

# OSPE

Foundation Block

Physiology team 441

## Team Leaders

- ★ Alanoud albawardi
- ★ Nawaf alshehri

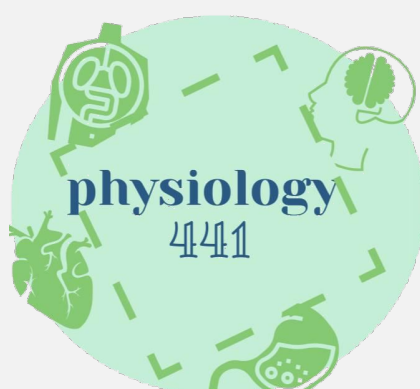
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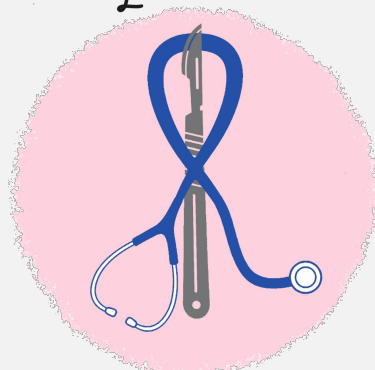
- Main Text
- **Important**
- Dr's notes
- Female
- Male
- Extra



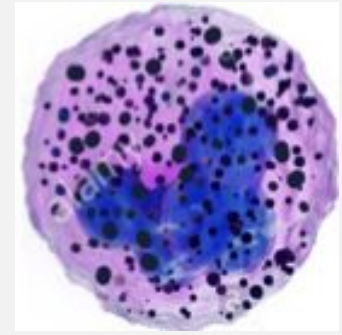
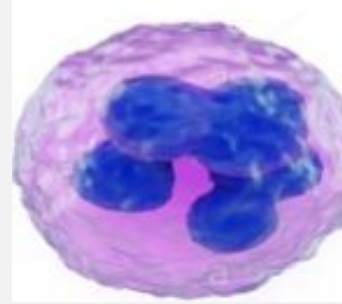
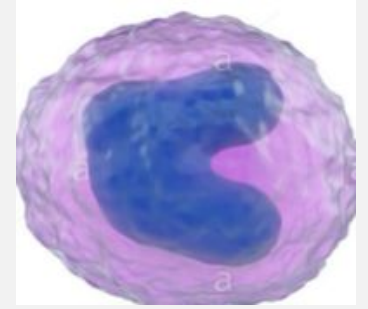
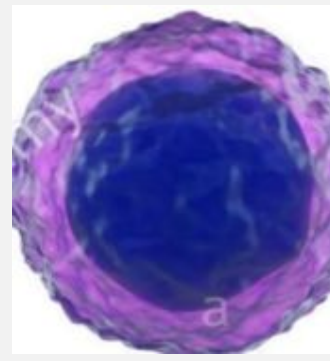
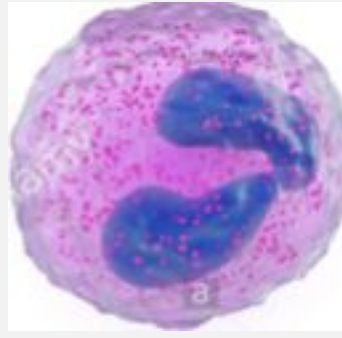
**MED441**  
KING SAUD UNIVERSITY



Abdulaziz & Bahammam  
Faye Wael Sendi



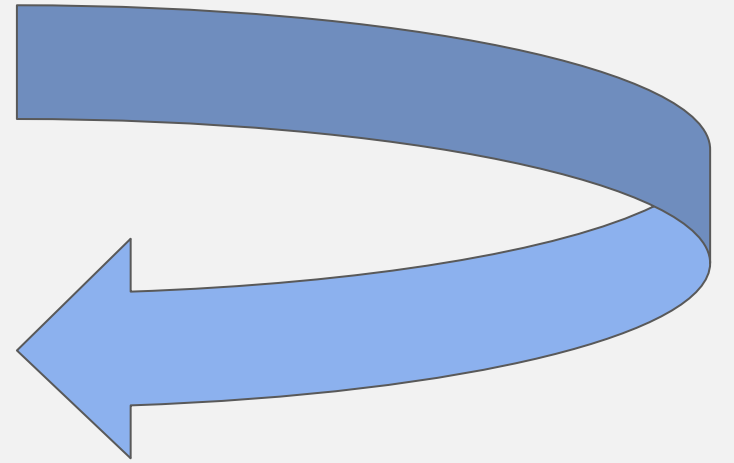
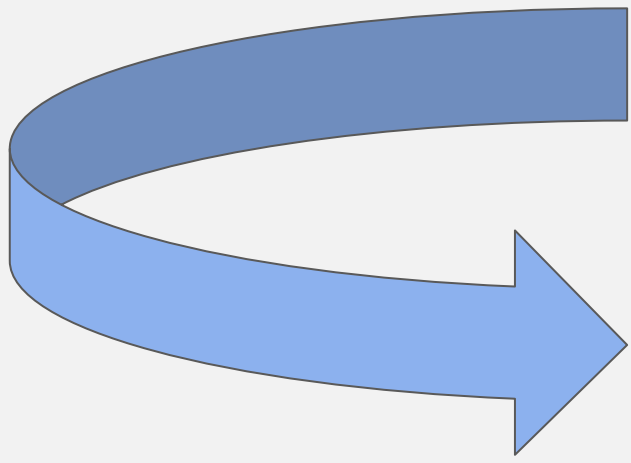
# White blood cells (WBC)



## Objectives :

- 1- To be able to identify the different types of leukocytes under the microscope.
- 2- To know the normal values expected for the differential white cells count .
- 3 - To practice the procedure for differential leucocyte.
- 4-To understand the clinical relevance of the differential white cell count in the diagnosis of disease.

# WBC

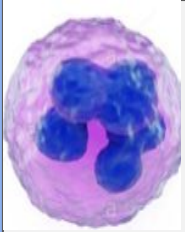


Granulocytes

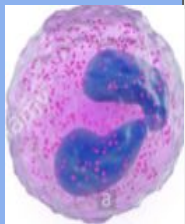
Agranulocytes



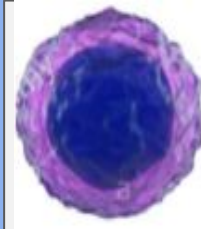
Basophils



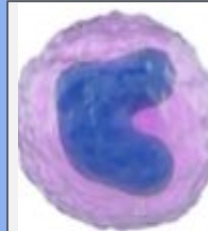
Neutrophils



Eosinophils

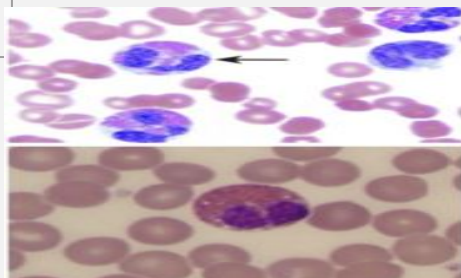
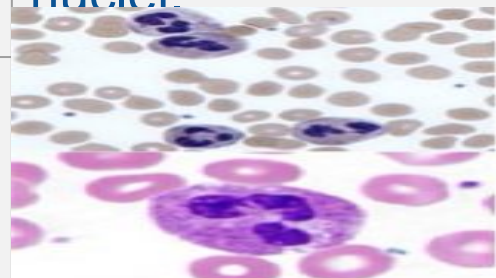
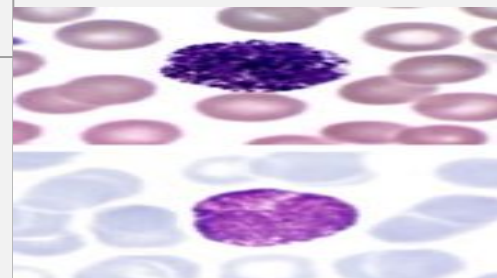


