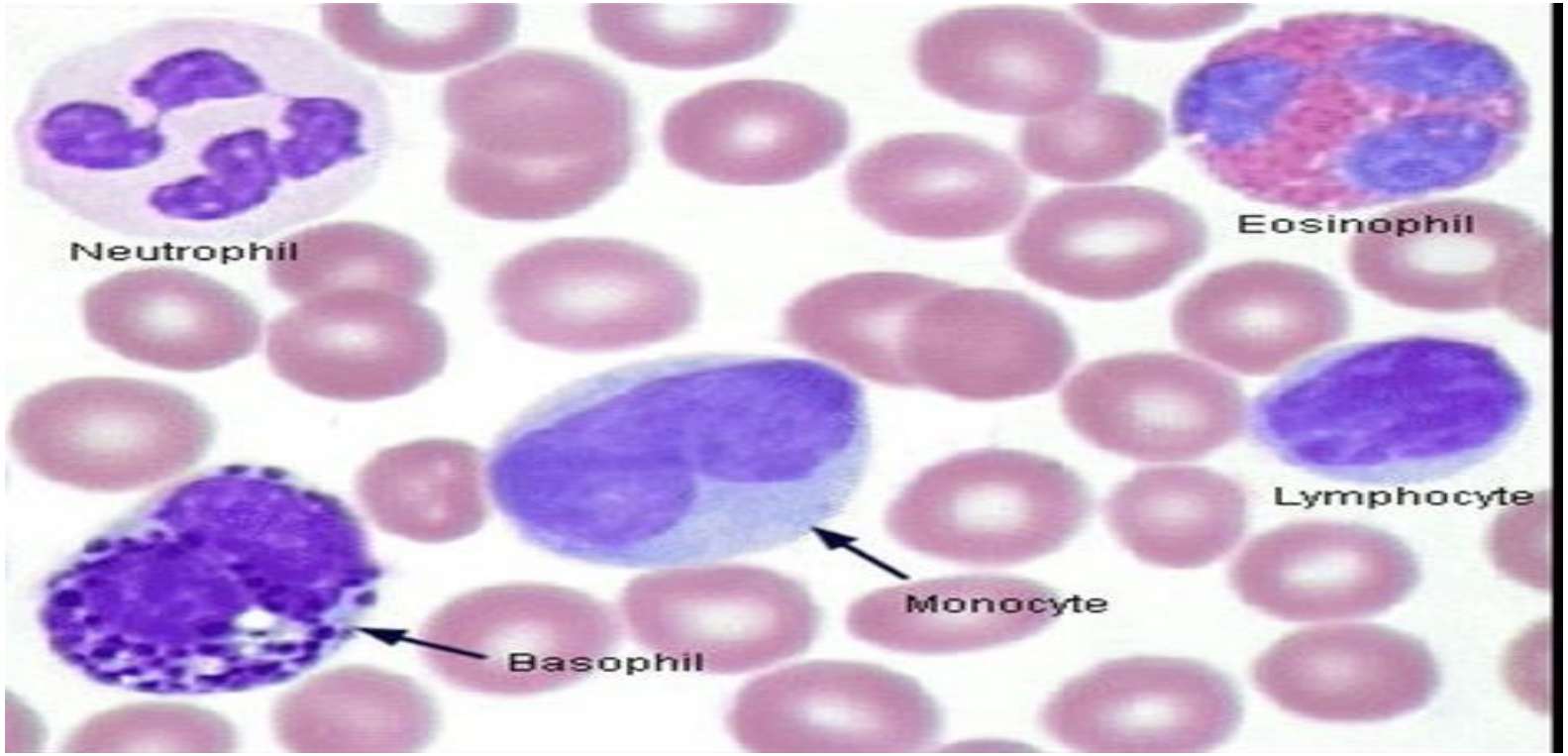
3-Wash off with dist. water and dry.

The dried and stained film will be examined.

A total of 100 cells should be counted in which every white cell seen must be recorded in a table

WBC ESTIMATION UNDER 40X

- Using the × 40 high dry with no oil.
- Choose a portion of the peripheral smear where there is only slight overlapping of the RBCs.



