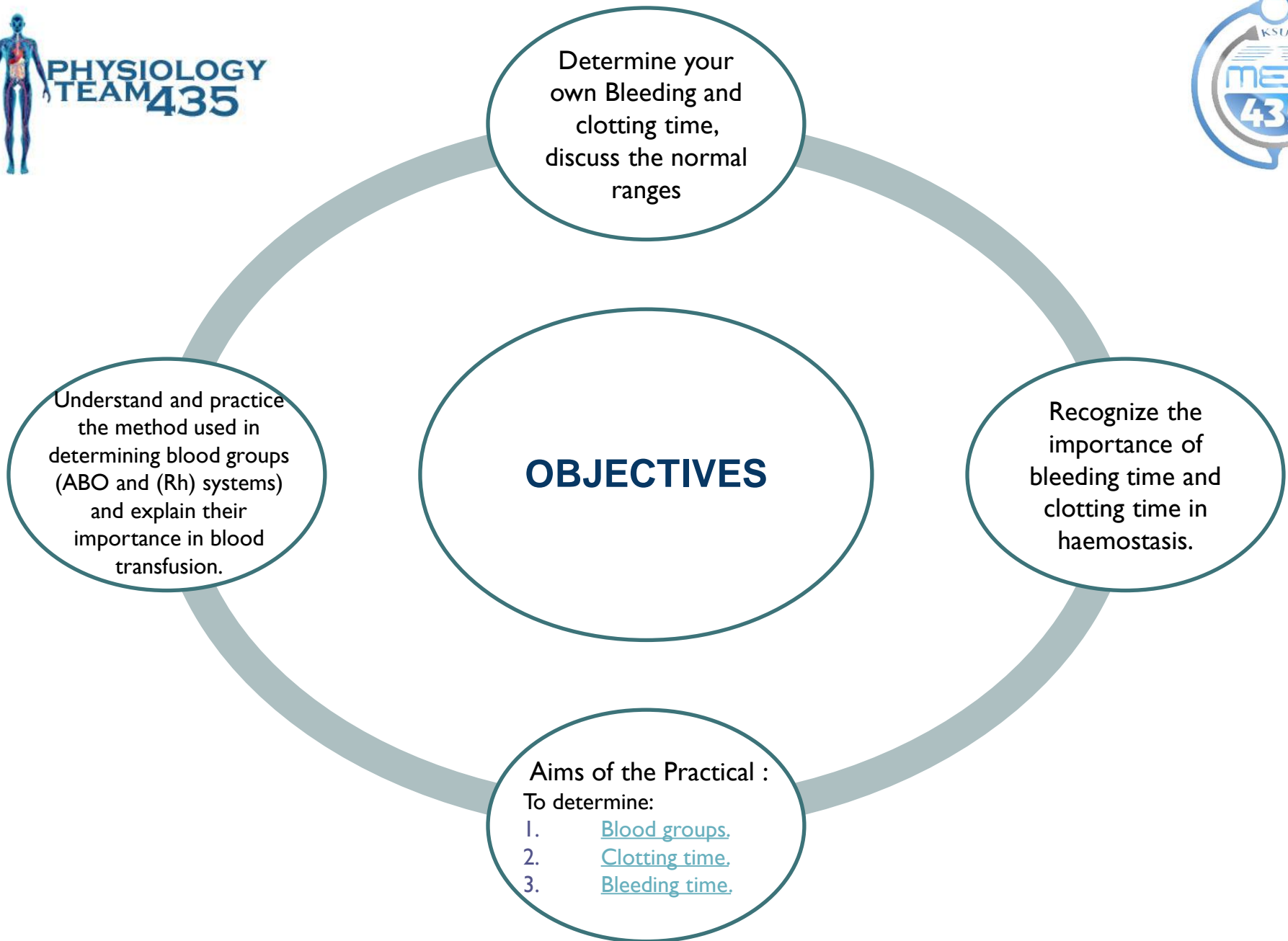


PRACTICAL-3

Blood Groups , Clotting Time , and Bleeding Time

- Very important
- Extra information
- Terms

Time is of the essence



Blood Groups

ABO System

Group A: Antigen A on RBC membrane, Anti B in plasma.