Lymphocytes

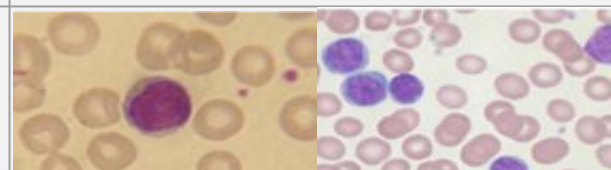



Monocytes

# Granulocytes

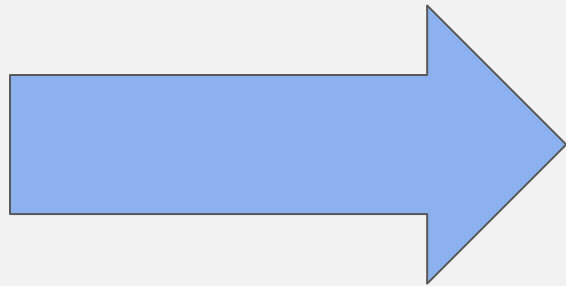
<u>Types:</u>	Eosinophils	Neutrophils ( segmented and band)	Basophils
<u>Amount in the blood:</u>	Less common (1%-3%).	Most common (50%-70%).	The rarest of the blood cells (0.4%-1%).
<u>Describe the shape of cell/nucleus and the granule colors:</u>	<b>Dumbbell</b> - shaped nucleus and large , prominent red , <b>orange</b> granules ( <b>eosinophilic</b> ) .	Small <b>violet pink or purple</b> granules with various stains such as Wright's stain ( have small cytoplasmic granules and complex).	Large cell granular with densely packed <b>dark violet/blue</b> granules ( <b>basophilic</b> ).  Large granules—> <b>contain heparin and histamine.</b>
<u>Number of the lobes and type its:</u>	<b>Bi-lobed</b> nuclei , 2-3 lobes.	<b>Multilobed</b> nucleus,2-6 lobes.  <b>Usually thin filaments present connecting the nuclei.</b>	<b>Small non-segmented</b> nucleus but often hardly visible amongst the dark granules.
<u>Pictures:</u>			






# Agranulocytes

<u>Types:</u>	Lymphocytes	Monocytes
<u>Amount in the blood:</u>	25%-35%	4%-6%
<u>Describe the shape of nucleus /cytoplasm and the granule colors:</u>	Agranular cytoplasm  <b>Small spherical cells</b> with <b>large, round , blue / violet stained nucleus</b> which covers a large part of the cytoplasm ( <b>Ring</b> ) ' leaving only thin rim of cytoplasm around it .	<b>Largest</b> of blood cells no granules in the cytoplasm ( <b>but some practicals may be seen like ; small red dust.</b>  <b>Kidney shaped or horse shaped nucleus.</b>
<u>Pictures:</u>		

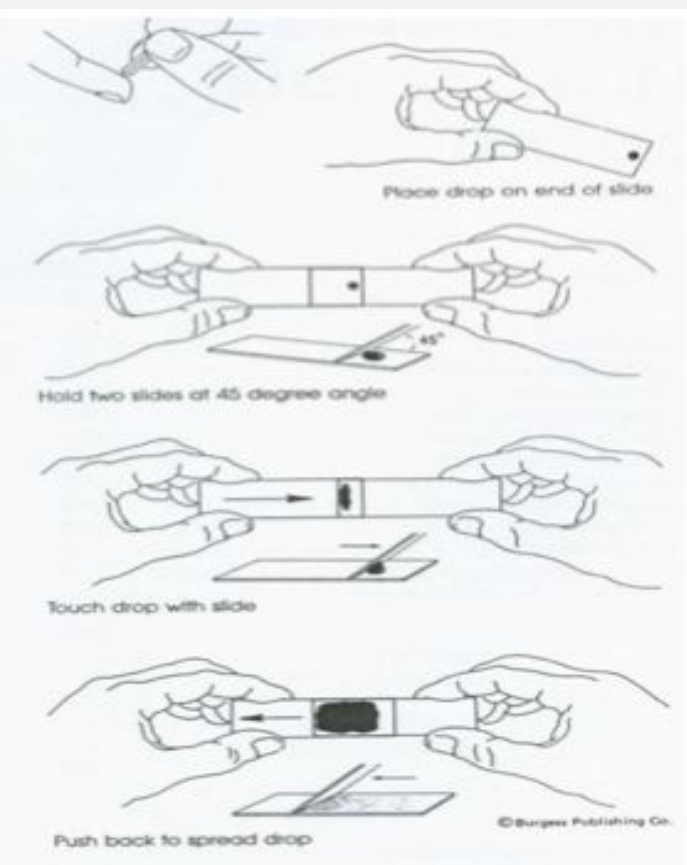
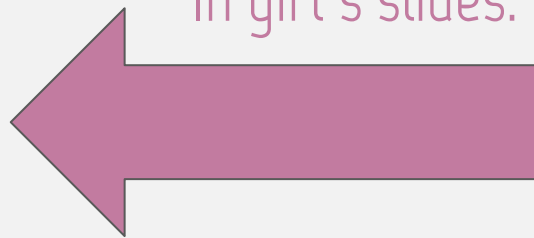


In the boy's slides :



Cell type	Function
<b>Leukocytes</b> (white blood cells, or WBCs) <b>Granulocytes</b> <ul style="list-style-type: none"> <li>• Neutrophils</li> </ul> 	Active phagocytes; number increases rapidly during short-term or acute infections
<ul style="list-style-type: none"> <li>• Eosinophils</li> </ul> 	Kill parasitic worms; increase during allergy attacks; might phagocytize antigen-antibody complexes and inactivate some inflammatory chemicals
<ul style="list-style-type: none"> <li>• Basophils</li> </ul> 	Granules contain histamine (vasodilator chemical), which is discharged at sites of inflammation
<b>Agranulocytes</b> <ul style="list-style-type: none"> <li>• Lymphocytes</li> </ul> 	Part of immune system; one group (B lymphocytes) produces antibodies; other group (T lymphocytes) involved in graft rejection, fighting tumors and viruses, and activating B lymphocytes
<ul style="list-style-type: none"> <li>• Monocytes</li> </ul> 	Active phagocytes that become macrophages in the tissues; long-term "clean-up team"; increase in number during chronic infections such as tuberculosis

In girl's slides:



Reagents and apparatus:

- A microscope with an oil immersion objectives .
- Mineral or cedar oil .
- Various dyes for staining blood films( e.g. wright's and leishman's stain).

Procedure:

1. Prepare blood film and stain it with wright's stain .
2. Examine it under the oil immersion objective lens ( count about 100 cells of different leukocytes).

Clinical application:  
Differential count provides clues about certain illnesses.

Viral infections  
( infectious mononucleosis)

Chronic infection

In allergy and malignancy

Allergy and parasitic infection

Pyogenic illness  
( bacterial and fungal infection)

Lymphocytosis

Monocytosis

Basophilia

Eosinophilia

Neutrophilia

In girls slides

# Eosinophilia: Allergy and parasitic infection

Blood element	% of leukocytes	Size $\mu$	Cytoplasmic staining	Nucleus morphology
Erythrocyte	-	7-8	pink, no granules	none
Neutrophil	50-70	10-12	salmon-colored small granules	Segmented, 2-5 lobed
Lymphocyte	25-35	7-8	Light blue, scant amount, no granules	Single large Oval purple
Monocyte	4-6	16-18	Basophilic, no granules	Large, kidney shaped
Eosinophil	1-3	13-14	Bright red coarse granules	bilobed purplish
Basophil	0-4-1	14-15	Large, basophilic granules	Bilobed bluish black

In boys slides :

