

# HLS MODULE PHYSIOLOGY (LAB 2) BLOOD GROUPS & HEMOSTASIS TESTS

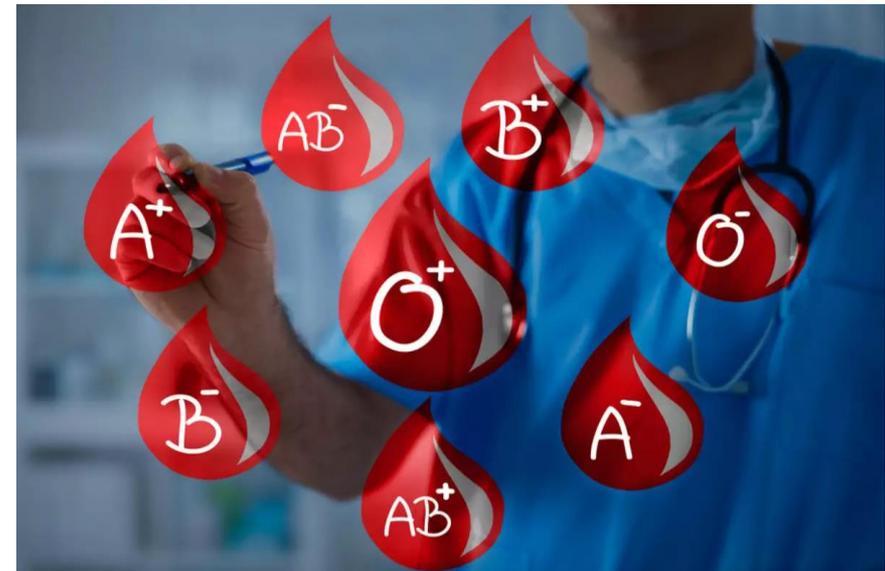
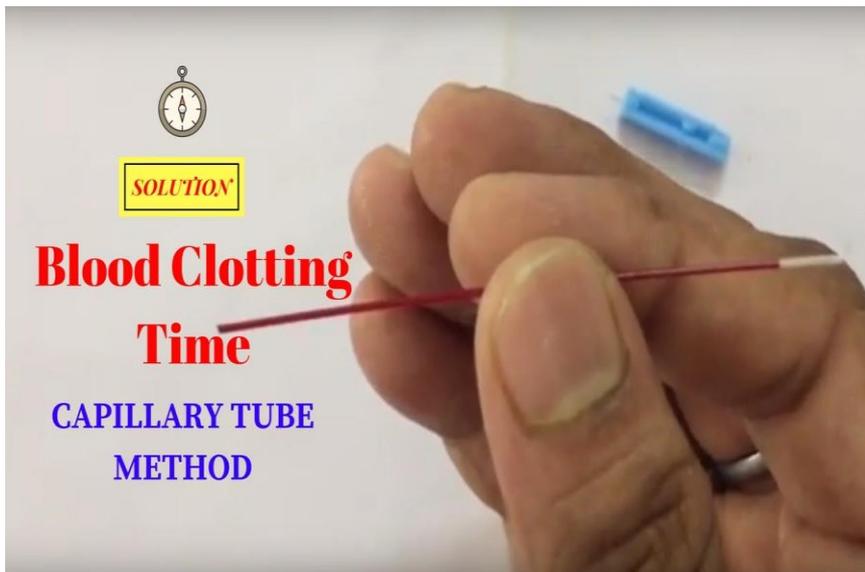
BY

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2024-2025



# Experiment 1

## BLOOD GROUPS

- There are 2 systems used to determine blood groups:
  1. The ABO system.
  2. The Rhesus (Rh) system.

# 1. The ABO System

- ✓ Human blood can be classified into four major groups A, B, AB, O.
- ✓ The classification is based on the **antigen nature** of red blood cell membrane.
- ✓ The membranes of RBCs contain antigens called agglutinogens. The most important agglutinogens are **A and B**.
- ✓ While, plasma contains specific antibodies for red cells antigens. These antibodies are called agglutinins;  $\alpha$  and  $\beta$  (alpha and beta).
- ✓ The blood of any person doesn't contain an agglutinogen (e.g A) and its corresponding agglutinin (anti-A;  $\alpha$ ), otherwise ..... Antigen – antibody reaction occurs which results in agglutination and hemolysis of RBCs.

