University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Blood Grouping and Cross Matching

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Outlines

- Objectives of blood grouping experiment
- Introduction, Mechanism, and principle

- Methods for determining blood group
- Procedure

- Some clinical implications

Objectives

- To determine the major blood groups (ABO system) and Rh type of the individual.
- To learn the mechanism and principle of the experiment .
- To get an idea about ABO & Rh incompatibility.

Introduction

- The surfaces of the RBCs contain numerous glycoprotein markers known as antigens (Ag) . There are many different types of antigens , but the most common are the A,B, & D antigens (D is also known as the Rh factor). According to the presence of these antigens , human blood can be classified into many groups.

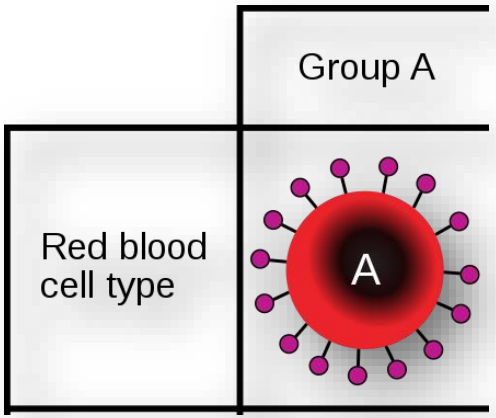
- About 6 months after birth , lymphocytes begin to produce certain antibodies (Ab) which are proteins that circulate in the blood plasma . These Ab are isolated from the plasma & used as anti-sera in the laboratory to determine blood types .

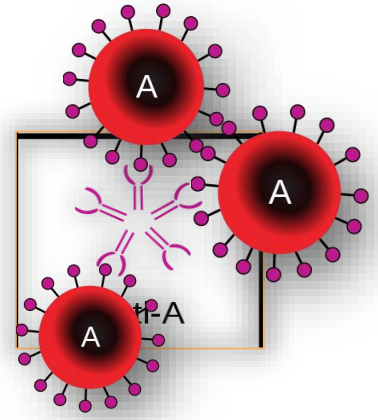
Introduction

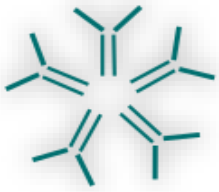
- ***Blood grouping :*** also called blood typing, is a test performed to define the individual blood group and it is performed by mixing anti-ABO and anti-Rh agglutinins (antibodies, anti-sera) with the RBCs .

- The erythrocytes of a person contain blood group antigens on the surface of the membrane. When these antigens are allowed to treat with corresponding antibodies (agglutinins), antigen-antibody reaction occurs and form ***agglutination***.

Agglutination







Anti-B

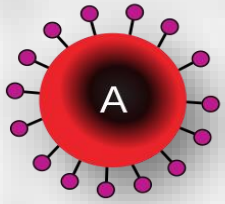
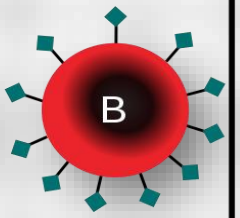
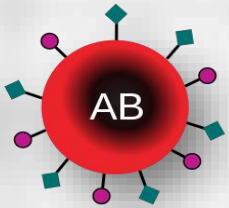
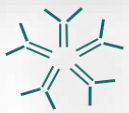
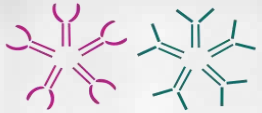
Introduction

- **There are two important blood group systems :**
  1. ABO system (the most important blood group system in blood transfusion)
  2. Rh system (the most important blood group system after ABO)

- The difference between ABO and Rh system is that in ABO , the agglutinins are formed spontaneously and can cause immediate reaction . While in Rh system ,a spontaneous formation of agglutinins does not occur which can cause only delayed reactions.