Conditions associated with increased counts

Normal ranges

CELL COUNT	TERMINOLOGY	CONDITION
Increase Neutrophils Count	Neutrophilia	Acute Bacterial Infection
Increase Eosinophils Count	Eosinophilia	Allergy Acute Parasitic infection
Increase Basophils Count	Basophilia	Allergy Malignancy
Increase Monocytes Count	Monocytosis	Chronic Bacterial/Viral Infection
Increase Lymphocytes Count	Lymphocytosis	Acute Viral Infection Chronic Infection

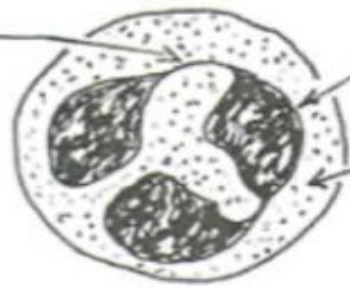
Blood Parameters	Normal Ranges
RBC Count	Males: $4.7 - 5.2 \times 10^6 / \mu\text{L}$ Females: $3.8 - 4.8 \times 10^6 / \mu\text{L}$
WBC Count	$4 - 11 \times 10^3 / \mu\text{L}$
Platelets Count	$150 - 400 \times 10^3 / \mu\text{L}$
Hemoglobin Concentration (Hgb)	Males: 13 - 18 g/dl Females: 11 - 16 g/dl
Hematocrit (Hct)	35 - 55 %
Neutrophils %	40 - 70 %
Eosinophils %	1 - 6 %
Basophils %	0 - 1 %
Monocytes %	5 - 10 %
Lymphocytes %	20 - 40 %



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### Neutrophil

Very thin filament



Pyknotic three lobed nucleus  
Very fine violet-pink granules

### Basophil

Numerous large, dark blue-violet granules that tend to be closely packed

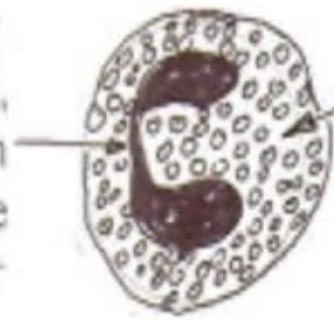


Nucleolus is smaller round, non-segmented and stains lighter than the remainder of the cell



### Eosinophil

Nucleus is rarely more than bilobed, but is pyknotic with a deep blue-purple color

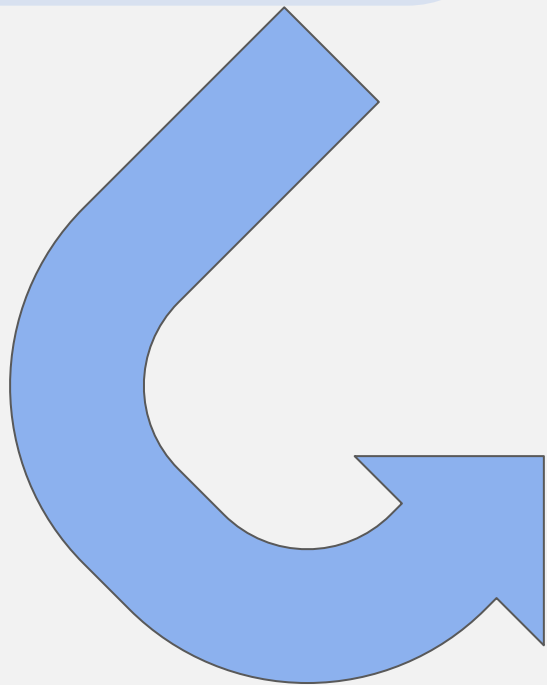


Numerous red-orange granules of uniform size



Granular

Agranular



### Large Lymphocyte

Abundant cytoplasm contains azurophilic granules, lighter in coloration than moderate size lymphocyte



Eccentric nucleus



### Small lymphocyte

Scanty cytoplasm (staining from sky-blue to darker hues)



Chromatin is homogenous with coarse appearance

Eccentric nucleus is round to oval



### Monocyte

Vacuoles

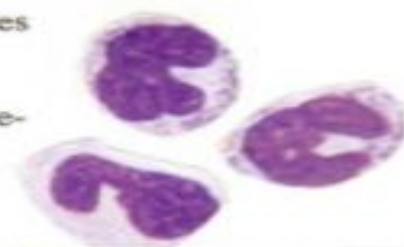
Nuclear crease

Pseudopod



Evenly dispersed red dust-like particles

Nucleus may be cerebriform or horse-shoe shaped, or an elongated band.

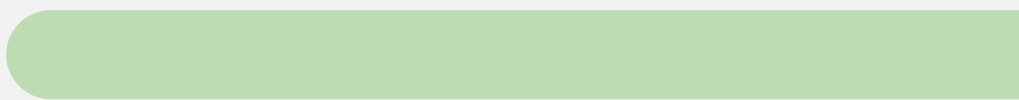






# CBC & ESR

## Objectives :

- 1-The procedure used for taking both capillary and venous blood
  - 2- The normal value recorded when taking these measurements
  - 3 - The methods used to measure the ESR and hematocrit
  - 4-The methods used to get CBC and assess RBCs indices including Mean corpuscular volume, Mean corpuscular hemoglobin, and Mean corpuscular hemoglobin concentration
- 

## ★ Complete Blood Count (CBC)



It is a **test panel** requested by a doctor or other medical professional that **gives information about the cells in a patient's blood.**

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
CBC With Differential/Platelet					
WBC	5.7		x10E3/uL	4.0-10.5	01
RBC	5.27		x10E6/uL	4.10-5.60	01
Hemoglobin	15.4		g/dL	12.5-17.0	01
Hematocrit	44.1		%	36.0-50.0	01
MCV	84		fL	80-98	01
MCH	29.2		pg	27.0-34.0	01
MCHC	34.9		g/dL	32.0-36.0	01
RDW	13.7		%	11.7-15.0	01
Platelets	268		x10E3/uL	140-415	01
Neutrophils	47		%	40-74	01
Lymphs	46		%	14-46	01
Monocytes	6		%	4-13	01
Eos	1		%	0-7	01
Basos	0		%	0-3	01
Neutrophils (Absolute)	2.6		x10E3/uL	1.8-7.8	01
Lymphs (Absolute)	2.6		x10E3/uL	0.7-4.5	01
Monocytes (Absolute)	0.4		x10E3/uL	0.1-1.0	01
Eos (Absolute)	0.1		x10E3/uL	0.0-0.4	01
Baso (Absolute)	0.0		x10E3/uL	0.0-0.2	01
Immature Granulocytes	0		%	0-1	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01

## ★ Coulter Counter Principle

1 It counts and measures the **size of the cells**. *How?* by **detecting and measuring electrical resistance when a liquid pass through aperture.**