**BLOOD TYPE**

**TYPE A**

**TYPE B**

**TYPE AB**

**TYPE O**

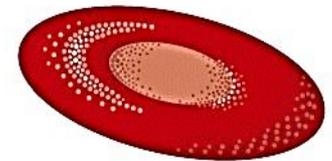
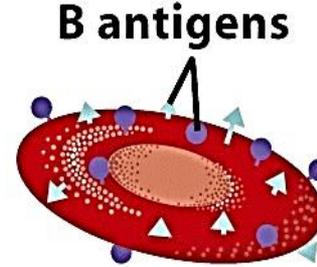
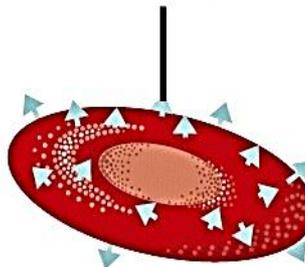
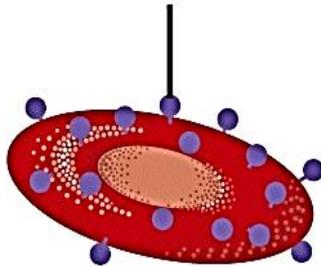
**A antigen**

**B antigen**

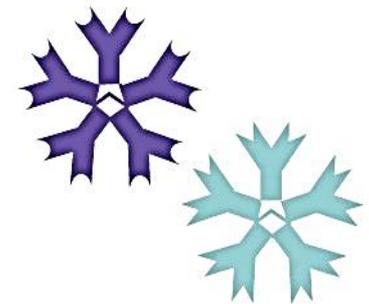
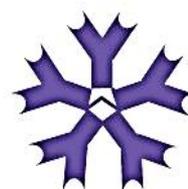
**Both A and B antigens**

**Neither A nor B antigen**

**Red blood cells**



**Plasma**



**Anti-B antibody**

**Anti-A antibody**

**Neither antibody**

**Both anti-A and anti-B antibodies**

<b>Blood group</b>	<b>RBCs membrane agglutinogens (Antigens)</b>	<b>Plasma agglutinins (Antibodies)</b>
<b>A</b>	<b>A</b>	<b>Anti-B (<math>\beta</math>)</b>
<b>B</b>	<b>B</b>	<b>Anti-A (<math>\alpha</math>)</b>
<b>AB</b>	<b>AB</b>	<b>None</b>
<b>O</b>	<b>None</b>	<b>Anti-A &amp; Anti-B (<math>\alpha</math> and <math>\beta</math>)</b>

- ✓ **Group O is called universal donor** (no agglutinogens and so no agglutination occurs when given).
- ✓ **Group AB is called universal recipient** (no agglutinins and so no agglutination occurs).

N.B. **Agglutination** of RBCs occurs between the **agglutinogens on the RBCs of the donor's blood** and **agglutinins of the recipient's plasma** this is because agglutinins in the donor's plasma:

1. Markedly diluted by the recipient's plasma.
2. Markedly neutralized by free agglutinogens present in the recipient's blood (produced by the daily destruction of human RBCs).

<b>Donor</b>		<b>Recipient</b>
<b>A</b>		<b>B</b>
A agglutinogen	×	B agglutinogen
β agglutinin		α agglutinin

## ABO Type Frequencies

ABO Type	Per Cent of population
O	45%
A	40%
B	10%
AB	5%

## The possible transfusions among blood groups:

- ✓ Group AB is a universal recipient and gives only group AB.
- ✓ Group O is a universal donor and can receive blood only from group O.
- ✓ Group A can receive blood from groups A & O and gives blood to groups A & AB.
- ✓ Group B is can receive blood from groups B & O and gives blood to groups B & AB.

<b>Blood Group</b>	<b>Antigens on cell</b>	<b>Antibodies in plasma</b>	<b>Transfuse with group</b>
<b>A</b>	A	Anti-B	A or O
<b>B</b>	B	Anti-A	B or O
<b>AB</b>	A and B	none	AB, A, B or O
<b>O</b>	None	Anti-A & Anti-B	O

## 2. The Rh System

**Rh factor (D antigen)** is an **agglutinogen** which was discovered on the RBCs of **Rhesus monkeys** (hence the name).

- It is present in RBCs of **85%** of people (called Rh positive "**Rh +ve**") and is absent in **15%** of people (called Rh negative "**Rh -ve**").

- Normally, there is no anti-Rh antibody (in either Rh -ve or +ve), **however**, it could be formed (Rh agglutinins are small sized, so they can cross the placenta) in the blood of Rh -ve persons **only by two methods:**

1. Blood transfusion from Rh +ve person to Rh -ve person.
2. Pregnancy of Rh -ve female with an Rh +ve baby.



## **Importance of blood groups:**

### **In blood transfusion ;**

To avoid dangers of incompatibility.