Cell Type	Count/ 100 WBCs
Neutrophil	
Lymphocyte	
Monocyte	
Eosinophil	
Basophil	

Platelet Count

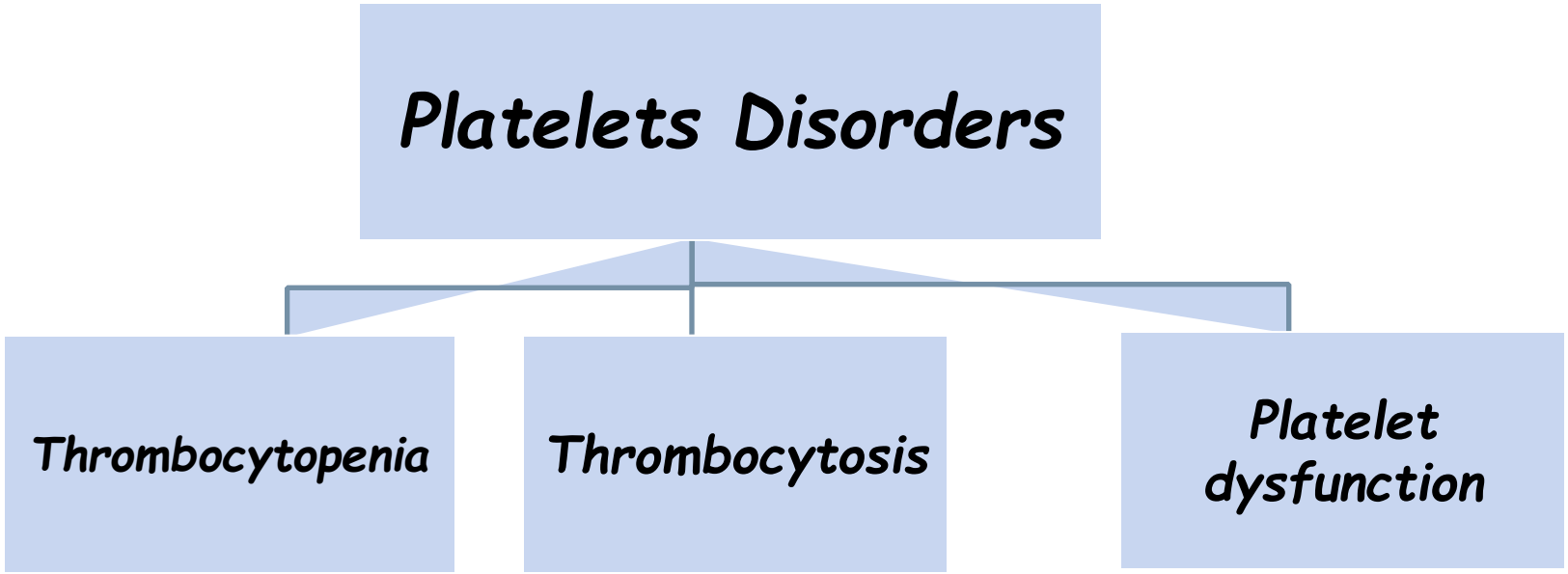
- Platelet Count can be determined by improved Neubauer's counting chamber with RBC pipette & 1% ammonium oxalate.
- They can be seen as tiny diameter, well seprated, highly refractile rounded bodies with silvery appearance.
- N : ***150.000 : 400.000 c / ml***
- Leishman stain : in clumps, blue cytoplasm, reddish purple granules, no nucleus.

Dilution factor / 1:20

No. platelets in undiluted blood

= no of platelets in 5 squares x 1000

Normal range ***150.000 : 400.000 c / ml***



THROMBOCYTOPENIA

- **Defined as reduced in the platelet count< 150, 000µL**
- **The risk of bleeding depends on the level of the platelet count:**

- **Mild thrombocytopenia (platelet <150 000 cells/µL)**
- **Moderate thrombocytopenia (platelet 20 000 - 50 000 cells/µL)**
- **Severe thrombocytopenia (platelet <20 000 cells/µL)**

THROMBOCYTOSIS

There is increased number of circulating platelets , the elevation is in the range of 500,000_1000,000/cubic mm, but may be higher.