2 While passing the aperture (Hole), the cells impedes (block) the current (التيار) and causes a measurable pulse.

3 Number of pulses → Number of particles

4 Height of pulses → Volume of particles.

## ★ RBC, WBC cell count, HB

### Puncture

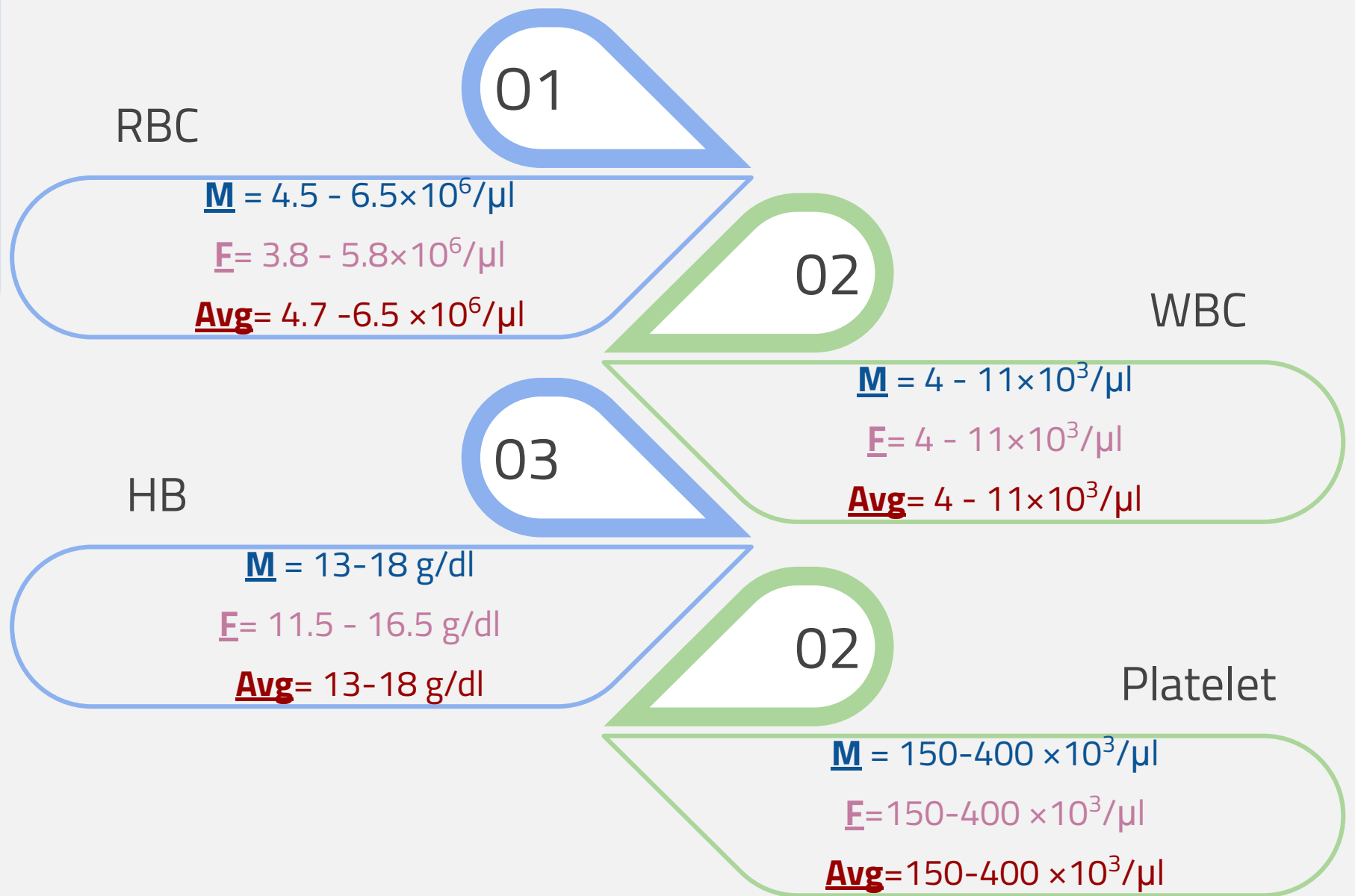
a small hole in a tire resulting in an escape of blood.



### Tourniquet

Used to stop blood flow through a vein or artery, usually by compressing the limb with a cord or an airtight bandage.

★ Normal Values



★ Clinical Terms

Low RBC	<b>Anemia</b>
High RBC	<b>Polycythemia</b>
Low WBC	<b>Leucopenia</b>
High WBC	<b>Leukocytosis</b>
Low platelets	<b>Thrombocytopenia</b>
High platelets	<b>Thrombocytosis</b>



## ★ Clinical Application

Low RBC	Low WBC	Thrombocytopenia (Low Platelets)
<b>-Blood loss when:</b> → Hemorrhage → Anemia (Various type)	<b>-Presence of cytotoxic substance</b>	<b>Thrombocytopenia, possible results:</b>  -Aplastic anemia, -Chemotherapy
<b>-Bone marrow failure, causes:</b> → Radiation → Fibrosis → Toxin → Tumor	<b>-Bone marrow failure, causes:</b> → Infection → Fibrosis → Tumor	
<b>-Erythropoietin deficiency results:</b> → Secondary to renal disease	<b>-Autoimmune/collagen-vascular disease, such as:</b> → Lupus erythematosus	
<b>-Hemolysis (RBC destruction)</b>	<b>-Disease of the liver spleen.</b> <b>-Radiation exposure.</b>	

High RBC	High WBC	Thrombocytosis (High Platelets)
<b>-Low oxygen tension in the blood</b> → Congenital heart disease → Cor pulmonale → Pulmonary fibrosis	<b>-Infectious diseases</b>	<b>-Chronic myeloid leukaemia.</b>
<b>-Polycythemia rubra vera.</b>	<b>-Inflammatory disease (such as rheumatoid arthritis or allergy).</b>	
<b>-Dehydration (such as from severe diarrhea).</b>	<b>-Leukemia</b>	
<b>-Renal (kidney) disease with high erythropoietin production.</b>	<b>-Severe emotional or physical stress.</b> <b>-Tissue damage (burns).</b>	

★ Etiological (Causes) Classification of Anemia:  
We classify anemia depending on the causes of anemia

01

**Hemorrhagic Anemia**  
Blood loss

03

**Nutritional Anemia**  
Deficiency of:  
Iron, Folic Acid, Vit B12

02

**Aplastic Anemia**  
Bone marrow suppression by  
drugs or radiation

04

**Hemolytic Anemia**  
Increased destruction of RBCs  
such as sickle cell anemia

★ Hematocrit: Packed Cell Volume (PCV)

The ratio of  $\frac{\text{Volume of RBCs}}{\text{Volume of blood (plasma)}}$

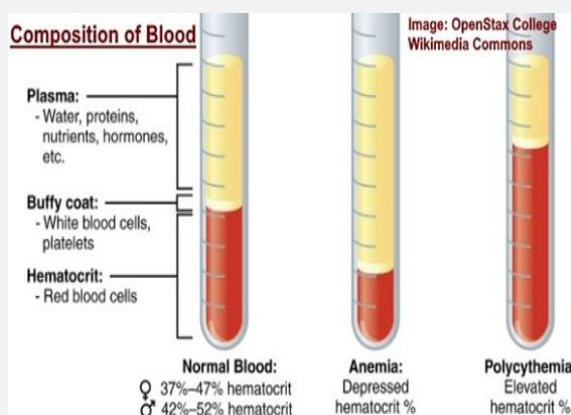
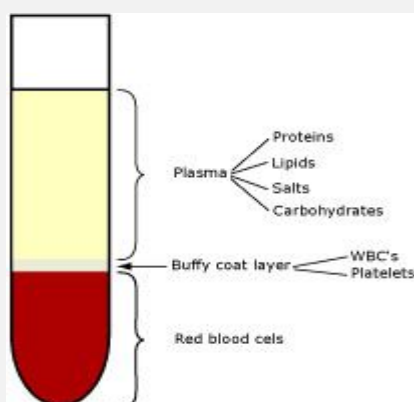
Normal Values of PCV:

**M** = 40 - 54 %

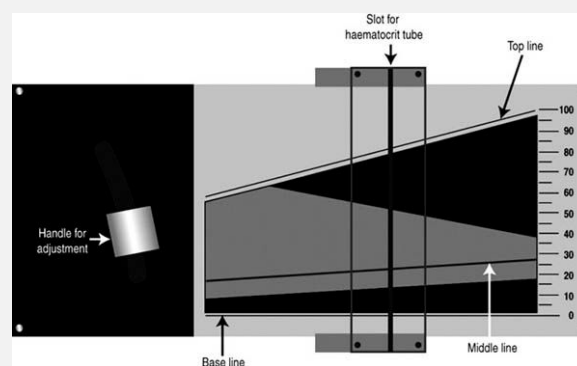
**F** = 35 - 47 %

**Avg** = 35 - 54 %

**Blood Composition**

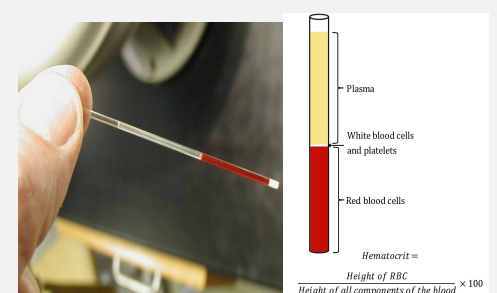
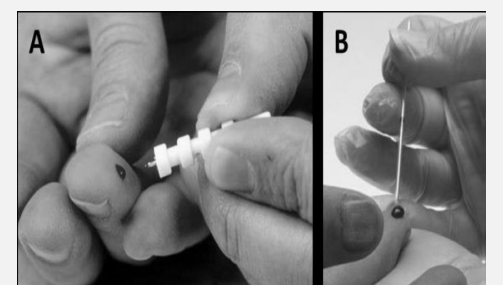