Group B: Antigen B on RBC membrane, Anti A in plasma.

Group AB: Antigen A and B on RBC membrane,
no Antibodies in plasma.


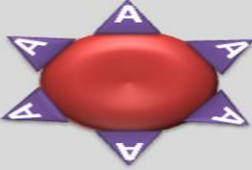


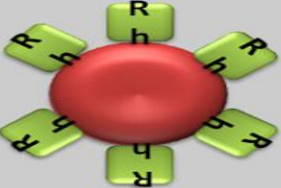
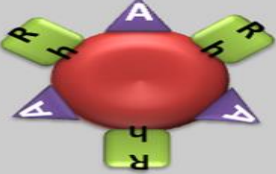

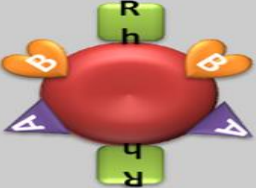
Group O: No Antigen on RBC, but there are Antibodies in plasma.

Rhesus Blood Group

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

Rh-ve(negative) No antigen D on RBC (2-4%).

General types of RBC

<p>O-</p> 	<p>A-</p> 	<p>B-</p> 	<p>AB-</p> 
<p>O+</p> 	<p>A+</p> 	<p>B+</p> 	<p>AB+</p> 

To identify the blood type in lab we have to bring the following materials:

1. Anti A (blue titer).
2. Anti B (yellow titer).
3. Anti D (to identify the rhesus antigen)
4. Microscopic slides and a microscope
5. Alcohol swab.
6. Grease pencil.
7. Tooth picks
8. Lancet or a pricker.



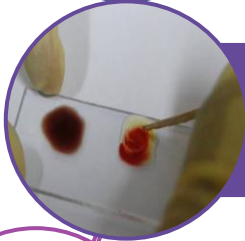
Blood group Procedure



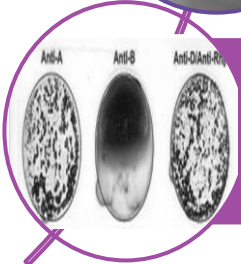
1. Prick a finger using a lancet after sterilizing it with alcohol swabs
2. place one drop of blood in each of the compartments A, B and D (these are clearly labeled on the microscope slides provided).



3. Quickly add a drop of anti-A, anti-B and anti-D sera to compartments A, B and D respectively.

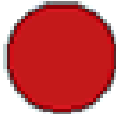








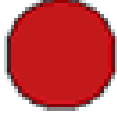

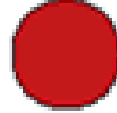

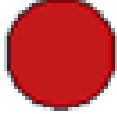
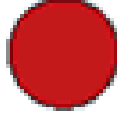

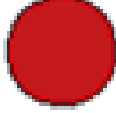













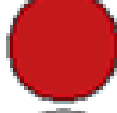
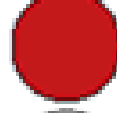






4. Mix the serum with the drop of blood by moving the slides gently for a minute or two, or with the help of different pieces of tooth picks



5. Examine the mixtures for signs of RBC agglutination or clump formation. If there is a doubt, examine the slides using the low power of a microscope.

HOW TO READ YOUR RESULTS

BLOOD TYPE	ANTI-A	ANTI-B	ANTI-D	CONTROL
O-POSITIVE				
O-NEGATIVE				
A-POSITIVE				
A-NEGATIVE				
B-POSITIVE				
B-NEGATIVE				
AB-POSITIVE				
AB-NEGATIVE				
INVALID				

