BLOOD GROUPING

Relevance

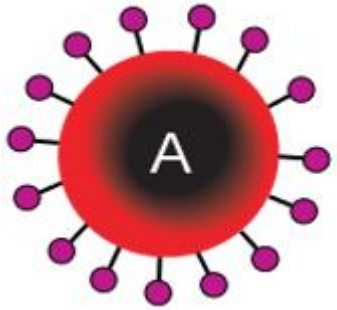
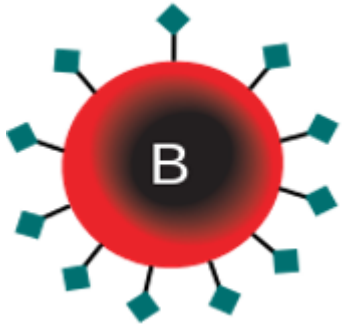
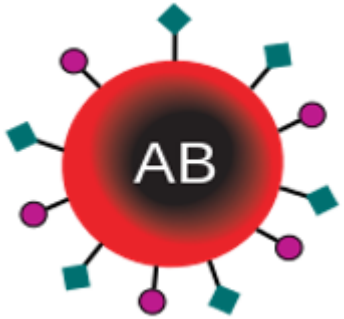
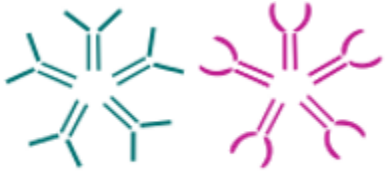
- Individuals can be divided into groups according to the presence or absence of antigen (agglutinogens) on the membrane of their red blood cells & other tissues;

1. Group A (antigen A)
2. Group B (antigen B)
3. Group AB (antigen A & B)
4. Group O (neither A nor B antigen)

Relevance

- These groups contains antibodies (agglutinin) of the opposite type in their plasma;

1. Group A (anti B antibody)
2. Group B (anti A antibody)
3. Group AB (neither anti A nor anti B antibody)
4. Group O (both anti A & anti B antibody)

Blood group	A	B	AB	O
RBC type				
Antigens in RBC	 A antigen	 B antigen	 A and B antigens	NONE
Antibodies in plasma	 Anti-B	 Anti-A	NONE	 Anti-A and Anti-B

Frequencies of blood group

ABO type	%
O	47
A	41
B	9
AB	3

Rh factor

- It is an antigen found on the red cells of 80–85% of humans also called Rh antigen.
- The Rh factor is so named because this antigen was discovered in the rhesus monkey by Landsteiner and Weiner in 1940.

Rh factor

- There are several varieties of Rh antigen (C, D, E) but the D antigen is the most common, and antigenically, the most potent.
- Therefore, Rh +ve persons are also called D+ve & Rh –ve are called D–ve.
- The antibody of D antigen is called anti-D antibody (anti-Rh antibody).

Rh factor

- There are no naturally occurring antibodies against Rh (D) antigen, but can be produced in Rh –ve persons in either of 2 ways:

1. When an Rh –ve person is given Rh +ve blood.

2. When an Rh –ve mother carries an Rh +ve fetus.

Principle

1. The surfaces of red cell membrane contain a variety of antigens (**agglutinogens**), while the plasma contains antibodies (**agglutinins**).

2. To determine the blood group of a person, RBC are made to react with commercially available antisera containing known agglutinins.

Principle

3. The slide is then examined under the microscope to detect the presence or absence of clumping and hemolysis (agglutination) of red cells which occurs as a result of antigen-antibody reaction.

Apparatus and materials

1. Microscope
2. glass dropper with a long nozzle, sterile blood lancet, sterile cotton/ gauze swabs, alcohol,

   5 ml test tube, & toothpicks
3. Clean, dry microscope slides
4. Normal saline

Apparatus and materials

5. **Blood group kit;**

   I. **Anti-A serum**

   - Contains monoclonal anti-A antibodies (anti-A or alpha (α) agglutinins).

   II. **Anti-B serum**

   - Contains monoclonal anti-B antibodies (anti-B or beta (β) agglutinins).

   III. **Anti-D (anti-Rh) serum**

   - Contains monoclonal anti-Rh (D) antibodies (anti-D agglutinins).