**Haematocrit Reader**



★ Procedure of finding PCV

1. Capillary blood obtained from pricking **finger tip** after cleaning it with **alcohol**
2. Fill a **heparinized** capillary tube, then seal (سد) one end by plasticine  
(قطعة مثل الصلصال). "Add heparin to a container of blood to prevent it from coagulation"
3. Centrifuge for **5 mins** to packed the **cells at one end** of the tube leaving **a clear plasma on the top**
4. Use the **hemocritic reader** to find the (PCV)



# Clinical Applications

High Hematocrit indicates:

Low Hematocrit indicates:

Elevated RBCs count, causes:

A person with low hematocrit referred to as being anemic, causes :

Polycythemia Rubra vera

Bone Marrow failure

↓ O<sub>2</sub> Tension

Dehydration

★ Radiation  
★ Fibrosis

★ Tumor  
★ Toxin

Blood loss

Hemolysis

★ Hemorrhage

Anemia or Leukemia

★ RBCs destruction (related to transfusion reaction)

- ★ Smoking
- ★ Congenital heart disease
- ★ Living at high Altitude

- ★ Burns
- ★ Diarrhea

- ★ Boxes= Causes
- ★ Under each box there are details related to it

## ★ The Calculation of Red Blood Indices

Mean Cell Volume (MCV)

Mean Cell Hemoglobin (MCH)

Mean Cell Hb Concentration (MCHC)

The average volume of red blood cell measured by femtoliters (fl)

The average weight of red Hb in a single RBC measured by picogram (pg)

Concentration of Hb per 100 ml of RBC measured in g/dl

★ a value that describes the average size of red blood cells (erythrocytes) in a blood sample.

★ A value refers to the average quantity of hemoglobin present in a single red blood cell.

★ Measure of the concentration of Hb in a given value of packed RBC *المعطي لنا هنا 100*

- ★ ↓ MCV → Microcytes
- ★ ↑ MCV → Macrocytes

- ★ ↓ MCH → Hypochromic
- ★ ↑ MCH → Hyperchromic

$$MCV (fl) = \frac{PCV}{RBC} \times 10$$

$$MCH (pg) = \frac{Hb}{RBC} \times 10$$

$$MCHC (\%) = \frac{Hb (100mg\ blood)}{PCV} \times 100$$

AVG= 78 - 98 μm<sup>3</sup> (fl)

AVG= 27 - 32 pg

AVG= 30 - 35 g/bl



# ★ Types of Anemia

	Case A	Case B
RBC	LOW	LOW
Hb	LOW	LOW
PCV	LOW	LOW
MCV	LOW	High
MCH	LOW	N/High
MCHC	LOW	N/LOW
Type of Anemia	Microcytic - Hypochromic	Macrocytic - Megaloblastic
Causes	Iron Deficiency	Vit B12 or Folic acid deficiency

★ **Macrocytic megaloblastic anemia** means that RBCs aren't produced properly so you have anemia =RBC are low.

★ **The size of RBCs are large** = macrocytic magaloblastic .  
MCHC is low due to low hemaglobin

## ★ A- Erythrocyte Sedimentation Rate

1

Is the rate at which RBCs sediment (precipitate) in a period of 1 hour.

2

ESR is a non-specific measure of inflammation. A faster-than-normal rate may indicate inflammation in the body.

## ★ B- RBCs Sedimentation

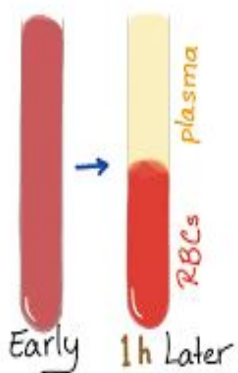
1

**Normal RBCs** have **-Ve** charges → they repel each other  
**Plasma proteins** (fibrinogen) have **+Ve** charges → promoting aggregation.

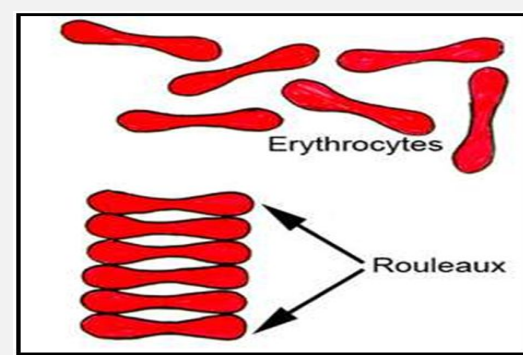
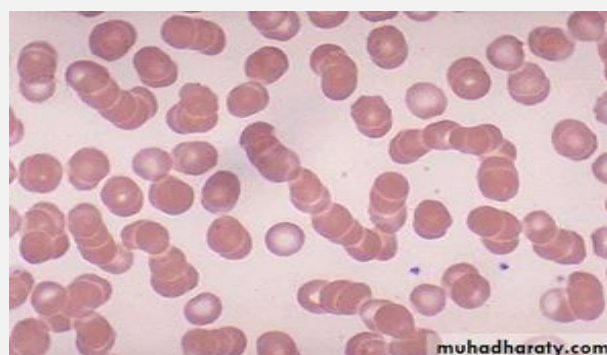
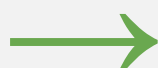
2

In inflammation, the **high fibrinogen level** causes RBCs to stick to each other. (Fibrinogen: is a clotting factor) **Why that happens?** due to aggregation to form stacks (**rouleaux**), which settle (sperate) faster.

ESR

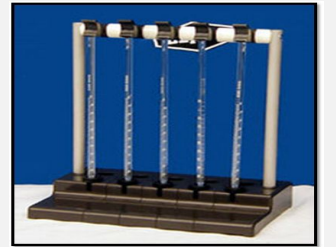


Formation of rouleaux



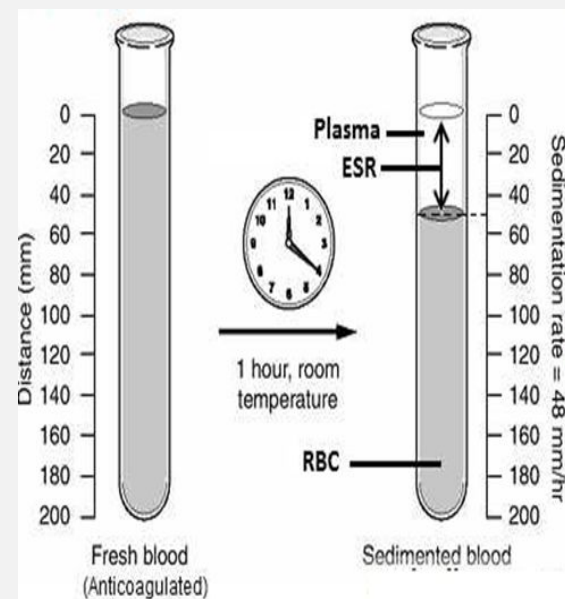
★ What do we need? Materials are:

