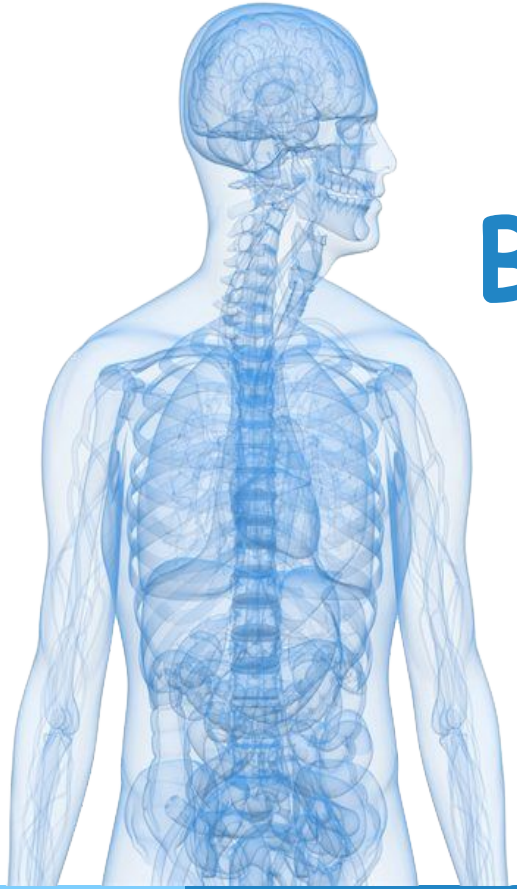

Practical

Blood groups, clotting and bleeding time

Team Leaders:
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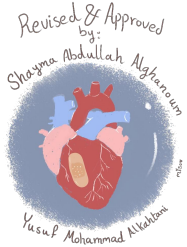
Abdulrahman Alswat



Red: Important
Black: In Male & Female slides
Blue: In male slides
Pink: In female slides
Green: Notes & extra information

Objectives

- At the end of this lab you should be able to:
- Understand and practice the method used in determining blood groups (ABO and Rhesus(Rh) systems).
- Determine your own Bleeding and clotting time.
- Recognize the importance of bleeding time and clotting time in haemostasis.

Aim of the practical

<u>To determine:</u>

- Blood groups
- Clotting time
- Bleeding time

ABO system

Antigen is what makes each blood group differ from each other ?

The presence of substances called. Antigens are like the cells identification tag . Antigens are located on the cell's membrane

A blood group: has A antigen on the cell membrane of RBCs and anti - B antibody in plasma

40% of people has blood group A

B blood group: has B antigen on the cell membrane of RBCs and anti - A antibody in plasma .