PATIENT NAME			Registered	02-07-2020 16:39:38	Collected	02-07-2020 16:41:14
الأستاذ/احمد ممدوح محمد حشيش.			Authenticated	03-07-2020 15:58:55	Printed	03-07-2020 05:01:56
Visit Number	Age	Gender	Referred By		Client ID	
28720510409	32 Year	Male	Prof : -		539	

Test Name	Result	Unit	Reference Range
Complete Blood Picture			
Haemoglobin	14.1	g/dl	12.5 - 17.5
Haematocrit (PCV)	42.0	%	41 - 52
RBCs Count	5.06	Millions / cmm	4.5 - 5.9
MCV	83.0	fl	80 - 100
MCH	27.9	pg	27 - 33
MCHC	33.6	g/dl	31 - 37
RDW-CV	13.2	%	11.5 - 15
Platelet Count (EDTA Blood)	286	thousands / cmm	150 - 450
Total Leucocytic Count (EDTA Blood)	5.9	thousands / cmm	4 - 11
	Percent Values		Absolute Values		
Neutrophils	58.9 %		3.48	x10^9/L	2 - 7
Lymphocytes	29.5 %		1.74	x10^9/L	1 - 4.8
Monocytes	10.0 %		0.59	x10^9/L	0.2 - 1
Eosinophils	1.4 %		0.08	x10^9/L	0.1 - 0.45
Basophils	0.2 %		0.01	x10^9/L	0 - 0.1
Other Cells					

Comment: NORMAL BLOOD PICTURE.

Visit Number	Age	Gender	Referred By	Client ID
28720508320	21 Year	Female	Prof : -	3416

Test Name	Result	Unit	Reference Range
Complete Blood Picture			
Haemoglobin	12.3	g/dl	11.5 - 15.5
Haematocrit (PCV)	37.6	%	36 - 45
RBCs Count	4.83	Millions / cmm	4 - 5.2
MCV	↓ 77.8	fl	80 - 100
MCH	↓ 25.5	pg	27 - 33
MCHC	32.7	g/dl	31 - 37
RDW-CV	12.9	%	11.5 - 15
Platelet Count (EDTA Blood)	229	thousands / cmm	150 - 450
Total Leucocytic Count (EDTA Blood)	4.2	thousands / cmm	4 - 11

	Percent Values		Absolute Values		
Neutrophils	29.0	%	1.21	x10^9/L	2 - 7
Lymphocytes	57.1	%	2.38	x10^9/L	1 - 4.8
Monocytes	12.5	%	0.52	x10^9/L	0.2 - 1
Eosinophils	0.7	%	0.03	x10^9/L	0.1 - 0.45
Basophils	0.7	%	0.03	x10^9/L	0 - 0.1

Other Cells

Comment:

RBCs show hypochromia and microcytosis.
Relative lymphocytosis.
Relative monocytosis.
Absolute neutropenia.
Follow up is recommended.

Blood Groups.

Introduction to Blood Group Systems

- 35 blood group systems are recognized
- Most important ABO blood group system and Rh system
- M-N system also has little importance
- Classification is based on inherited antigenic substances

ABO Blood Group System

- The most important and well studied blood group system

- Discovered by Karl Land
- Steiner(1900-1901)

Antigens Vs Antibodies

	GROUP A	GROUP B	GROUP AB	GROUP 0
Erythrocytes				
Antibodies	 Anti-B	 Anti-A	none	 Anti-A Anti-B
Antigenes	 A antigen	 B antigen	 A and B antigen	none

Rh Blood Group System

- This system also discovered by Karl Land Steiner(1940)
- Second important blood group system
- The main cause of hemolytic disease of new born(HDN)

Rh Blood Group System

- This system also discovered by Karl Land Steiner(1940)
- Second important blood group system
- The main cause of hemolytic disease of new born(HDN)

