

HLS MODULE PHYSIOLOGY LAB 2 BLOOD GROUPS & HEMOSTASIS TESTS

BY

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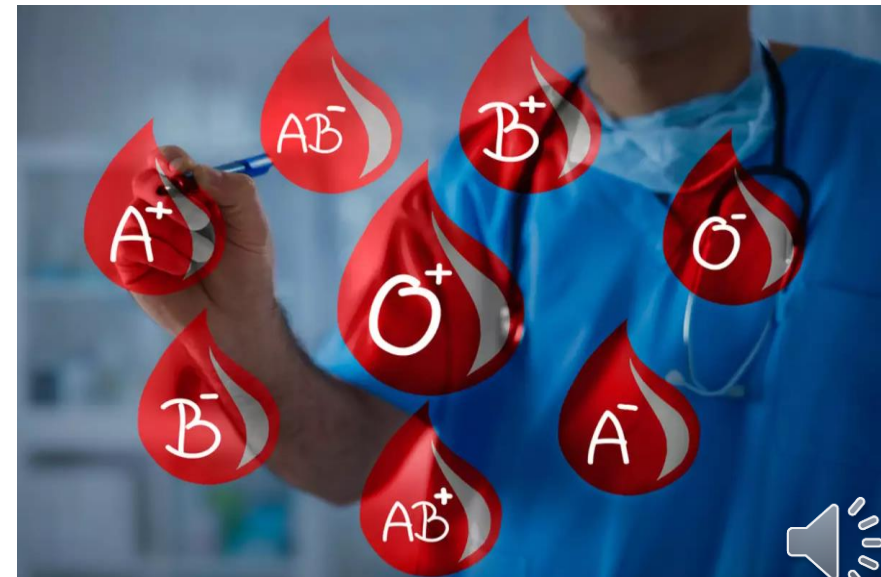
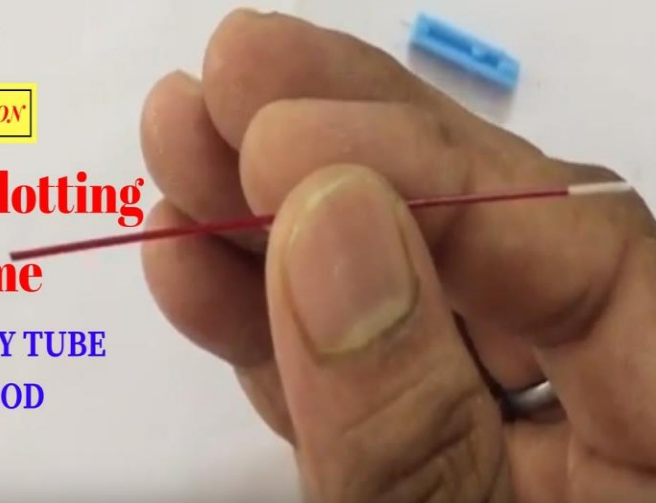
2023-2024



SOLUTION

**Blood Clotting
Time**

CAPILLARY TUBE
METHOD



Experiment 1

BLOOD GROUPS

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



ABO 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 (IgM).
- ✓ The blood of any person doesn't contain an agglutinogen (e.g A) and its corresponding agglutinin (anti-A; α), otherwise Antigen – antibody reaction occurs which results in agglutination and hemolysis of RBCs.



BLOOD TYPE

TYPE A

TYPE B

TYPE AB

TYPE O

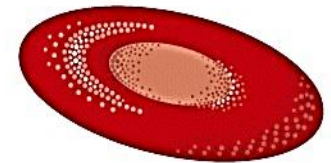
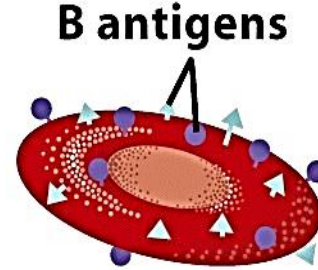
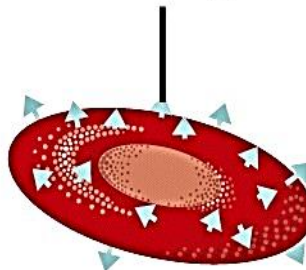
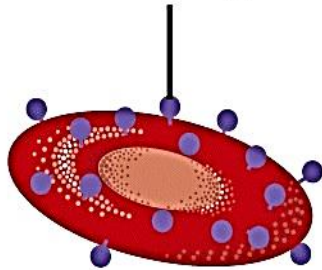
A antigen