Apparatus and materials

6. RBC suspension

- A suspension of red cells in saline should preferably be prepared and used instead of adding blood drops directly from the fingerpick to the antisera for the following reasons:

Apparatus and materials

1. Dilution of blood permits easy detection of agglutination if present.
   - RBC in undiluted blood tend to form large rouleaux and masses. These may be mistaken for agglutination.
2. Plasma factors likely to interfere with agglutination are eliminated.

Procedure

1. Using a glass-marking pencil, divide 2 slides, each into two halves by a line drawn down the middle.
   - Mark the slides as follow; anti-A, anti-B, anti-D & ‘C’ (for control).
2. Place one drop of saline on the slide marked ‘C’.

Procedure

3. Determination of Blood group; Put one drop of:

   I. **Anti-A serum on the slide** (marked anti-A)

   II. **Anti-B serum on the slide** (marked anti-B)

   III. **Anti-D serum on the slide** (marked anti-D)

Procedure

4. Add one drop of red cell suspension on each of; **anti-A sera, anti-B sera, normal saline.**

5. Add one drop of blood from a finger prick or venous blood on the **anti-D sera.**

Procedure

6. Mix the anti-sera with RBC suspension, and saline with RBC suspension on each slide by gently tilting it first one way and then the other a few times.

   - You may use separate toothpicks to transfer red cell suspension to the anti-sera & saline, and for mixing them.

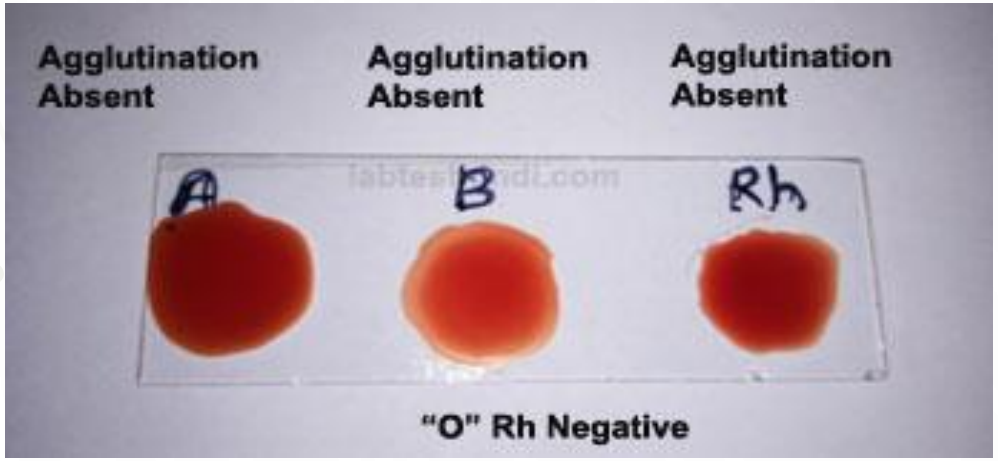
Procedure

7. Wait for 8–10 minutes, then inspect the antisera-red cell mixtures (“test” mixtures) and “control” mixture.

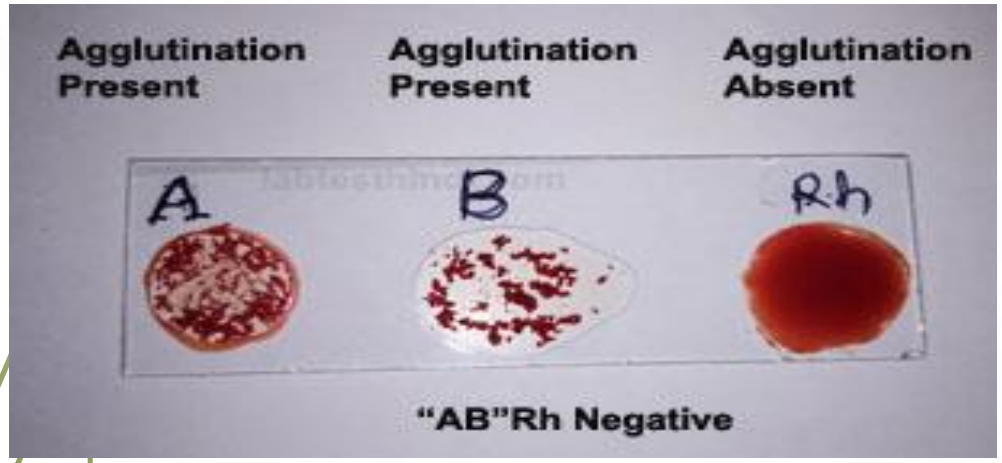
- First with the naked eye to see whether agglutination has taken place or not.
- Then confirm under low magnification microscope.