Introduction: ABO System

ABO blood grouping system divides blood into 4 groups (A,B,AB,O) based on the presence or absence of antigens A and B on the RBC surfaces and naturally occurring antibodies anti-A and anti-B on serum. In normal human blood, a reciprocal relationship exists between antigens and antibodies as described below:

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	A antigen	 B antigen	 A and B antigens	None

Introduction : Rh System

- There are 6 common types of Rh antigens these are C,D,E,c,d,e .
- The most common is the D antigen . *Any body that has D agglutinogens is Rh positive .*

- The antibodies to Rh antigens do not occur naturally but can occur after blood transfusion and during pregnancy .

- The percentage of people who are Rh+ is 75 – 85 % .

ABO and Rh Systems (Summary)

The underlying table shows the relation between blood group, the presence of agglutinogens and agglutinins, and their frequency.

Blood type	Antigens	Antibodies	Frequency
O	----------	Anti-A, Anti-B	46%
A	A	Anti-B	42%
B	B	Anti-A	9%
AB	A , B	----------	3%
Rh +	D-antigen		
Rh -	None		

Mechanism and Principle

Blood grouping is done **on the basis of agglutination** .

Agglutination means the collection of separate particles like RBCs into clumps or masses. Agglutination occurs if an antigen is mixed with its corresponding antibody . For example, when A antigen is mixed with anti-A serum, the anti-A bodies in the serum stick to the A antigens on the erythrocytes. ***This Ag-Ab reaction will give the slide a beaded appearance.***

Methods

Slide Method

- Requires less blood and less time to get the result.
- It is the method that we are going to use in today's lab.

Tube Method (Gold standard method)

- Allows longer incubation of Ag & Ab without drying.
- Tubes can be centrifuged to enhance the reaction.
- Can detect weaker antigen – antibody reaction.

Cross Matching

- This is a direct test of ***compatibility*** of donor's cells and recipient's serum . In this test the donors RBCs are mixed with the recipient serum on a slide . If agglutination occurs this means that the donor's blood is incompatible with the recipient blood .

- It is a prerequisite for blood transfusion to avoid reactions of mismatched transfusion .

Materials and Instruments

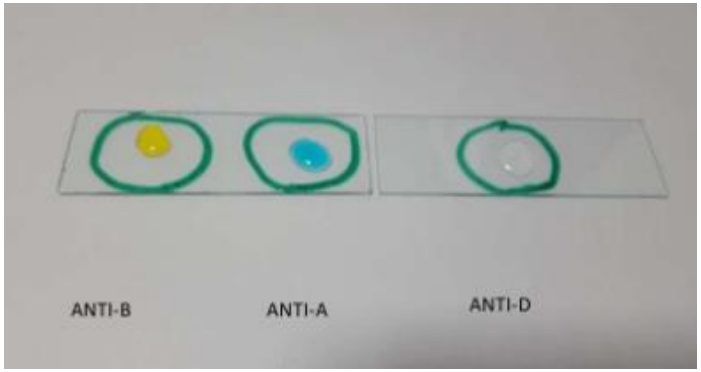
- Clean dry microscope slide.
- Antisera (anti-A serum, anti-B serum, and anti-D serum)
- Capillary blood.
- Toothpick for mixing.
- Lancet , alcohol 70%, cotton
- Wax pencil.

Procedure

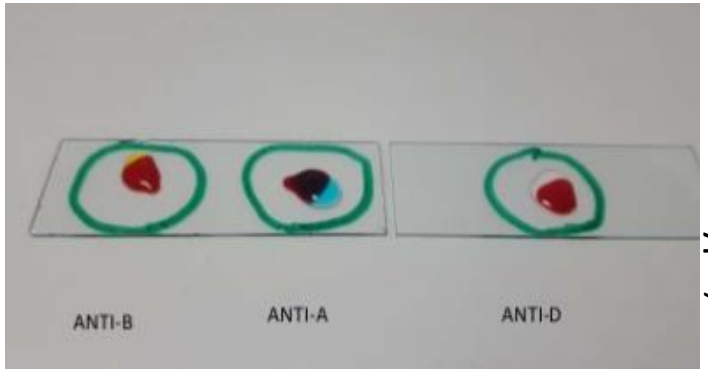
1. On a prepared microscope slide with three circles drawn with wax pencil and labeled A , B , and Rh, add anti-A serum to circle labeled A , Anti-B serum to the one labeled B , and anti-D (or anti-Rh) to circle labeled D . To make it easier and avoid any confusion, we will use two slides. On one slide put anti-A serum & anti-B serum and on the other slide put anti-D serum .
2. Place a small drop of blood in each circle (make sure you have enough blood , but do not completely fill the circle … if you do , you have too much blood on your slide) .
3. Mix the contents of each circle with a clean toothpick . The reaction will appear in **20-60 sec**.
4. Check for agglutination by naked eye or by microscope and the test should be performed in a warm room temperature to prevent the action of cold agglutinins which may cause a confusion . You can place slide on a white piece of paper to better view the agglutination process .