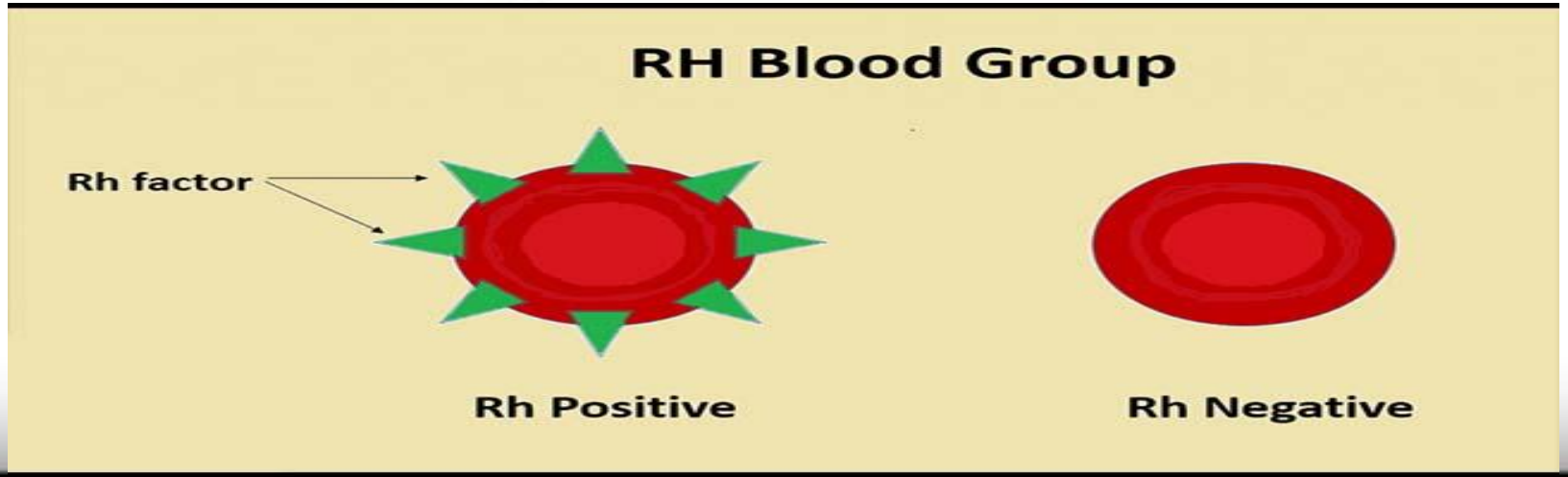
Types of Rh Blood Group System

- **Rh Positive:**

  Posses Rh antigen on surface of RBCs

- **Rh Negative:**

  Lack Rh antigen on surface of RBCs

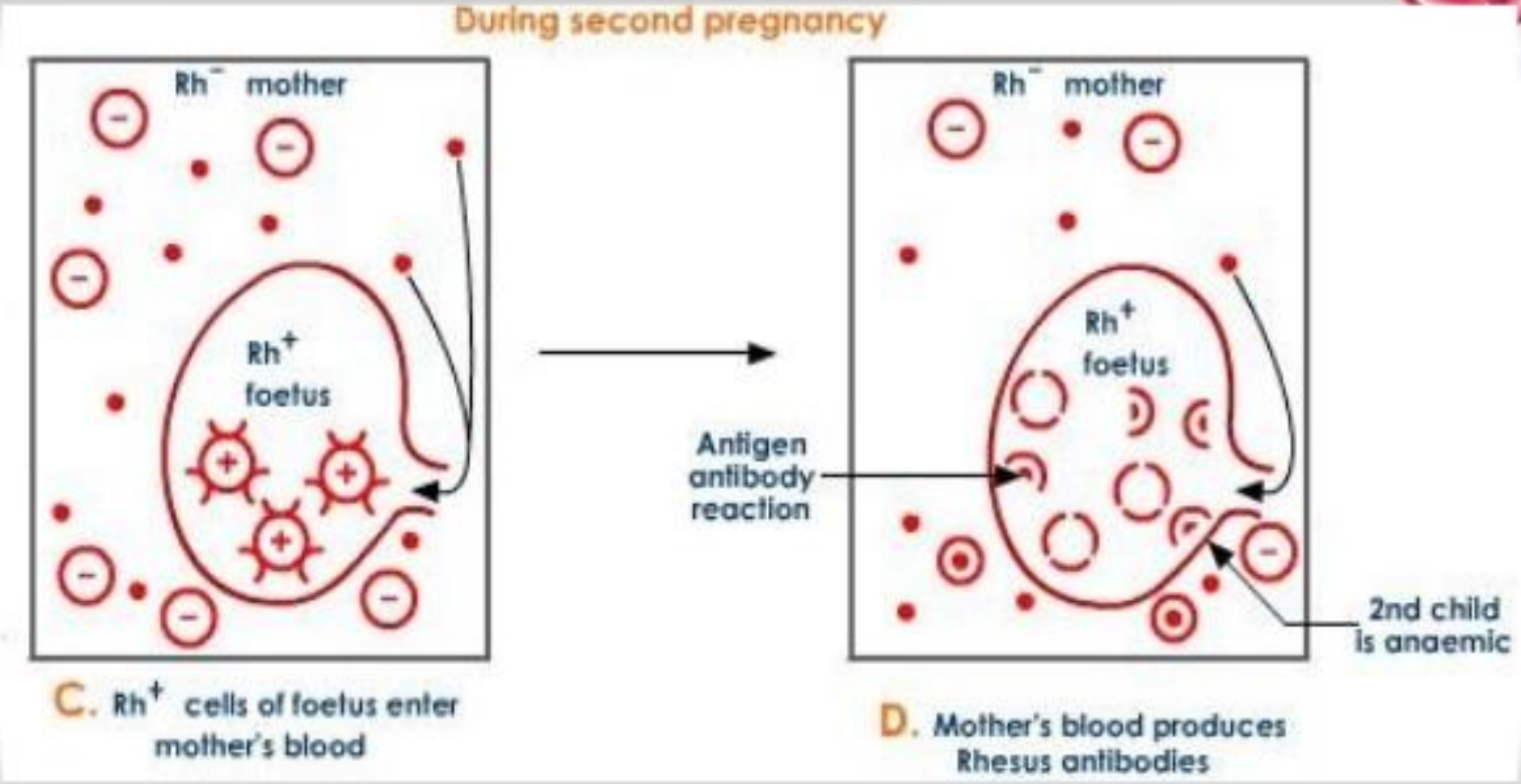


Erythroblastosis Fetalis

- Hemolytic disease of new born

Occurrence:

- If a mother with Rh^- have a fetus with Rh^+
- Mother develop Rh^- antibodies against fetus Rh^+
- These antibodies will react with subsequent Rh^+ fetus
- Lead to bursting of RBC's


During second pregnancy
Rh⁻ mother
Rh⁺ foetus
Rh⁻ mother
Rh⁺ foetus
Antigen antibody reaction
2nd child is anaemic
C. Rh⁺ cells of foetus enter mother's blood
D. Mother's blood produces Rhesus antibodies

Treatment For Erythroblastosis Fetalis

- Steps are taken to prevent antibodies production against fetus antigens
- Usually a shot of Rh antibodies are given to mother within 72 hours of delivery
- Blood Transfusion

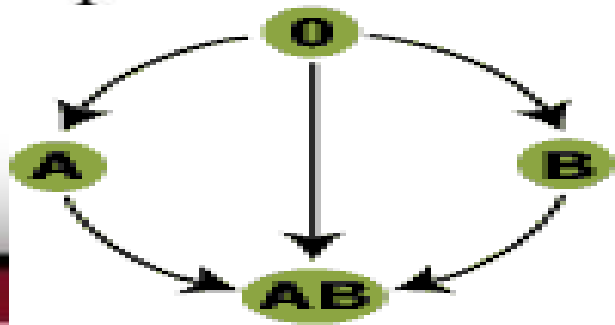
Universal Donors Vs Universal Acceptors

Universal Donor:

- Can donate their blood to anyone
- Have O negative blood group

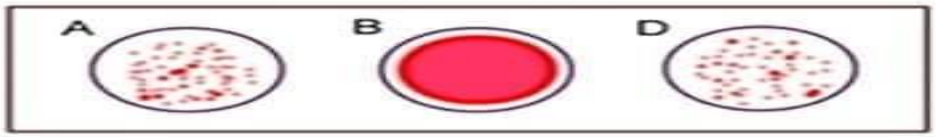
Universal Acceptors:

- Can accept blood from anyone
- Have AB positive blood group

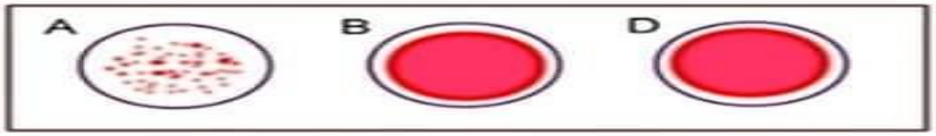



TransClone
ANTI-B
SANOFI DIAGNOSTICS
TransClone
ANTI-A+B
SANOFI DIAGNOSTICS
A
B
AB
O

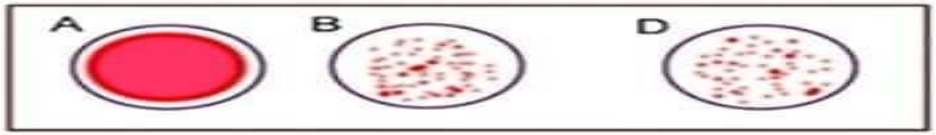
Reading Blood Grouping Results



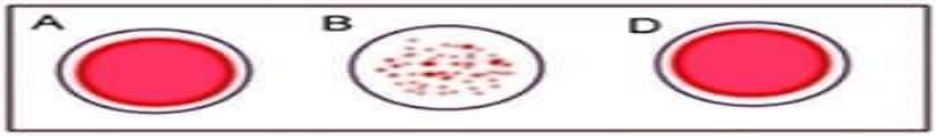
A Positive



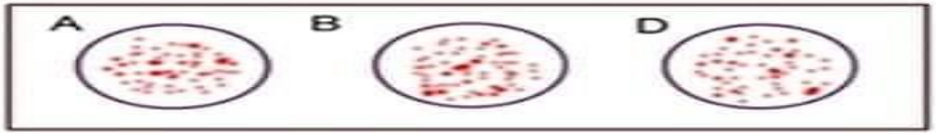