Blood samples on the slides

Important visible features

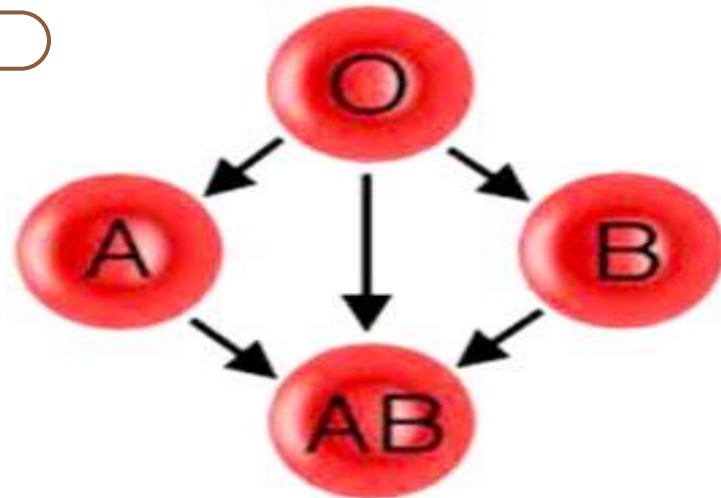
The same type

If there is a coagulation and reaction between the anti titer (antibody = agglutinins) and the blood sample (antigens = agglutinogens).

Another type

If there is no coagulation and reaction.

Note: more than 30 blood group systems have been identified other than classical ABO and Rh groups such as MNS system, Kell System, Lewis System etc.



Blood groups

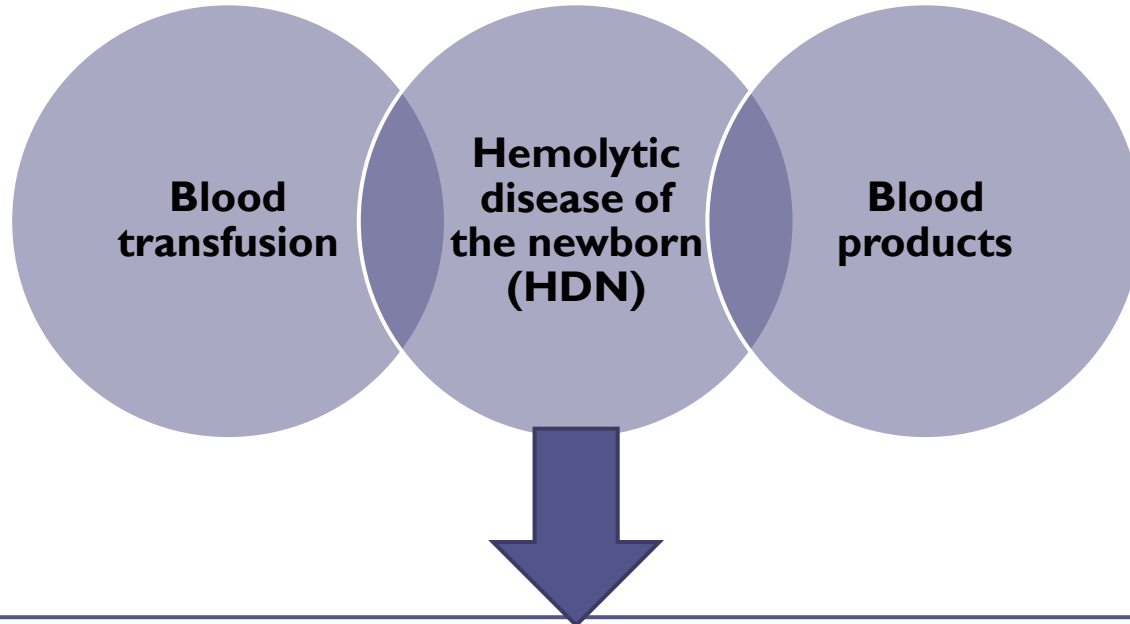
Blood type	Antigens on blood cells	Anibodies made by the immune system	Can donate blood to	Can receive blood from
O-	None	Anti-A, Anti-B, Anti-Rh	All blood types	O- only
O+	Rh	Anti-A, Anti-B	Any Rh+ blood types	O- or O+
A-	A	Anti-B, Anti-Rh	Any A or AB	O or A-
A+	A, Rh	Anti-B	A+ or AB+	Any O or A
B-	B	Anti-A, Anti-Rh	Any B or AB	B- or O-
B+	B, Rh	Anti-A	B+ or AB	Any O or B
AB-	A, B	Anti-Rh	Any AB	Any Rh-
AB+	A, B, Rh	None	AB+	All blood types

Distribution of the ABO and Rh blood groups in Saudi Arabia

O+	48%
A+	24%
B+	17%
AB+	4%
O-	4%
A-	2%
B-	1%
AB-	0.23%

- From the above table, we can easily conclude that about 93 % of Saudi population is Rh +ve and only about 7% is Rh –ve.
- The most common blood group in ABO system is O, followed by A, then B and the least common is AB among Saudis.
- Almost the same distribution is seen in Europe and America as in Saudi Arabia. The blood group “B” is more prevalent than blood group “A” in some Asian countries.