Procedure Steps in Pictures

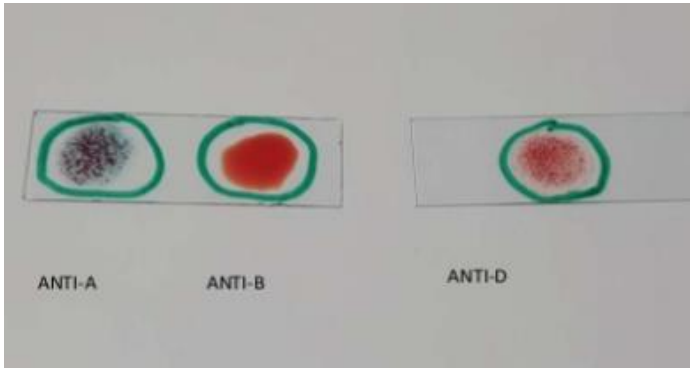
Step 1

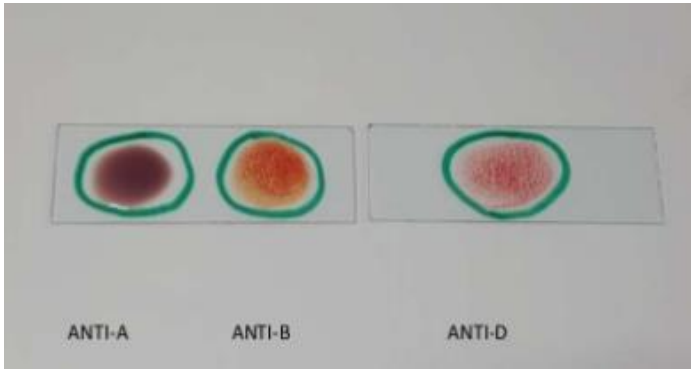


Step 2



After mixing ani-sera with the blood, you will get something as in the below slides (depending on the blood type)