A Negative



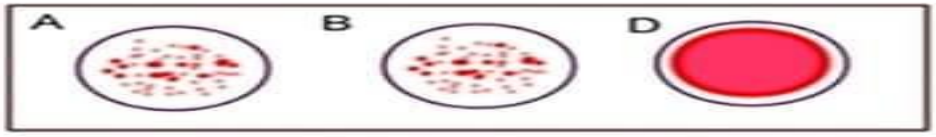
B Positive



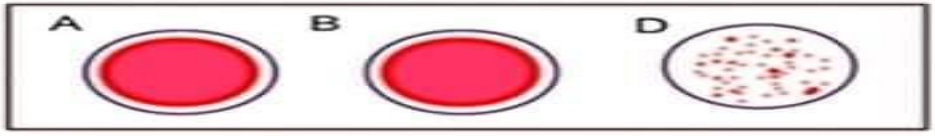
B Negative



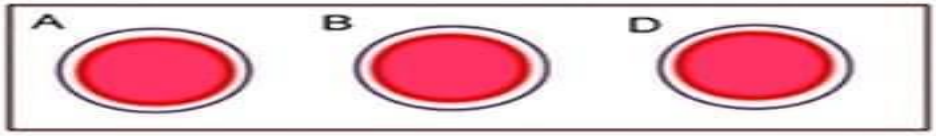
AB Positive



AB Negative

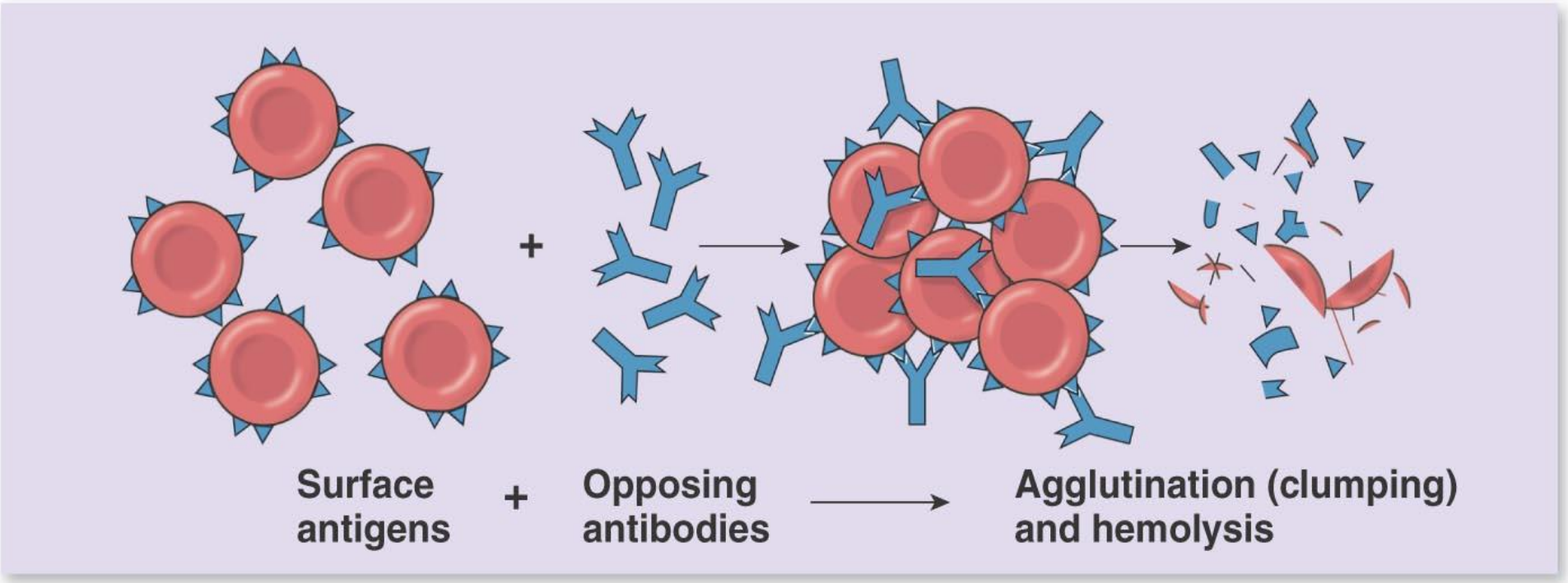


O Positive



O Negative

Mis-Matched Blood Transfusion



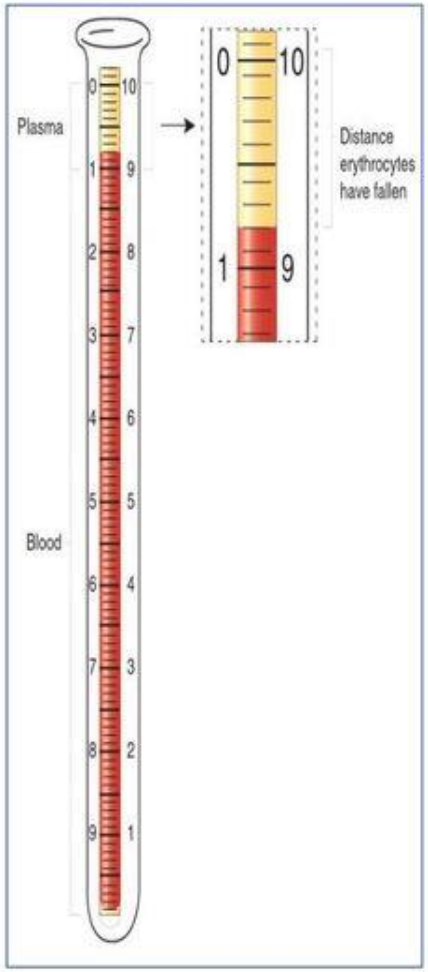
If a blood transfusion is given to a person who has antibodies to that type of blood, then the transfused blood will be attacked and destroyed (transfusion reaction)