11% of people has Blood group B

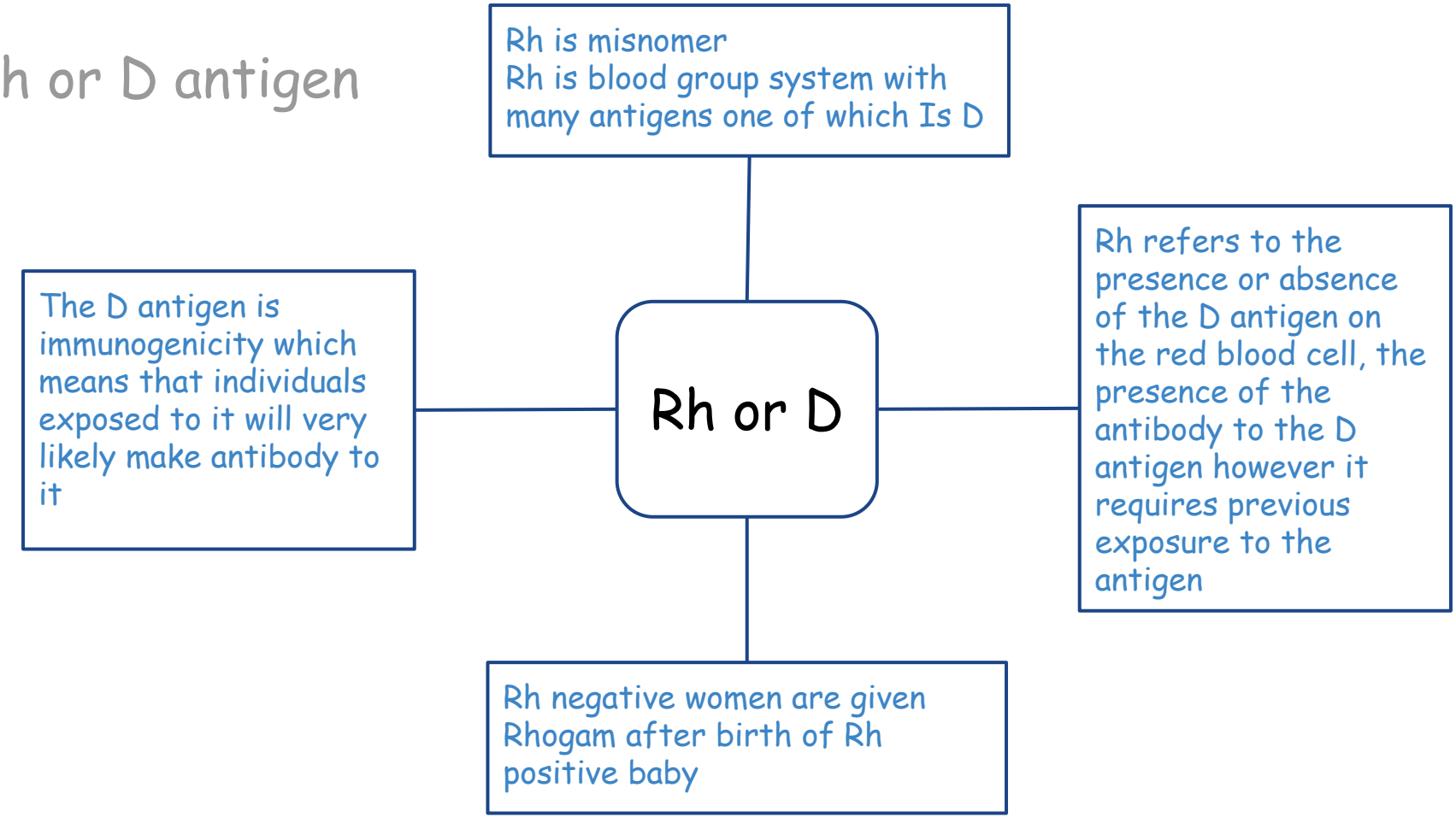
AB blood group: has both A and B antigen on the cell membrane of RBCs and NO antibodies in plasma .

4% of people has blood group AB

O blood group: has NO antigen on the cell membrane of RBCs and anti A and anti B antibodies in plasma

45% of people has blood group O

Rh or D antigen



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

Blood groups Antigens

Materials

- A grease Pencil.
- High titer anti-A ,anti-B and anti-D sera.
- Microscope slides.
- Alcohol swab and pricker.

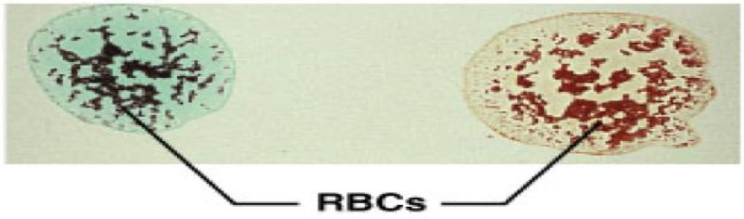
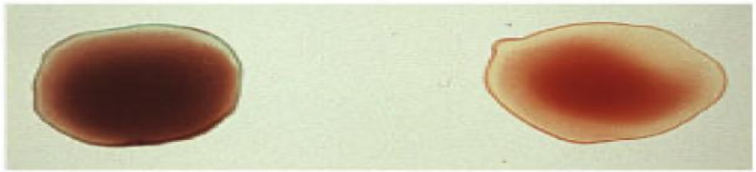
Procedure:

1. Prick a finger and place one drop of blood in each of the compartments A,B and D (these are clearly labeled on the microscope slides provided)
2. Quickly add a drop of anti-A , anti-B and anti-D sera to each compartment
3. Mix the serum with the drop of blood by moving the slides gently for a min or two , then examine the mixtures for signs of RBC agglutination or clump formation