B antigen

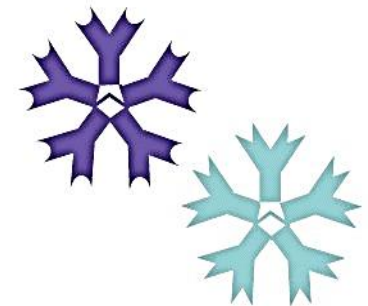
Both A and B antigens

Neither A nor B antigen

Red blood cells



Plasma



Anti-B antibody

Anti-A antibody

Neither antibody

Both anti-A and anti-B antibodies



| Blood group | RBCs membrane agglutinogens (Antigens) | Plasma agglutinins (Antibodies) |
|--------------------|---|---|
| A | A | Anti-B (β) |
| B | B | Anti-A (α) |
| AB | AB | None |
| O | None | Anti-A & Anti-B (α and β) |



- ✓ **Group O is called universal donor** (no agglutinogen and so no agglutination occurs when given).
- ✓ **Group AB is called universal recipient** (no agglutinin 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 blood.
2. Markedly neutralized by free agglutinogens present in the recipient's blood (produced by the daily destruction of human RBCs).

| | | |
|----------------|---|------------------|
| Donor | | Recipient |
| A | | 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.

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



Rh System

2. Rhesus (Rh) blood group system:

Rh factor (D antigen) ; it 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*).

Medico-legal importance; disputed paternity



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)



RBCs

Type B (contains agglutinin B)



Type A (contains agglutinin A)



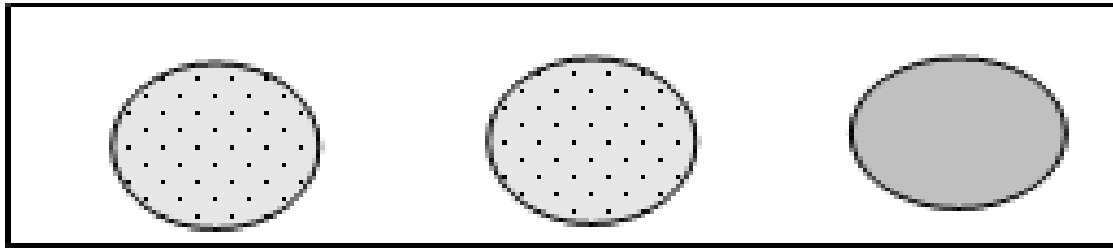
Type O (contains no agglutinogens)



Figure 19-8 Blood Type Testing

| Anti-A | Anti-B | Anti-D | Blood type |
|---|---|---|-----------------|
|  |  |  | A ⁺ |
|  |  |  | B ⁺ |
|  |  |  | AB ⁺ |
|  |  |  | O ⁻ |