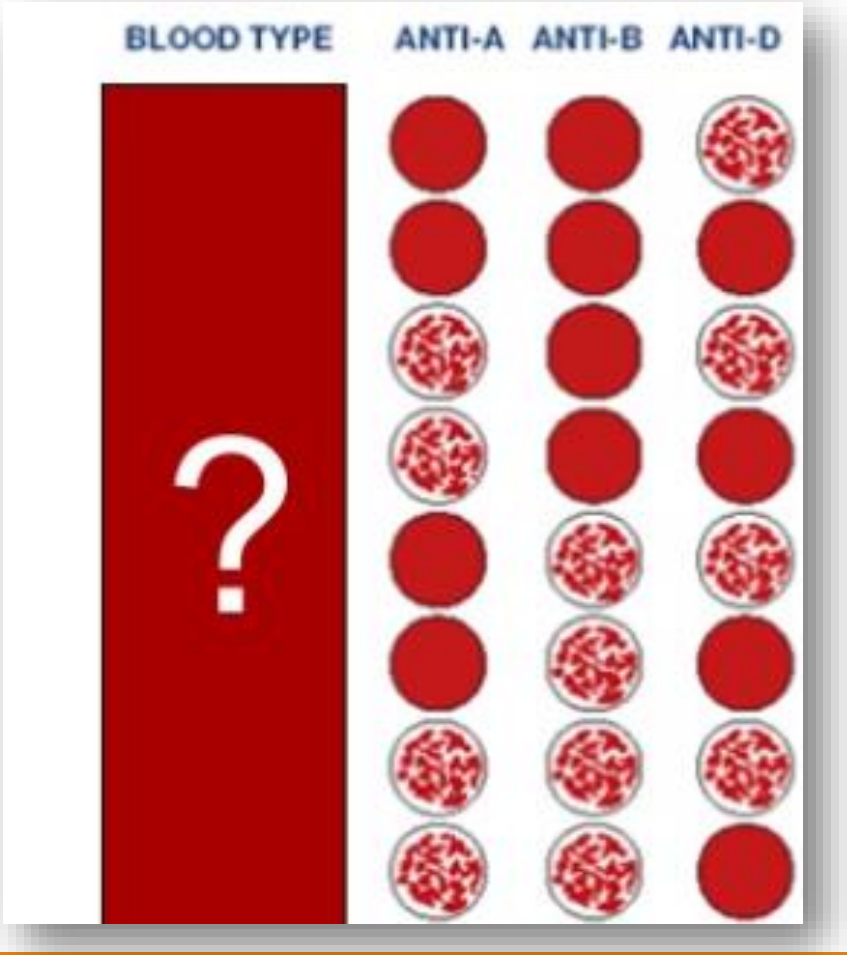




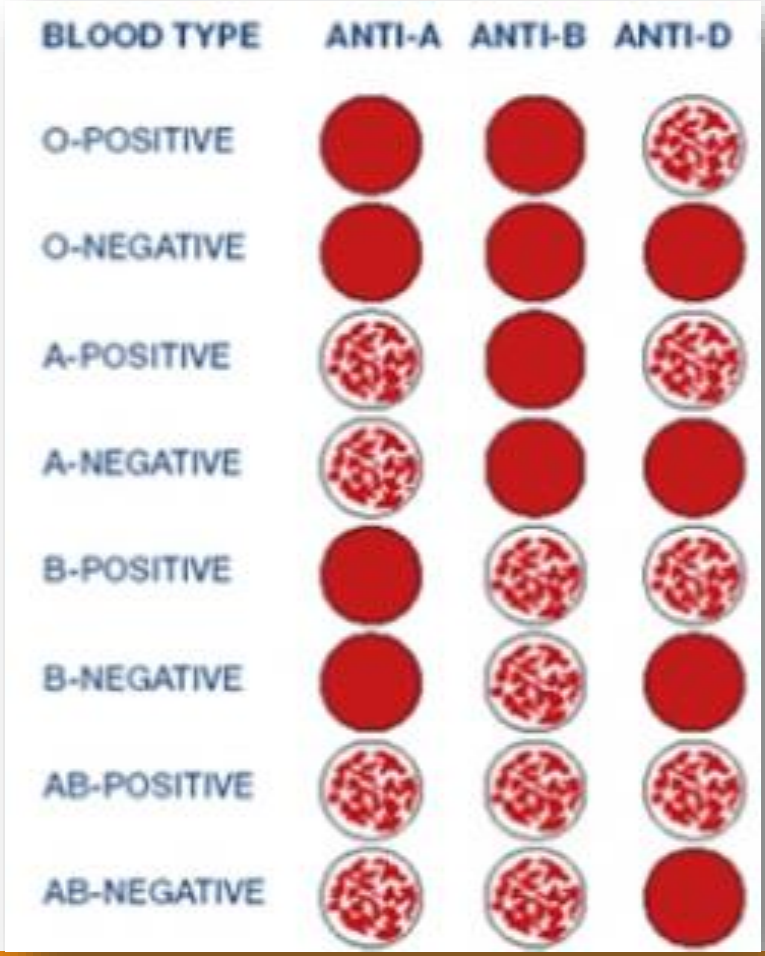
Important Note

Be sure to have a clean microscope slide (slides) with three circles drawn in wax pencil and the circles labeled A ,B , and Rh (or D) available ***before*** you stick yourself with lancet !