1. Westergren's sedimentation apparatus
2. Anticoagulant (EDTA)
3. Disposable sterile syringes and needle

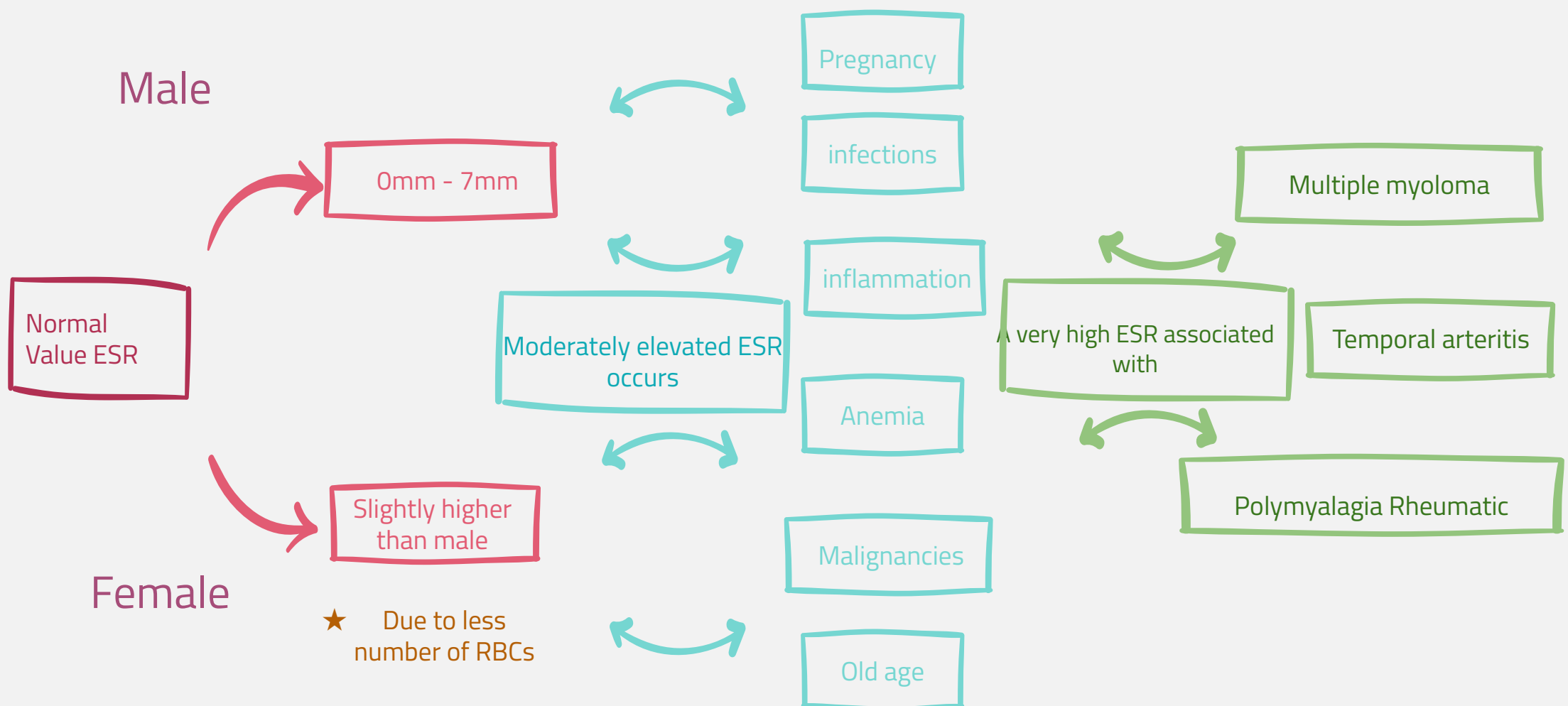


★ Procedure:

1. Using a sterile syringe draw **1.6ml of blood** from a suitable vein.
2. Transfer it to **a test tube (containing EDTA)** and then draw up blood into **a Westergren tube** exactly to the zero mark.
3. Place the tube upright in the stand and leave undisturbed.
4. The **height of the column** of clear plasma at the top of the tube is **noted at the end of an hour**, this will be **ESR reading**.



★ ESR Results



## ★ Clinical application of ESR

Nonspecific test

Prognostic **not diagnostic**

Monitor disease activity and response to therapy.

-ESR is nonspecific marker of **inflammation**  
-It's affected by other factors.  
-ESR results must be used along with other **clinical findings**

## ★ C-reactive protein & ESR

- ❑ C-reactive protein: is an **acute phase protein** produced by the **liver** during an inflammatory reaction.
- ❑ Since C-reactive protein levels in the blood **rise** more quickly after the inflammatory or infective process begins, **ESR is often replaced with C-reactive protein measurement.**



# Test yourself

## Q1: What is the clinical importance of knowing the red blood cells indices?

RBC indices are part of the complete blood count (CBC) test. They are used to help diagnose the cause of anemia. **The indices include:**

Average RBC volume (MCV),  
Hemoglobin amount per RBC (MCH),  
The concentration of Hb per 100 ml of RBC (MCHC)

## Q2: Discuss briefly the etiology classification of anemia?

Hemorrhagic Anemia → Blood loss

Aplastic Anemia → Bone marrow suppression by drugs or radiation.

Nutritional Anemia → Iron, Folic acid, Vit B12 deficiency

Hemolytic Anemia → Increased destruction of RBCs such as sickle cell anemia

## Q3: What is meant by rouleaux formation? Why does rapid rouleaux formation increase the ESR?

Stacks or aggregations of red blood cells (RBCs) due to high fibrinogen level.

## Q4: What is the clinical significance of ERS?

It considered as non-specific measure of inflammation

## Q5: What conditions are associated with an increased ESR?

Multiple myeloma, polymyalgia Rheumatic, temporal arteritis.

## Q6: Peripheral blood parameters of two adult males (Subject A and Subject B) are shown in the table below. Using the information shown in the table answer the questions a & b.

Laboratory parameter	Subject A	Subject B
RBC Count	$3.6 \times 10^6 /\text{mm}^3$	$2.4 \times 10^6 /\text{mm}^3$
Hb concentration	7.2 g/dl	8 g/dl
Hct	25%	25%

a. Calculate MCV, MCH, and MCHC for each of these subjects.

b. What are the RBC abnormalities seen in these men. List possible causes for each of these abnormalities?



## ★ Blood groups and Rhesus blood group

**01 Group A**  
antigen A on RBC  
membrane anti B in  
plasma.

**04 Group B**  
Antigen B on RBC  
membrane Anti A in  
plasma.

**02 Group AB**  
Antigen A and B on RBC  
membrane NO  
antibodies in plasma.

**05 Rh +ve**  
Antigen D on RBC  
(96-98%).

**03 Group O**  
NO antigen on RBC  
membrane both Anti A  
and Anti B in plasma.

**06 Rh-ve**  
NO Antigen D on RBC  
(2-4%).

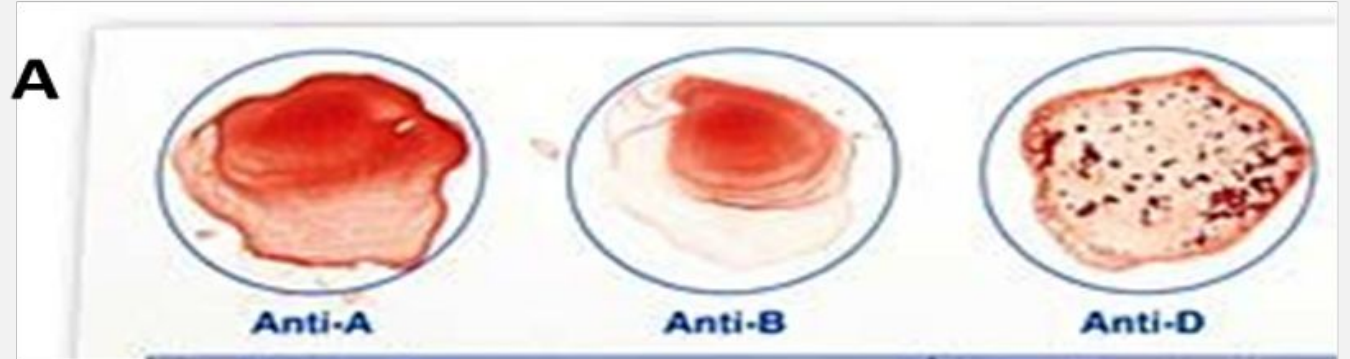
## ★ Materials

1. High titer anti-A, anti-B and anti-D sera.
2. A microscope.
3. Tooth picks.
4. Microscope slides.
5. Alcohol swabs.
6. Lancet.