### **In marriage ;**

- To avoid Rh incompatibility (**erythroblastosis fetalis** or **hemolytic disease of the newly born**).

# Determination of blood groups (blood typing)

***This is usually carried by slide technique:***

- *Three drops of blood under test are placed separately on a glass slide.*
- *A drop of Anti-A serum, a drop of Anti-B serum and a drop of Anti-D serum are added to the three blood drops.*
- *Anti-A serum is then mixed with the first drop while Anti-B serum is mixed with the second drop and Anti-D serum is mixed with the third one.*
- *After 2-3 minutes, the drops are examined for antigen – antibody reaction (agglutination).*

## ***Results :***

- If agglutination occurs with Anti-A serum only → the subject is group A.
- If agglutination occur with Anti-B serum only → the subject is group B.
- If agglutination occurs with both sera → the subject is group AB.
- If no agglutination occurs with both sera → the subject is group O.
- If agglutination occurs with Anti-D serum → the subject is Rh positive.
- If no agglutination occurs with Anti-D serum → the subject is Rh negative.

# Blood group determination

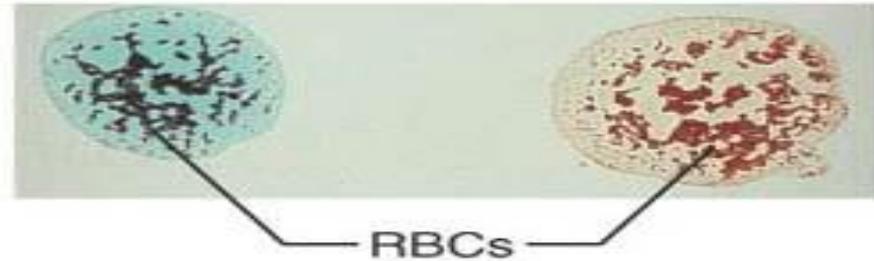
**Blood being tested**

**Serum**

Anti-A

Anti-B

Type AB (contains agglutinogens A and B)



Type B (contains agglutinin B)



Type A (contains agglutinin A)



Type O (contains no agglutinogens)



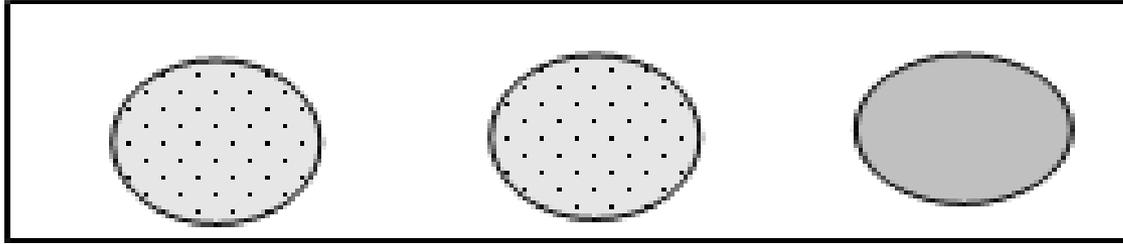
Figure 19-8 Blood Type Testing

Anti-A	Anti-B	Anti-D	Blood type
			A <sup>+</sup>
			B <sup>+</sup>
			AB <sup>+</sup>
			O <sup>-</sup>



# Summary of Slide Typing

<b>Anti-A</b>	<b>Anti-B</b>	<b>Anti-D</b>	<b>Blood Group</b>
Negative	Negative	Positive	O +ve
Positive	Negative	Negative	A -ve
Negative	Positive	Positive	B +ve
Positive	Positive	Negative	AB -ve

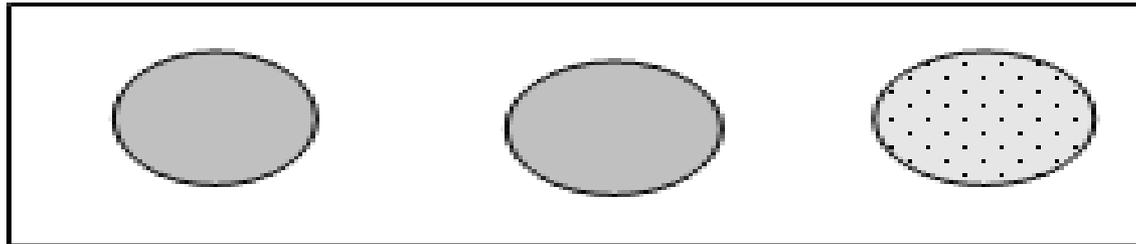


Anti-A serum

Anti-B serum

Anti-D serum

**AB negative**



Anti-A serum

Anti-B serum

Anti-D serum

**O positive**

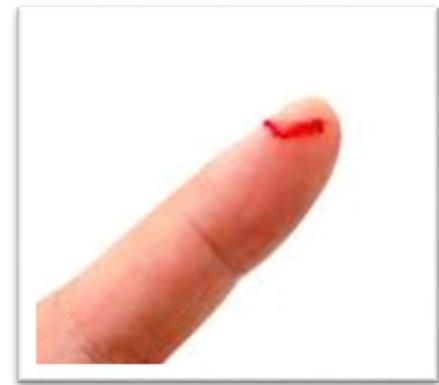
## Experiment (2) (Hemostasis tests)