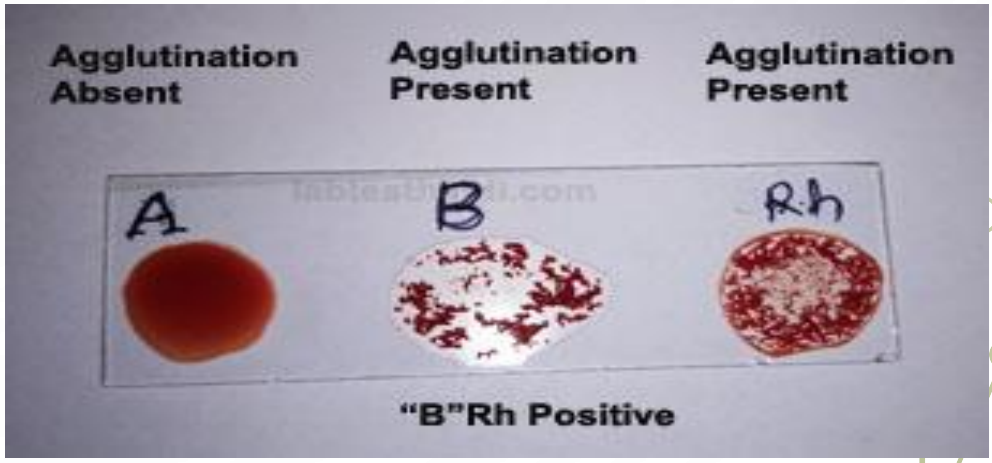
Observations and results

1. If agglutination occurs, it is usually visible to the naked eye.
    - The hemolysed red cells appear as isolated (separate), dark-red masses (clumps) of different sizes and shapes.
2. Under LP objective, the clumps are visible as dark masses and the outline of the red cells cannot be seen.


Agglutination Absent
Agglutination Absent
Agglutination Absent
A
B
Rh
"O" Rh Negative


Agglutination Present
Agglutination Absent
Agglutination Present
A
B
Rh
"A" Rh Positive


Agglutination Present
Agglutination Present
Agglutination Absent
A
B
Rh
"AB"Rh Negative


Agglutination Absent
Agglutination Present
Agglutination Present
A
B
Rh
"B"Rh Positive

Universal donor & recipient

- **Type O** persons are called “universal donors” because they do not have either A or B antigens on their red cells, therefore, their blood can, be given to all 4 blood types.

- **Type AB** persons are called “universal recipients” because they do not have circulating agglutinins in their plasma and can, therefore, receive blood of any type.

Compatibility of blood transfusion

	Recipient				
Donor		O	A	B	AB
	O	✓	✓	✓	✓
	A		✓		✓
	B			✓	✓
	AB				✓

		Recipient							
		O^-	O^+	A^-	A^+	B^-	B^+	AB^-	AB^+
Donor	O^-	✓	✓	✓	✓	✓	✓	✓	✓
	O^+		✓		✓		✓		✓
	A^-			✓	✓			✓	✓
	A^+				✓				✓
	B^-					✓	✓	✓	✓
	B^+						✓		✓
	AB^-							✓	✓
	AB^+								✓

Cross matching

- The process of testing the donor red cells with the recipient plasma, & the donor plasma against the red cells of the recipient. If there is no agglutination in either case, the donor blood can safely be given to the recipient.
    - The red cells and the plasma of the donor and recipient blood can be separated by centrifugation.

Importance of blood grouping

1. Blood transfusion for treatment purposes.
2. Determination of Rh incompatibility between the mother and child.
3. Paternity disputes.
4. Choice of a donor in tissue/organ transplantation.
5. Genetic studies.
6. Medico legal use.
7. Susceptibility to disease.

PRECAUTIONS

1. The slides should be dry, dust-free and grease-free.

2. Identify and mark all slides, containers, and test tubes clearly and legibly.

   Double-check every step of the procedure.

3. The droppers supplied with the antisera bottles should not be interchanged.

PRECAUTIONS

4. Examine the slides with the naked eye and then under the microscope after 8–10 minutes but before the sera-blood mixtures dry up.

5. Do not add undiluted blood from the finger-prick directly on to the antisera.

6. A control should always be used to exclude false positive result.