## ★ Procedure

1. Take 3 microscope slides and label them clearly as "A", "B" and "D".
2. Sterilize the fingertip with an alcohol swab.
3. Prick the finger using a lancet and place one drop of blood in each of the 3 microscope slides.
4. Quickly add a drop of anti-A, anti-B and anti-D sera to slides labeled as "A", "B" and "D" respectively.
5. Stir the mixture on each slide with the help of different pieces of tooth picks for a minute or two.
6. Examine the mixtures carefully for the signs of red blood cell agglutination. When red blood cells clump together (agglutination), they have a speckled or peppered appearance. If there is a doubt, examine the slides using the low power of a microscope.

Sample A: O+ blood type



Sample B : B+ blood type



Sample C : A+ blood type



Blood Group	Antigens	Antibodies	Can give blood to	Can receive blood from
AB <sup>+</sup>	A, B, Rh	None	AB <sup>+</sup>	All blood groups
AB <sup>-</sup>	A, B	Anti-Rh	AB <sup>-</sup> , AB <sup>+</sup>	AB <sup>-</sup> , A <sup>-</sup> , B <sup>-</sup> , O <sup>-</sup>
A <sup>+</sup>	A, Rh	Anti B	A <sup>+</sup> , AB <sup>+</sup>	A <sup>+</sup> , A <sup>-</sup> , O <sup>+</sup> , O <sup>-</sup>
A <sup>-</sup>	A	Anti B, Anti Rh	A <sup>-</sup> , A <sup>+</sup> , AB <sup>-</sup> , AB <sup>+</sup>	A <sup>-</sup> , O <sup>-</sup>
B <sup>+</sup>	B, Rh	Anti A	B <sup>+</sup> , AB <sup>+</sup>	B <sup>+</sup> , B <sup>-</sup> , O <sup>+</sup> , O <sup>-</sup>
B <sup>-</sup>	B	Anti A, Anti Rh	B <sup>-</sup> , B <sup>+</sup> , AB <sup>-</sup> , AB <sup>+</sup>	B <sup>-</sup> , O <sup>-</sup>
O <sup>+</sup>	Rh	Anti A, Anti B	O <sup>+</sup> , A <sup>+</sup> , B <sup>+</sup> , AB <sup>+</sup>	O <sup>+</sup> , O <sup>-</sup>
O <sup>-</sup>	None	Anti A, Anti B, Anti Rh	All blood group	O <sup>-</sup>

### ★ Rh (D) antigen

- Of next importance is the Rh type.
  - >Term "Rh" is a misnomer.
  - > Rh is a blood group system with many antigens, one of which is "D".
- Rh refers to the presence or absence of the D antigen on the red blood cell. The presence of the antibody to the "D" antigen however requires previous exposure to the antigen.
  - Production of antibody to D requires exposure to the antigen.
  - The D antigen is immunogenic, i.e. individuals exposed to it will very likely make an antibody to it.
  - For this reason all individuals are typed for D, if -ve must receive Rh (D) -ve blood
- The most important patient population to consider is females of child-bearing age.
  - If immunized to Rh (D) antigen the antibody can cross the placenta and destroy Rh (D) positive fetal cells resulting in death.
  - This is why Rh negative women are given (Rhogam) after birth of Rh positive baby



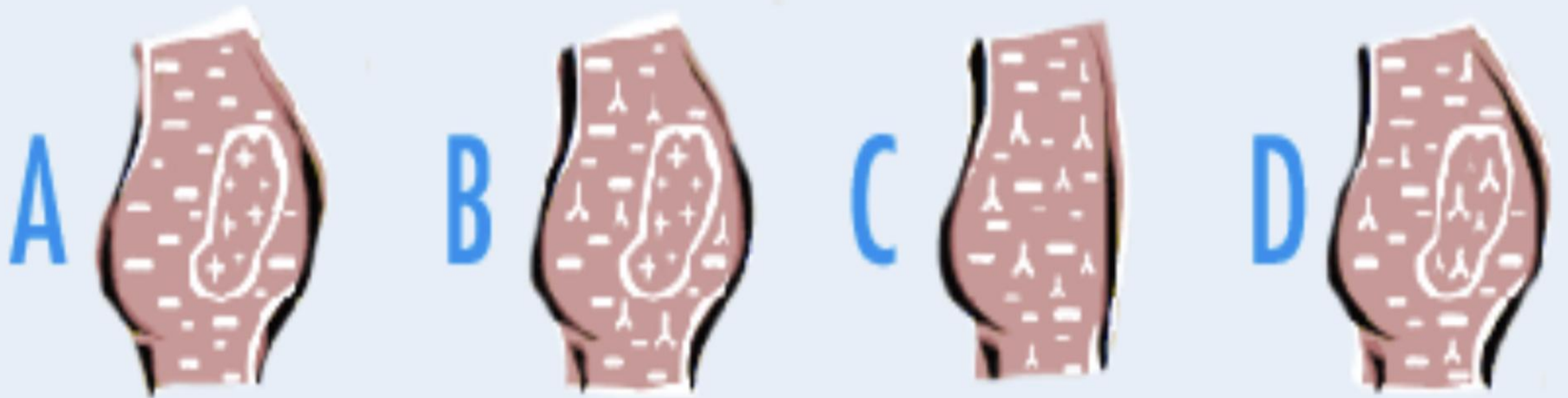
## ★ Clinical applications

1. Blood transfusion
2. Hemolytic disease of newborn (HDN)Explained in detail in the upcoming slide
3. Blood products

### 2. Hemolytic disease of newborn (HDN) (Erythroblastosis Fetalis)

- If a child is Rh positive.
- Then during pregnancy fetal Rh positive RBC's escape into maternal circulation
- Mother produces antibodies to Rh (D) antigen.
- Second or subsequent pregnancies with Rh (D) positive child results in destruction of fetal D positive RBCs.

### Hemolytic Disease of the Newborn How Rh Sensitization occurs



#### Interpretation of Slide Typing Testing with **Anti-B** Anti-Serum

- If an RBC contains the **"B"** antigen the red blood cells will be agglutinated by **anti-B**, (a positive reaction).
- If an RBC does not have the B antigen there will be **no clumping by anti-B**, (a negative reaction).

#### Interpretation of Slide Typing Testing with **Anti-A** Anti-Serum

- if an RBC contains the **"A"** antigen the red blood cells will be agglutinated by **anti-A**, (a positive reaction).
- If an RBC does not have the A antigen there will be **no clumping**, (a negative reaction).



## ★ Clotting time

The time required for blood to form a clot.

1

The normal coagulation time in glass tubes is 3 to 10 minutes.  
5 to 15 min in boys slides

2

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

3

Used in diagnosis of **hemophilia (bleeding disorder)**

4

Its chief application is in monitoring anticoagulant therapy.

## ★ Materials

1. Capillary tubes of uniform size (non heparinized)
2. A petri-dish.
3. Alcohol swabs.
4. Cotton wool.
5. Plasticine.
6. A water bath set at 37°C.
7. A watch

## ★ Procedure

1. Prick a finger of the subject observing the usual precautions and note the time at which the prick is made.
2. Wipe away the first drop of blood.
3. Then while the blood is still freely flowing, place one end of the capillary tube on it and let the tube fill with it by the capillary action.
4. Close both ends of this filled capillary tube with the plasticine.
5. Place this capillary tube in the water bath.
6. Repeat all the above steps with many capillary tubes.
7. Two minutes after making the prick, break a capillary tube and separate the two halves slowly and look for a thread like clot between the two broken halves of the tube.
8. Repeat step 7 at 30 seconds interval with the remaining tubes until you see a thread-like clot between the broken halves of one of the capillary tubes.
9. Note the time. The time from pricking the finger to the appearance of the clot is the clotting time.