### ***1- Bleeding time***

**Definition:** It is the time between the start of bleeding from an injured small blood vessel until its complete stoppage without formation of a blood clot.

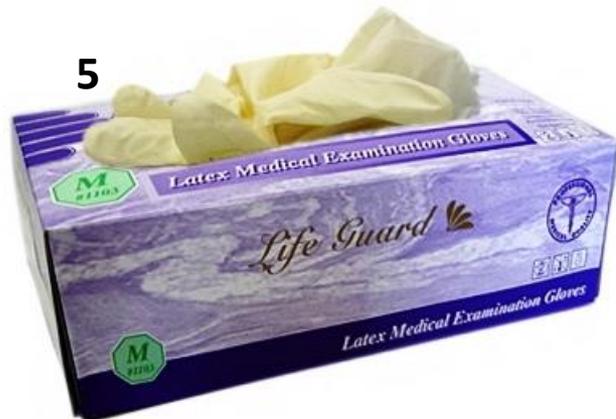
### ***Principle:***

Determination of bleeding time tests the efficiency of hemostatic mechanisms other than blood clotting (**vascular spasm and platelet plug formation**).



## Materials:

1. Sterile, disposable lancet
2. Stopwatch
3. Filter paper
4. Alcohol pads
5. Gloves
6. Bandage



## ***Procedure (Duke's method):***

- Clean the tip of finger or earlobe with alcohol pad.
- Prick the fingertip or earlobe with the lancet.
- The oozing blood is removed by a filter paper every about 30 seconds.
- The time elapsing between the prick and stoppage of bleeding is recorded by a stopwatch.

## DUKE'S METHOD

- Easy to perform
- Requires minimal equipment
- Requirements-alcohol,sterile lancet,stopwatch,filter paper



20

## Duke Method

With the Duke method, the patient is pricked with a special needle or lancet, preferably on the earlobe or fingertip.

The prick is about 3–4 mm deep. The patient then wipes the blood every 30 seconds with a filter paper.



## ***Results:***

**Normal bleeding time ranges between 1-4 minutes.**

## ***Causes of prolonged bleeding time :***

1. Purpura: Thrombocytopenia (decrease in the number of platelets below 50,000/ microliter or cubic mm; mm<sup>3</sup>).
2. Vitamin C deficiency ( Scurvy).
3. von Willebrand disease (due to deficiency of vWF).
4. Prolonged use of aspirin.

## ***2- Clotting (Coagulation) time:***

- It is the time needed for the blood to clot after withdrawal from the body (until fibrin thread is seen).
- It is measured from the time of blood withdrawal till a firm clot is formed.

### ***Method:***

**Non-heparinized capillary tube method.**

### ***Principle:***

It depends on the availability of the clotting factors required for blood clotting by **the intrinsic pathway of prothrombin activator.**

## Materials:

- 1- Sterile, disposable lancet
- 2- Non heparinized capillary tubes
- 3- Stopwatch
- 4- Alcohol pads
- 5- Gloves
- 6- Bandage



## ***Procedure (Non-heparinized Capillary tube method):***

1. Sterilize the fingertip with alcohol, allow to dry and then prick with lancet.
2. The oozing blood is withdrawn into a long glass non- heparinized capillary tube.
3. Short pieces of the tube are broken every 0.5 (half) minute until threads of fibrin are seen between the two ends (clot is formed).
4. The time between blood withdrawal and clot formation is recorded using a stopwatch.



# RESULTS

## *Results:*

**Normal clotting (coagulation) time ranges from 3-10 minutes.**

## **Causes of Prolonged clotting time:**

- A severe liver disease ( in which most clotting factors are deficient).
- Vitamin K deficiency as in newborns, prolonged use of antibiotics, liver diseases and obstructive jaundice.
- Congenital abnormality as in : **Hemophilia** due to deficiency of factor VIII (A) or IX (B).
- Administration of anticoagulants.



**This is a test of ...clotting (coagulation) time .....**



**This is a test of ...bleeding time.....**

Thank

You

Best

Wishes