Erythrocyte Sedimentation Rate

(ESR test)

Erythrocyte Sedimentation Rate (ESR)

- ESR is the mm of plasma separated per hour.
- It is used clinically as a **non-specific** screening test to:
  - detect the presence of infection in the body in general.
  - monitor the status of chronic inflammatory disease such as rheumatoid arthritis.
- ESR is **not diagnostic** of any particular disease, but rather is an indication that a disease process is ongoing and must be investigated.

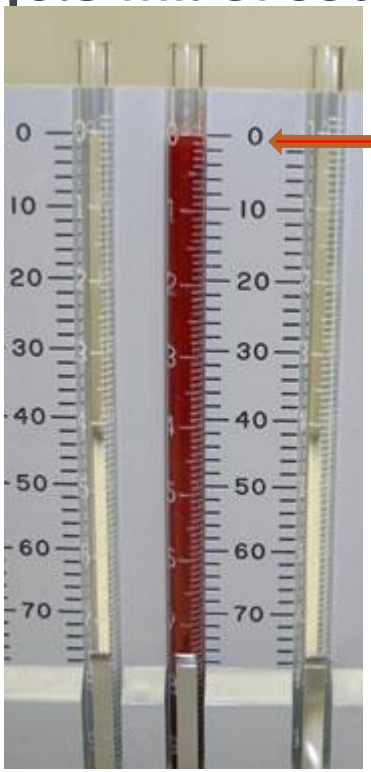


3-How is the test performed?

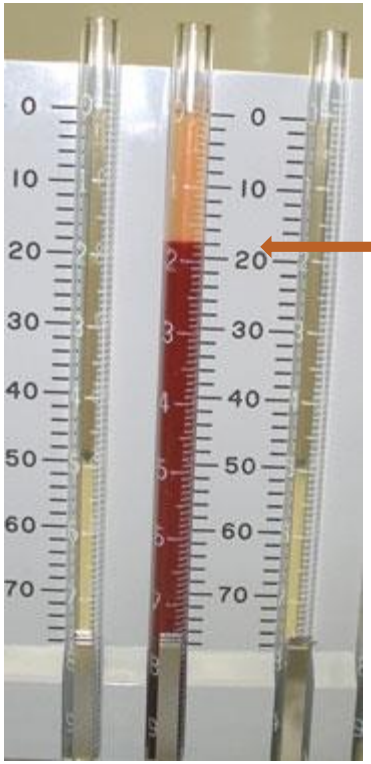
Apparatus : Wistergren tube (graded from 0 =200 mm.)

1- Fill with blood

(0.5 ml. of sodium citrate solution is added to 2ml blood {1-4



2- Fill the Wisergren tube to the mark 0 and put the tube in the stand vertically.



3- Read the height of the clear plasma column on the top of the RBCs after one hr.

The results are expressed as ESR - .....mm/ 1st hour

Factors affecting ESR

Physiological factors

- Plasma factors
- Red cell factors
- Rouleaux formation
- Age
- Sex
- Pregnancy

Normal range

- Male=0-10 mm in 1st hour
- Female=0-20 mm in 1st hour

laboratory factors

- Temperature
- Time
- Anticoagulants
- Tube factor
- Tilting of tube
- Vibration
- Sunlight

FACTORS THAT INCREASE ESR

Physiological Factors	• Old age • Female • Pregnancy
Pathological Factors	• Anemia • Elevated fibrinogen levels • Infection • Inflammation • Malignancy
Mechanical Factors	• Technical factors • Dilution problems • Increased temperature of specimen • Tilted ESR tube

Sedimentation rate key facts:

- A high sedimentation rate signals high levels of inflammation in the body
- Most people with an autoimmune disease will have a high sed rate, but the test can't help diagnose a specific disease
- The sed rate test can help evaluate how well your treatments are working