## ★ Results

Prolong clotting time seen in deficiencies in the intrinsic coagulation pathway.

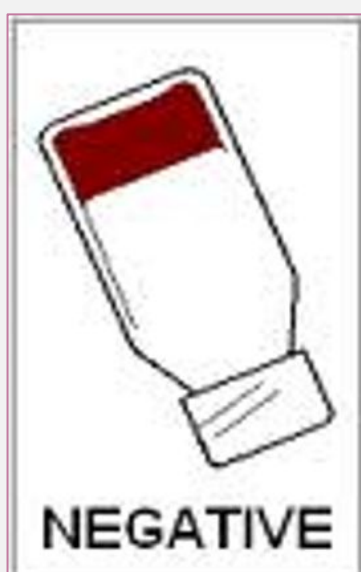
Usually the clotting time measured by this method is in the **range 3-10 minutes.**

Example: **Hemophilia** due to deficiency of Factor VIII (8).

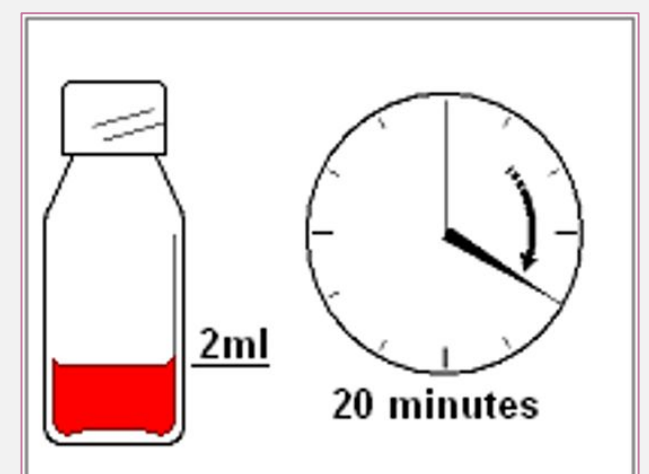
## ★ Clotting Time using Test Tube Method

- 1 Place 2 ml blood into non heparinized test tube incubated in water bath.
- 2 Every 30 second invert gently to check for clot formation.
- 3 Time from pricking finger to clot formation is **clotting time.**
- 4 Normally 6-10 min by this method
- 5 Time from pricking finger to clot formation is **clotting time.**
- 6 Normally 6-10 min by this method

Clot formed



NO Clotting







## ★ Bleeding time

**1** The time taking for bleeding to stop (time for a platelet plug to form).

**2** Bleeding time is a test of **platelet function**.

**3** Bleeding time is prolonged in cases of **thrombocytopenia**

## ★ Materials

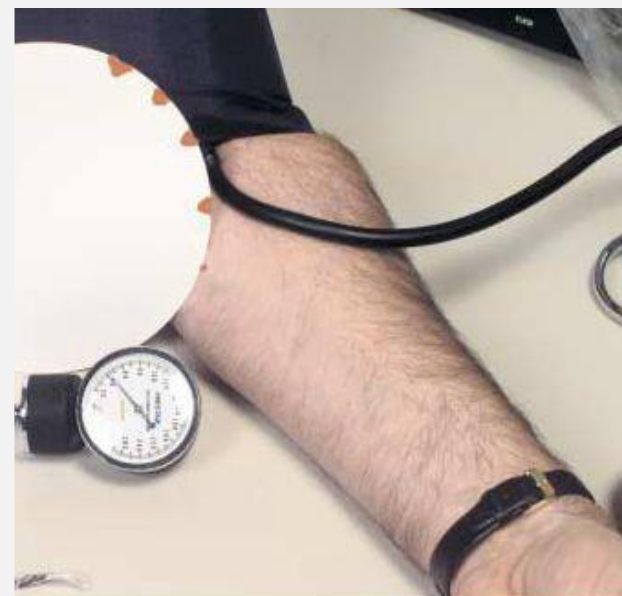
- a. Blotting paper.
- b. Stop watch.
- c. Alcohol swabs.
- d. Lancets.

## ★ Procedure

1. Prick a finger of the subject observing the usual precautions and note the time at which the prick is made. (The pricked skin should not be touched until the experiment is over.)
2. Apply a piece of filter paper (blotting paper) to the emerging drop of blood from the pricked skin every 30 seconds until the bleeding stops.
3. Note the time when the bleeding stops. The time from pricking the finger to the stop of bleeding is the bleeding time.

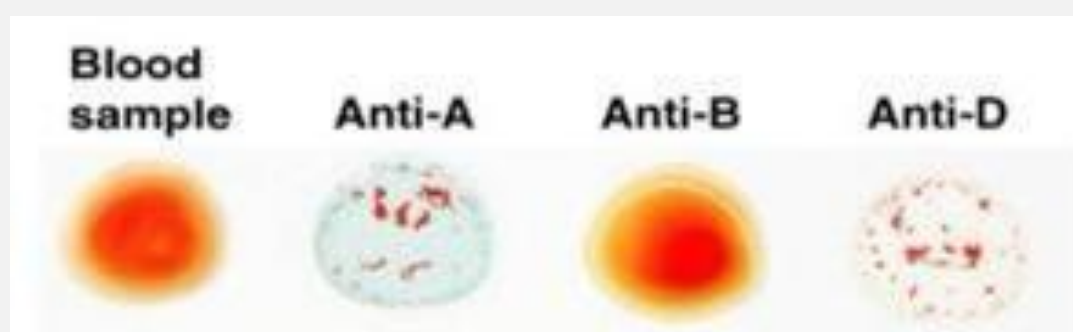
★ The standardized template method

1. A **sphygmomanometer** cuff is applied to the subject's arm and inflated to 40mmHg.
2. The volar surface is cleaned with 70% alcohol.
3. A sterile metal template with a linear slit (11mm long) is pressed firmly against the skin.
4. A scalpel blade, with a guard, is carefully introduced so that it protrudes 1mm through the template slit. An incision, 1mm deep and 9mm long can then be made.
5. Blood is gently, but completely removed with filter paper at 15 second intervals until the bleeding stops.
6. Normal bleeding times determined with this method are in the range **2.5-9.5 minutes**.



## Test yourself

1. What is the blood type seen in this sample?

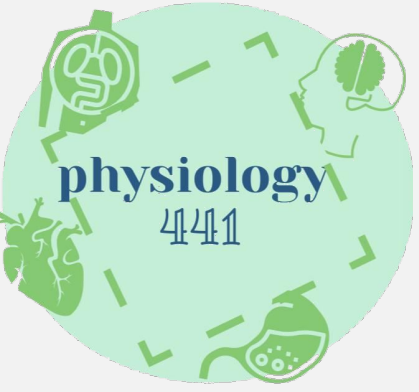


2. You did a bleeding test to a patient, it took 17min to form a clot, is normal?

- 1) A+ , it contain A antigens, it contain D+
- 2) The normal range it is from 3- 10 min, we suspect haemophilia



**MED441**  
KING SAUD UNIVERSITY



Foundation Block

Physiology team 441



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Rayan Ali  
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Fahad Alkhatabi  
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Naif Alfahed  
Faisal Alshuaibi  
Bader Alshahrani  
Bader Rajeh



## Female Members

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Munira Alsharif  
Shimah Alsalhi  
Albandry Bin Habda  
Raaoum Jabor  
Arwa Alenzi  
Ayah Sayed  
Lama Aleyadhy  
Lujain Alkhalaf  
Layan Almasri  
Deema Almuhammel  
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- ★ Nawaf alshehri

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