How to Read Your Results



How to Read Your Results



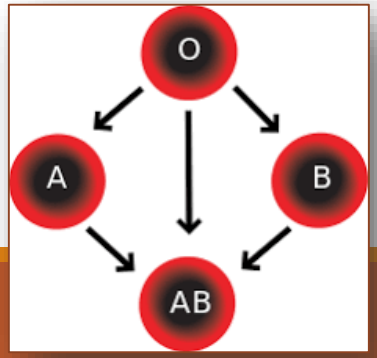
Universal Recipient Vs Universal Donor

Universal Recipient

- Plasma of ***AB group*** has no antibody. This does not cause agglutination of RBCs from any other blood group. People with AB group can receive blood from persons with any blood group
- Thus, people with this blood group are called ***universal recipients*** .

Universal Donor

- RBC of ***O group*** has no antigen (no Ag-A/ Ag-B) and so agglutination does not occur with any other blood group .
- Thus, "O " group blood can be given to any blood group persons and people with this blood group are called ***universal donors .***



Source of Errors

- Inadequate cleaning of the microscope slide. Wet slide can cause an error as well.
- Circles not labeled properly.
- Very low room temperature .
- Drying of reaction giving a false positive results .
- Large drops of blood will lead to overflow the circle to other one.

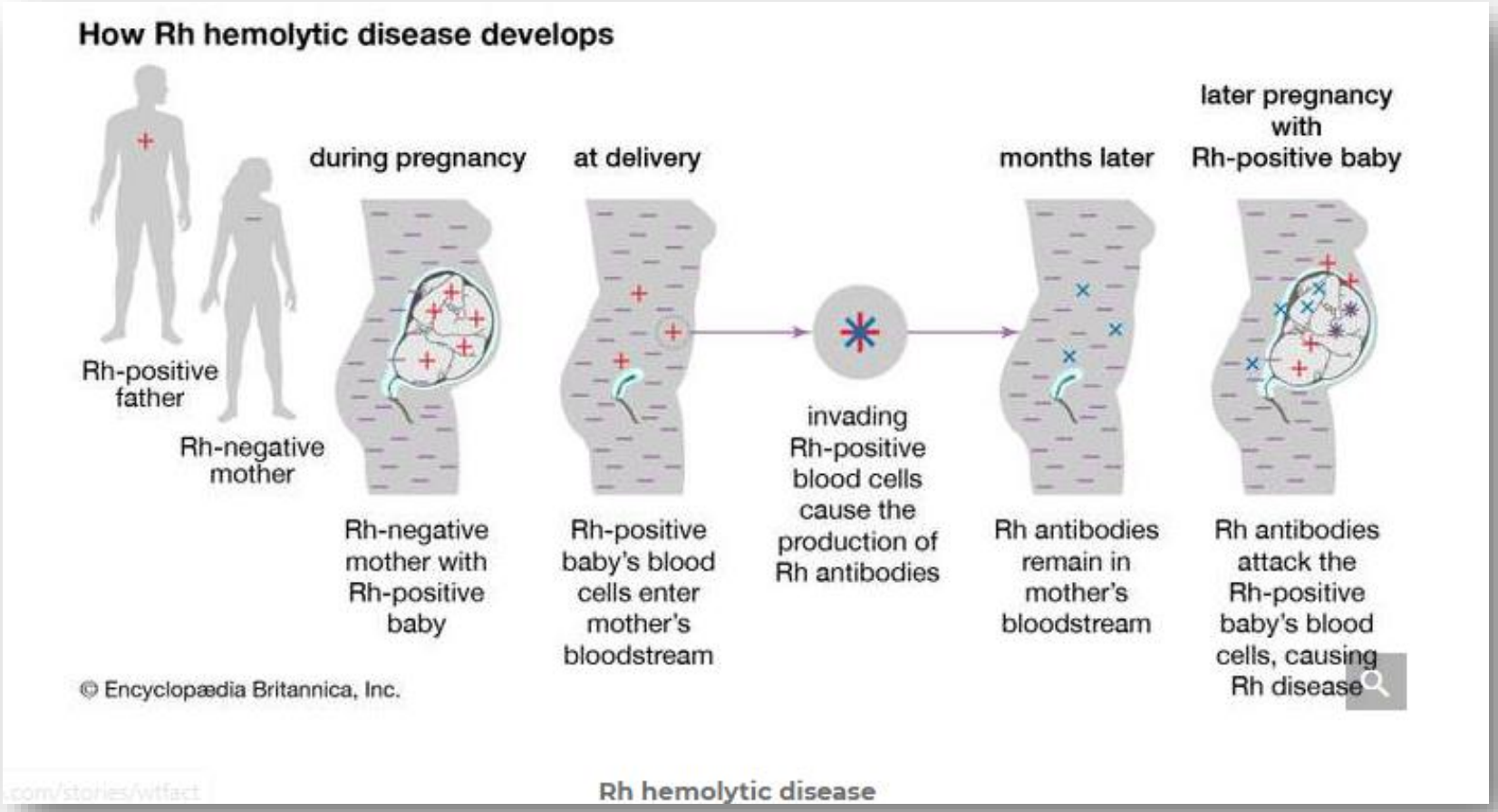
Advantages and Disadvantages of Slide Method