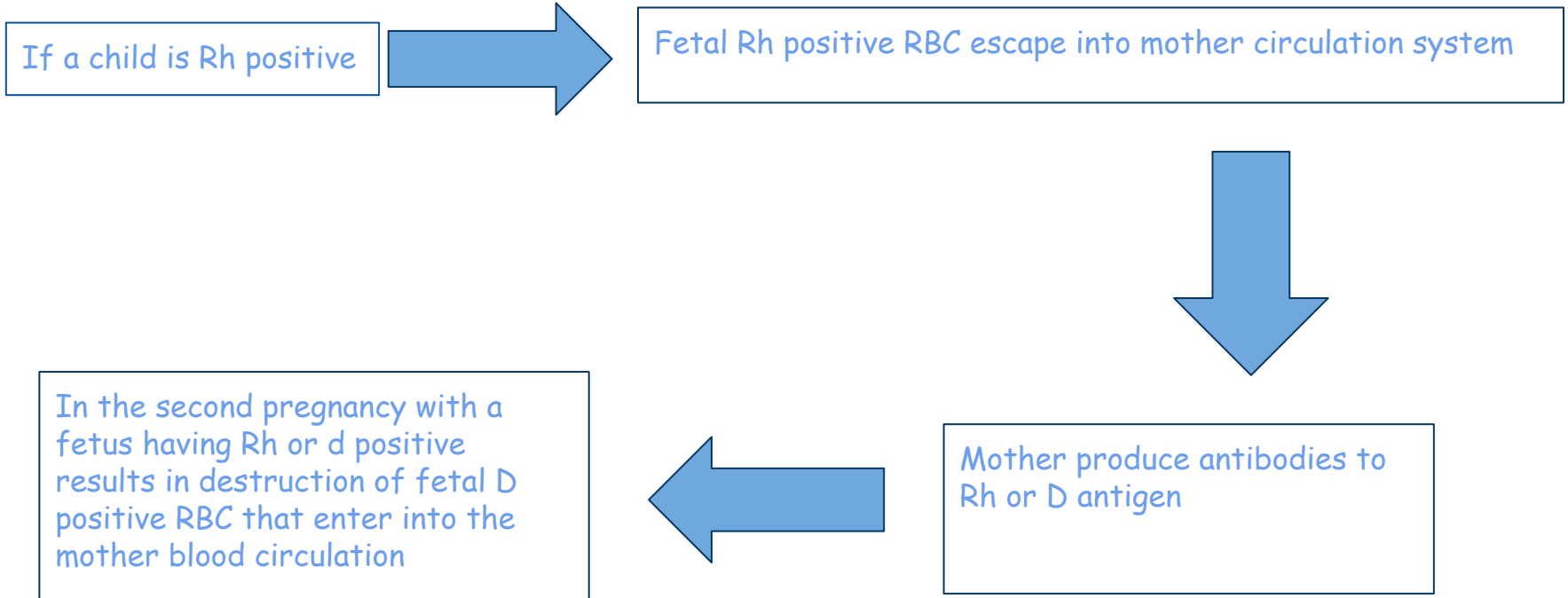
Clinical applications

Important in the following conditions:

- Blood transfusion
- Blood products
- Hemolytic disease of the newborn (HDN)

Blood being tested	Serum	
	Anti-A	Anti-B
Type AB (contains agglutinogens A and B)		
Type B (contains agglutinogen B)		
Type A (contains agglutinogen A)		
Type O (contains no agglutinogens)		

Hemolytic disease of the Newborn



Interruption of slide typing

- If we have A blood group on a slide and we add anti A antibody it will coagulate
- If we have a B blood group on a slide and we add anti B antibody it will coagulate
- If we have AB blood group on a slide and we add anti A or anti B antibodies it will coagulate in both cases
- If we have O blood group on a slide it will not coagulate with any of the ant A or anti B antibodies
- If we add anti rhesus Rh to a blood group and it coagulate then the blood group has Rh or D antigen if it didn't coagulate then there is no D antigen hence it is Rh negative

Clotting Time

- The time required for blood to form a clot.
- Gives a rough measure of all intrinsic clotting factors in the absence of tissue factors.
- Normal coagulation time in glass tubes: 3-10 min , 5–15 min
- Used in diagnosing hemophilia
- Chief application: monitoring anticoagulant therapy. (Heparin, Warfarin)

Materials

- Capillary tubes of uniform size (non heparinized)
- A petri-dish
- Alcohol swabs
- Cotton wool
- Plasticine
- A water bath set at 37˚

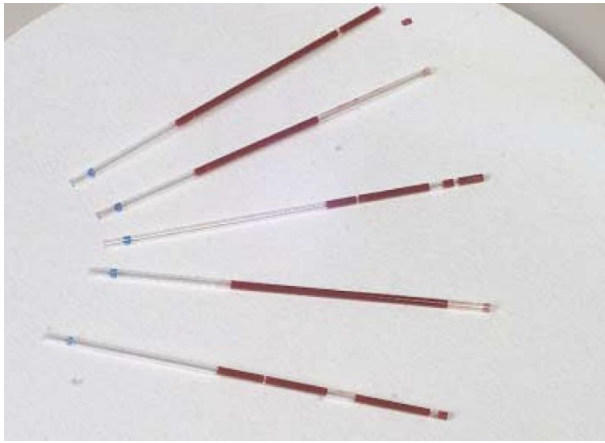
Procedure:

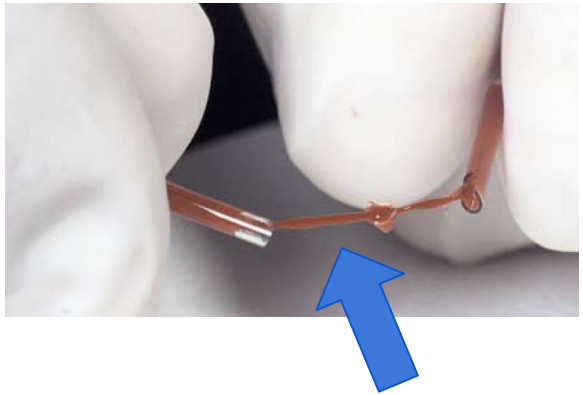
1. Clean finger with alcohol swap, prick it with lancet and note the time that the prick is made.
2. Wipe away the first drop of blood. Then while the blood is still flowing freely place one end of a capillary tube in the blood. Holding the tube horizontally let it fill by capillary action, fill more than one tube.
3. Close the end of the capillary tube with plasticine. Place the tube in the water bath.
4. Two minutes after making the puncture, break a capillary tube and separate the two halves slowly.
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

Clotting Time

Result:

- Usually the clotting time measured by this method is in the range 5-15 min , 3-6 min
- Prolong clotting time seen in deficiencies in the intrinsic coagulation pathway.
- Example: hemophilia due to deficiency of Factor VIII (8).





- Coagulation: also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It results in the cessation of blood loss, therefore maintains homeostasis.
- Hemophilia: a rare disorder in which your blood doesn't clot normally because it lacks sufficient blood-clotting proteins (intrinsic clotting factors).

Alternative method

Clotting Time using Test Tube method

Procedure:

- Place 2 ml blood into non heparinized test tube incubated in water bath.
- Time from pricking finger to clot formation is clotting time.
- Every 30 second invert gently to check for clot formation.
- Normally 6-10 min by this method.
- Measurement of the clotting factors are better used.

Bleeding Time

- The time taking 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.

Materials

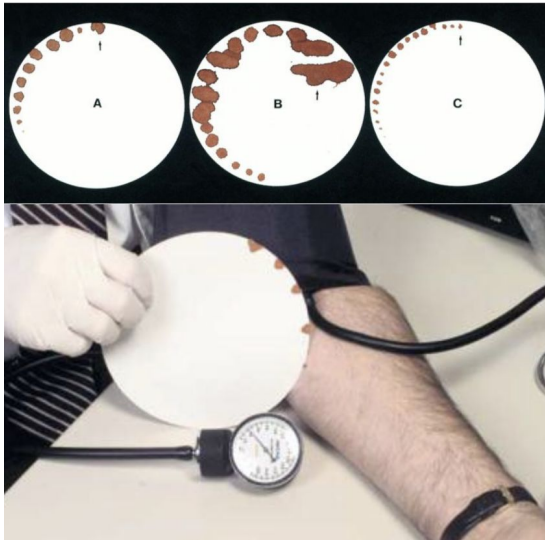
- Alcohol swabs.
- Filter paper.
- A stopwatch.
- A stylette to prick an earlobe

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 within 2-5 minutes.

Bleeding Time

The Standardized Template Method



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

Bleeding Time

Note:

•If the bleeding time exceeds 15 minutes:

- Stop the procedure.
- Apply pressure to stop the bleeding.
- Report as greater than 15 min.

Clinical applications

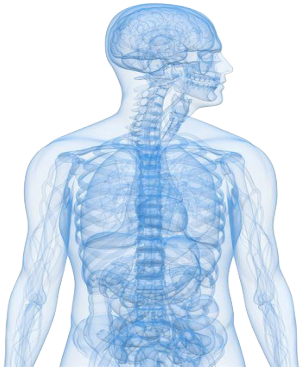
Bleeding time is prolonged in these conditions:

- Platelet dysfunction
- Thrombocytopenia
- Vitamin K deficiency
- Medications: aspirin
- Von Willebrand disease

Click for:

- Useful videos
- Laboratory File

Good luck!



Thank You

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