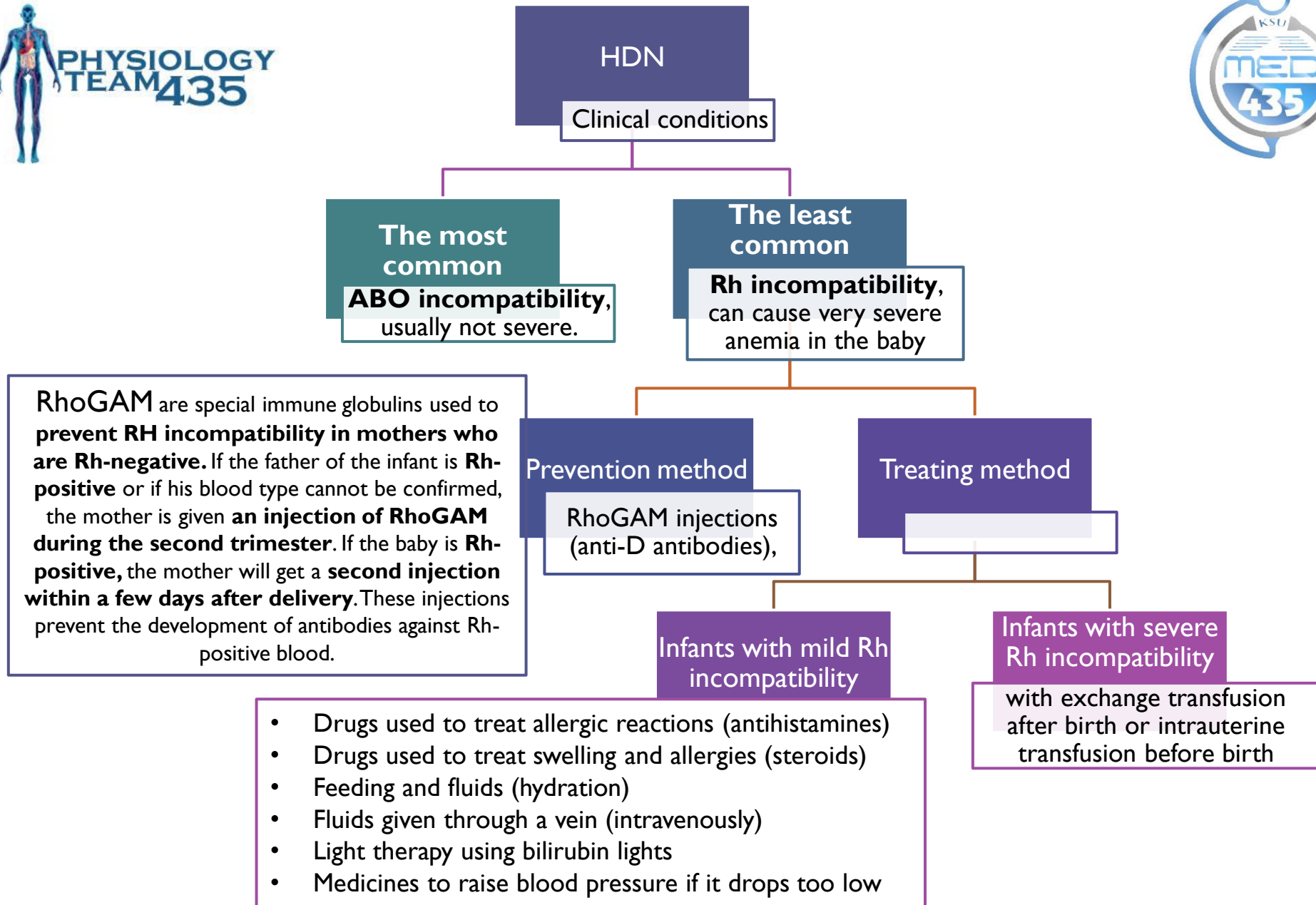
Important Clinical Applications of blood groups



Hemolytic disease of the newborn (HDN)

*It is a blood disorder in a fetus or newborn infant. HDN may develop when a mother and her unborn baby have different blood types (called "incompatibility").