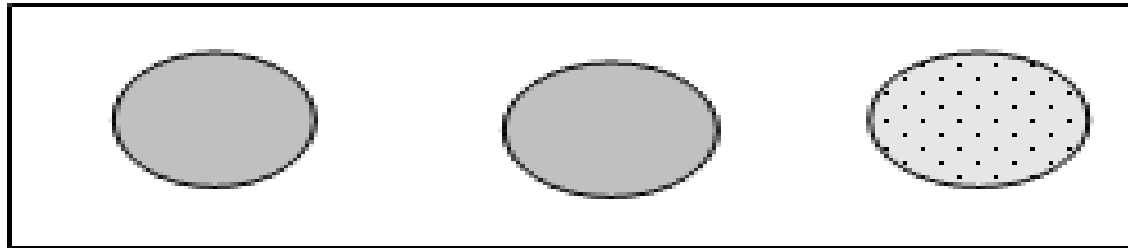


Anti-A serum

Anti-B serum

Anti-D serum

AB negative



Anti-A serum

Anti-B serum

Anti-D serum

O positive



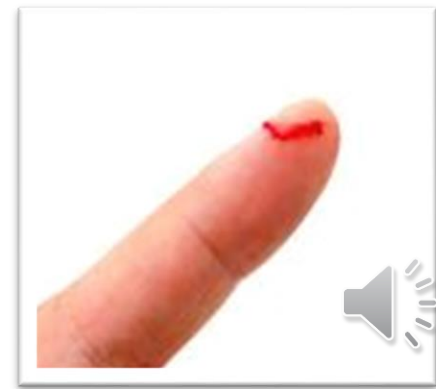
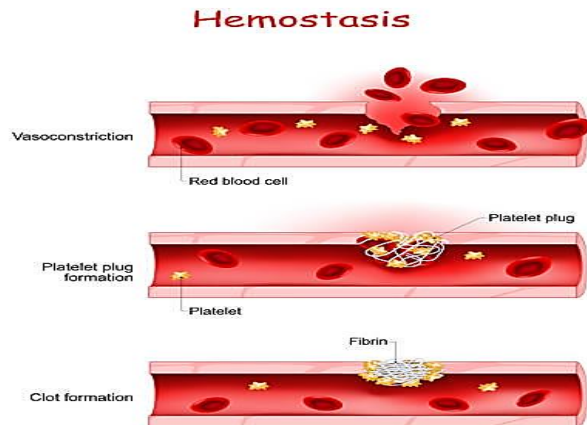
Experiment (2) (Hemostasis tests)

1- Bleeding time

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

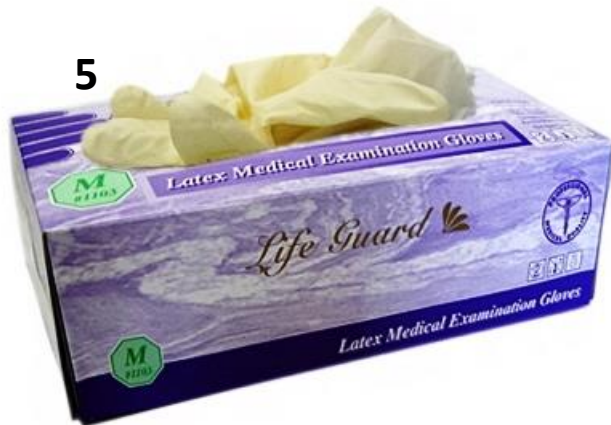
Principle:

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



Materials:

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



Procedure (Duke's method):

- Clean the tip of finger or lobule of ear with alcohol pad.
- Prick the tip of finger or lobule of ear with blood lancet (make sure your pricking is rapid, sharp, deep).
- The oozing blood is removed by a filter paper every about 30 seconds without touching the skin.
- 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



Results:

Normal bleeding time ranges between 1-4 minutes.

Causes of prolonged bleeding time :

1. Purpura: Thrombocytopenic (decrease in the number of platelets below $40.000/\text{mm}^3$) and thromboasthenic purpura (defect in platelets function).
2. Vitamin C deficiency (scurvy) : due to weak vascular walls.
3. Prolonged use of aspirin (due to defect in platelet aggregation).



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 active thromboplastin.**



Materials:

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



Procedure (Non-heparinized Capillary tube method):

1. Sterilize the tip of finger with alcohol, allow to dry and then prick with a sterile pin.
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 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 (antihemorrhagic vitamin) deficiency (due to deficiency of factors II, VII, IX and X) as in newborns, prolonged use of antibiotics, liver diseases and obstructive jaundice.
- Congenital abnormality as in : Hemophilia (due to deficiency of factor VIII or IX or XI) and Para hemophilia (due to lack of factor V)
- Administration of anticoagulant.





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



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





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THANK YOU

THANK YOU