Advantages	Disadvantages
✓ Small sample volume ✓ Fast method ✓ Preliminary typing test ✓ Easy	✓ Less sensitive ✓ Drying of reaction giving to false positive results

Clinical Implications: Hemolytic Disease of Newborn

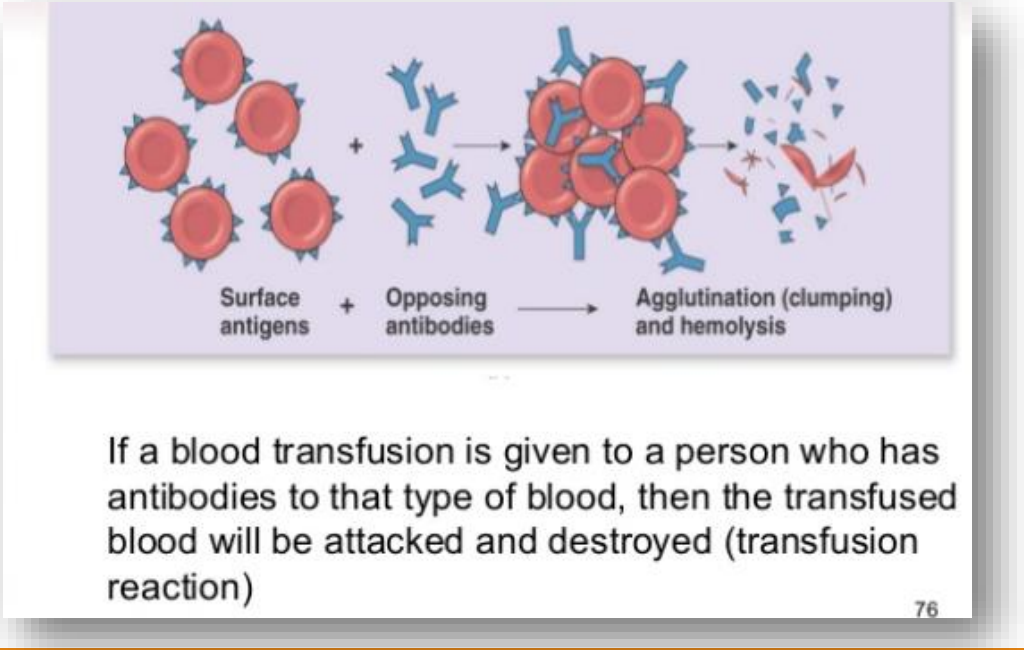
- ✓ During pregnancy for the Rh+ offspring of Rh-incompatible parents, when the mother is Rh- and the father is Rh+. The first child of such parents is usually in no danger unless the mother has acquired anti-Rh antibodies by virtue of incompatible blood transfusion.
- ✓ During delivery, however, a small amount of the fetus's blood may enter the mother's bloodstream. The mother will then produce anti-Rh antibodies, which will attack any Rh-incompatible fetus in subsequent pregnancies. This process produces ***erythroblastosis fetalis***, or ***hemolytic disease of the newborn*****,** which can be fatal to the fetus or to the infant shortly after the birth.
- ✓ The disease can be avoided by vaccinating the mother with ***Rh immunoglobulin*** after delivery of her firstborn if there is Rh-incompatibility. The Rh vaccine destroys any fetal blood cells before the mother's immune system can develop antibodies.