*The mother produces substances called antibodies that attack the developing baby's red blood cells



Rh incompatibility

Rh incompatibility is a condition that develops when a pregnant woman has Rh-negative blood and the baby in her womb has Rh-positive blood inherited from the Rh-positive father.

During pregnancy, red blood cells from the unborn baby can cross into the mother's bloodstream through the placenta.

Because the mother is Rh-negative, her immune system treats Rh-positive fetal cells as if they were a foreign substance and makes antibodies against the fetal blood cells. These anti-Rh antibodies may cross back through the placenta into the developing baby and destroy the baby's circulating red blood cells.

When red blood cells are broken down, they make bilirubin. This causes an infant to become jaundiced.

Because it takes time for the mother to develop antibodies, firstborn infants are often not affected unless the mother had past miscarriages or abortions that sensitized her immune system. However, all children she has afterwards who are also Rh-positive may be affected.

 all about blood types in 3 minutes

Clotting Time

Definition:

It is the time required the blood to form a clot.

The Normal time for clotting:

Almost 3-10 min.

Importance of identifying the clotting time:

1. For surgery.
2. For Diagnosis of bleeding disorders.

Substances used as anticoagulant:

1. Heparin.
2. Warfarin.
3. Calcium Oxalate.
4. Sodium Citrate.
5. EDTA (Ethylene Diamine Tetra-butyrlic Acid).

The source of heparin in the body is:

1. Mast cells.
2. Basophils.
3. Liver.
4. Lungs.

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

***Used in diagnosis of hemophilia, when the clotting time is higher than normal.**

***Its chief application is in monitoring anti-coagulant therapy.**

Materials

petri-dish.



**Alcohol
swabs**



Plasticine



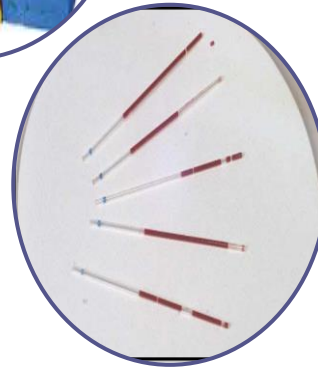
**Cotton
wool**



**water bath
37°C**




**Capillary
tubes of
uniform size**



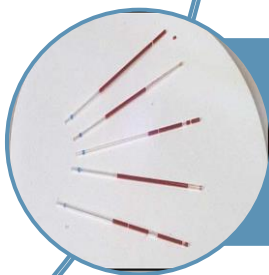
Clotting time Procedure



1. Clean finger with alcohol swab, prick it with lancet and note the time that the prick is made.



2. Wipe away the first drop of blood. Then while the blood is still flowing freely place one end of a capillary tube in the blood. Holding the tube horizontally let it fill by capillary action.




3. Close the end of the capillary tube with plasticine. Place the tube in the water bath. Repeat all the above steps with many capillary tubes.



4. Two minutes after making the puncture, 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.



5. Repeat the procedure at 30 second intervals with the remaining tubes.



6. When the blood forms a continuous thread-like clot between the broken ends of the tube, the end-point has been reached, note the time.

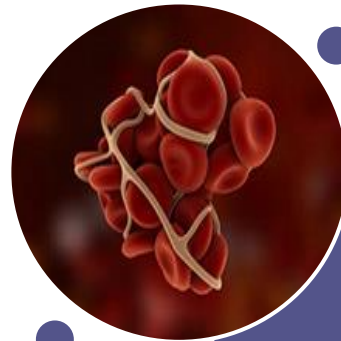


7. The time from pricking the finger to the appearance of the clot is the clotting time

Results



Usually the clotting time measured by this method is in the **range 5-15 minutes**.



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



Clinical condition:
Hemophilia: a medical condition in which the ability of the blood to clot is severely reduced by the lack of coagulation factor.

*Deficiency of factor 8 (**VIII**) leads to disease known as **Hemophilia A**.

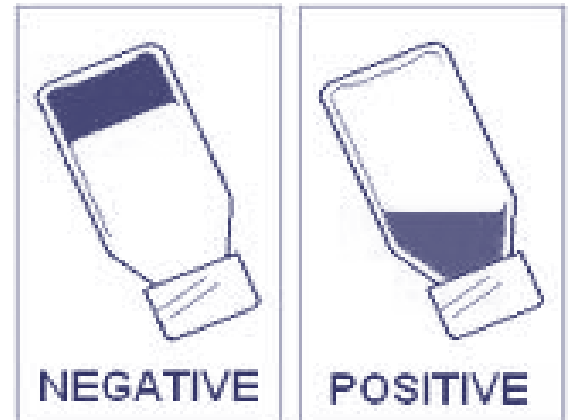
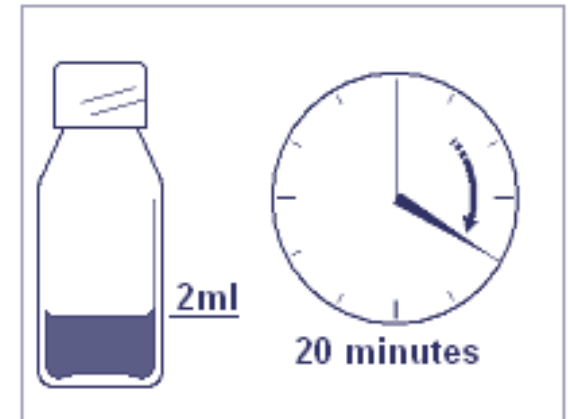
*Deficiency of factor 9 (**IX**) or **Christmas** factor leads to disease known as **Hemophilia B**.

Clotting Time using Test Tube Method

Place 2 ml blood into non heparinized test tube incubated in water bath.

Every 30 second invert gentle to check for clot formation.

Time from pricking finger to clot formation is **clotting time**.



Bleeding time

*Definition: The time taken for bleeding to stop (time for a platelet plug to form).

*Bleeding time is a test of platelet function.

*The template bleeding time is used when the test is performed by standard template method.

*The normal range of bleeding time is 2 – 5 minutes

Materials

Filter paper



A **stylette** to prick an ear lobe



Alcohol swabs



stop-watch.




I. Bleeding time Procedure



1. Clean the lobe of the ear with an alcohol swab.



2. When it is dry, make a single puncture with a stylette (about 3mm deep).
3. Note the time at which the puncture is made.
4. The skin of the ear should not be touched once the puncture has been made until the experiment is over.



5. Apply a piece of filter paper to the blood-drop every 30 seconds until the bleeding stops.
6. The bleeding time estimated by this method of a normal subject is: 2-5 minutes.

2. The Standardized Template Method



A sphygmomanometer cuff is applied to the subject's arm and inflated to 40mmHg.



The volar surface (palm) is cleaned with 70% alcohol.
A sterile metal template with a linear slit (11 mm long) is pressed firmly against the skin.



A scalpel blade, with a guard, is carefully introduced so that it protrudes 1 mm through the template slit. An incision, 1 mm deep and 9 mm long can then be made.



Blood is gently, but completely removed with filter paper at 15 second intervals until the bleeding stops.



Normal bleeding times determined with this method are in the range 2.5-9.5 minutes.



If the bleeding time exceeds 15 minutes:
Stop the procedure. Apply pressure to stop the bleeding. Report as greater than 15 min.

Clinical Application

Bleeding time is prolonged in the following conditions:

- Platelet dysfunction.
- Blood vessel wall disorders.
- Haemophilia.
- Von Willebrand Disease.
- Thrombocytopenia.
- Vitamin K deficiency.
- Medications: Aspirin.



Thank you

You don't have to be a doctor to save lives.



Just donate blood.

Do you know that just a pint of blood can save up to 3 lives?
Donating blood is safe. It's painless, simple, and noble.

Physiology practical team



Practical videos to determine:

1. [Blood groups.](#)
2. [Clotting time.](#)
3. [Bleeding time.](#)