Clinical Implications: Hemolytic Disease of Newborn



Clinical Implications: Transfusion Reaction

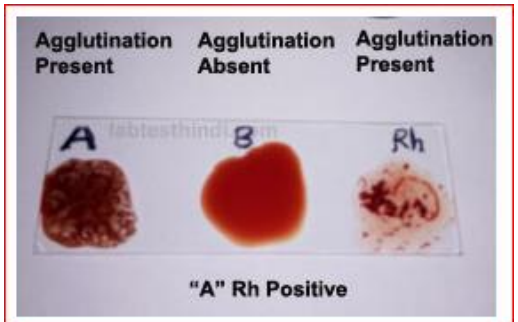
Transfusion Reaction occurs due to transfusion of incompatible blood . The reactions may be mild causing only fever & hives (skin lesions characterized by itching) or may be severe leading to renal failure , shock and death.

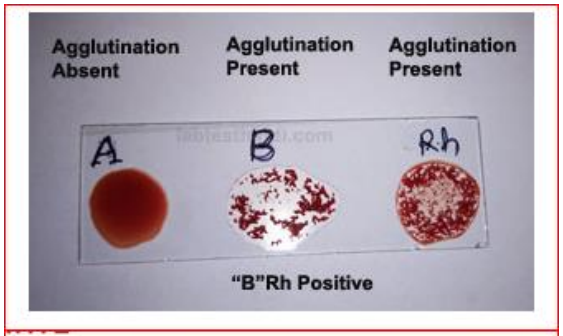


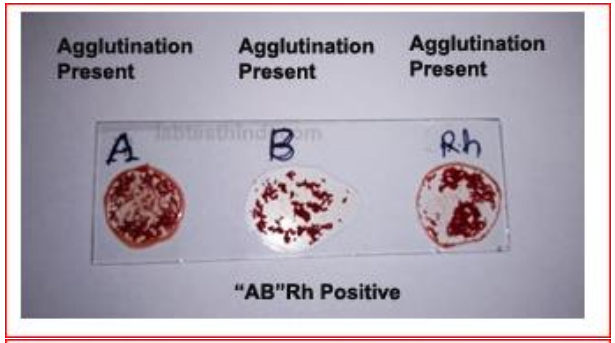
Clinical Implications: Kidney Failure

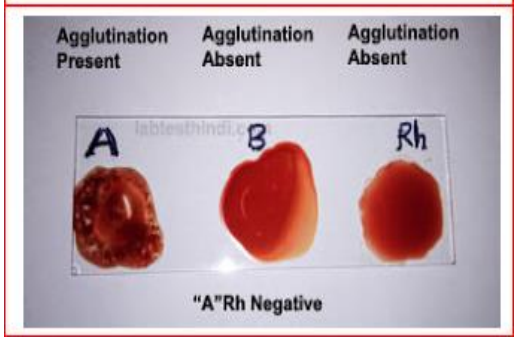
- Because the arms of the ***Y-shaped antibodies*** attach randomly to more than one nonself erythrocyte surface, they form clumps of erythrocytes. This process is called ***agglutination.***
- The clumps of erythrocytes block small blood vessels throughout the body, depriving tissues of oxygen and nutrients.
- As the erythrocyte clumps are degraded, ***in a process called hemolysis***, their hemoglobin is released into the bloodstream. This hemoglobin travels to the kidneys, which are responsible for filtration of the blood. However, the load of hemoglobin released can easily overwhelm the kidney's capacity to clear it, and the patient can ***quickly develop kidney failure***.

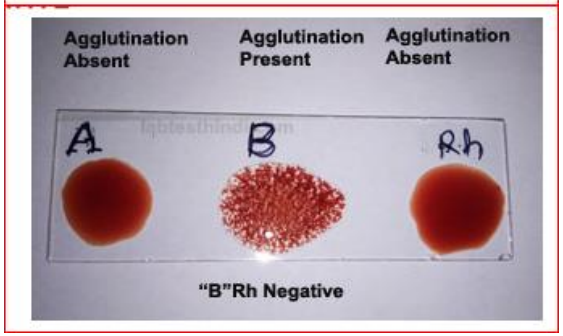
Summary of all the results you may get:

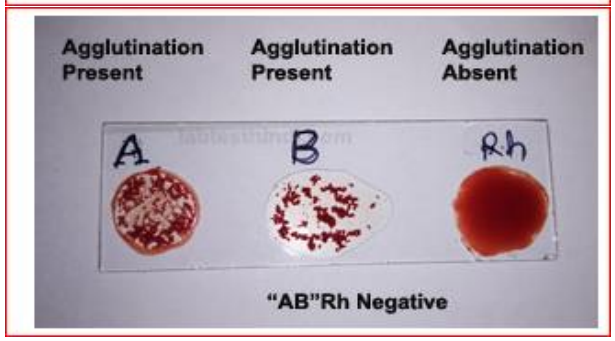


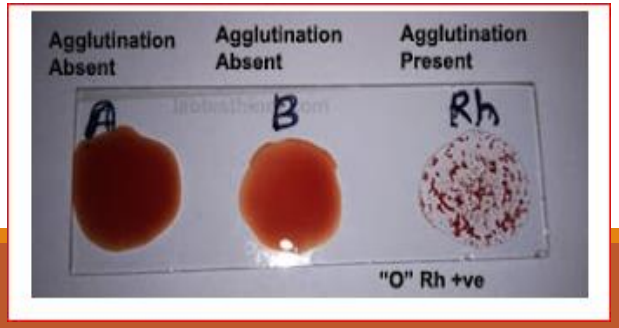


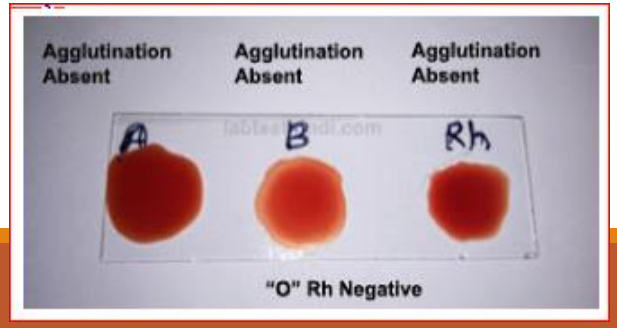












Take Home Assignment #1: Fill the Blank Rectangular Spaces

Blood Group	A	B	AB	O
Surface antigens Please draw the surface antigens present on the red blood cells of each blood type				
Antibodies in the blood Please draw the shape of the antibodies present in the blood of each blood type			none	
Can accept blood from Please write down the blood types each blood type can accept blood from				
Can give blood to Please write down the blood types each blood type can give blood to				

Take Home Assignment #2: Case Study

Ahmed is a 17-year-old who was admitted to hospital following a car crash. He suffered a crushing injury to his right leg. He is booked for surgery and his blood group is ***Type AB***. Who in your group can give blood to Ahmed ? Explain briefly?

BLOOD TYPE A

PERSONALITY TRAITS

by Psychología.co

- kind
- compassionate
- sensitive
- calm
- curious
- loyal
- idealistic
- deep
- committed

BLOOD TYPE B

PERSONALITY TRAITS

by Psychología.co

- friendly
- outgoing
- energetic
- expressive
- curious
- creative
- imaginative
- independent
- spontaneous

BLOOD TYPE AB

PERSONALITY TRAITS

by Psychología.co

- independent
- outgoing
- spontaneous
- energetic
- fun loving
- quick
- adaptable
- creative
- problem solver

https://psychologia.co/blood-type-personality/

BLOOD TYPE O

PERSONALITY TRAITS

by Psychología.co

- responsible
- practical
- strategic
- organized
- determined
- decisive
- objective
- success-oriented
- logical

https://psychologia.co/blood-type-personality/

Thank you