

Aim: To determine the blood group and Rh factor of an individual

Principle: In 1900 Karl Landsteiner, an Austrian scientist, reported the presence of 2 antigens A and B on the surface of human blood cells. ABO and Rh are the main systems of blood groups currently in use.

Antigens on the surface of RBCs	Antibodies in the serum	Blood Group	Genotype
A	Anti B	A	$I^A I^A$ or $I^A I^O$
B	Anti A	B	$I^B I^B$ or $I^B I^O$
A and B	absent	AB	$I^A I^B$
absent	Anti A, Anti B	O	$I^O I^O$

The associated Anti A and Anti B antibodies usually belong to IgM and IgG class of immunoglobulins. The antigens are glycoproteins with oligosaccharide chains that project above the RBC surface. These chains are attached to proteins and lipids that lie in the RBC plasma membrane. The I^A allele encodes a glycosyl transferase that produces the A antigen (N-acetyl galactosamine is the immunodominant sugar), and the I^B allele encodes a glycosyl transferase that creates the B antigen (D galactose is its immunodominant sugar). The I^O allele encodes an enzyme with no function and neither A nor B antigen is produced, leaving the underlying precursor unchanged.

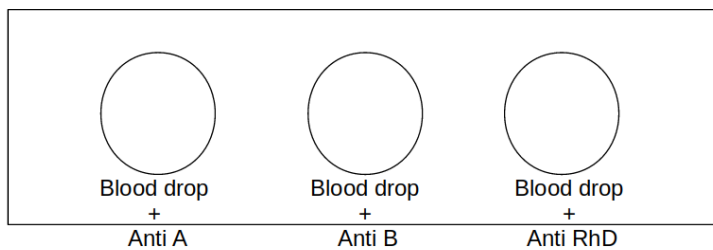
The Rhesus system (Rh) is the second most important blood group system in humans. The most significant and immunogenic Rh antigen is the RhD antigen. Individuals carrying Rh antigen are considered to have Rh positive blood group while those that lack this antigen are considered to have Rh negative blood group. Rh antigens are transmembrane proteins with loops exposed at the surface of RBCs. They appear to be used for transport of CO₂ and NH₃ across the plasma membrane. It was first discovered in Rhesus macaques and hence the name Rh came about.

The ABO and Rh blood grouping system is based on agglutination reaction. When RBCs carrying one or both the antigens are exposed to the corresponding antibodies, they react to form agglutinates – visible clumps. People with AB blood group do not produce any antibodies against A or B antigens and hence can receive blood of any type – they are called universal recipients. People with O blood group do not have either A or B antigens and hence can donate blood to persons of any blood group – they are called universal donors. Rh positive person can receive both Rh positive as well as Rh negative blood. But if Rh positive blood is given to a Rh negative person, anti Rh factor develops in the blood in 10-12 days. As a result in case of a second transfusion of Rh positive blood to this Rh negative person causes hem-agglutination. The hemolytic disease of newborn infants called erythroblastosis foetalis results from Rh incompatibility between Rh negative mother and Rh positive foetus. The first child is usually not affected, but a subsequent pregnancy with Rh positive foetus causes a massive secondary reaction of maternal immune system.

Requirements: HiPer® Blood Grouping Teaching Kit (HIMEDIA product code: HTI008) containing Anti A, Anti B and Anti RhD sera, cavity slide, disposable mixing stick, and blood lancet, rectified spirit, cotton.

Procedure:

1. Hand dangled down to increase the flow of blood in the fingers.
2. A fingertip (ring or middle finger) cleaned with rectified spirit.
3. The fingertip pierced with a sterile lancet and one drop of blood placed in each of the 3 cavities in a cavity slide.
4. One drop of antiserum added into each cavity as shown below:



5. Each blood drop and the corresponding antiserum mixed with a fresh mixing stick.
6. Agglutination observed in the form of fine red granules within 30 sec. Anti RhD may take slightly longer time to agglutinate as compared to Anti A and Anti B.

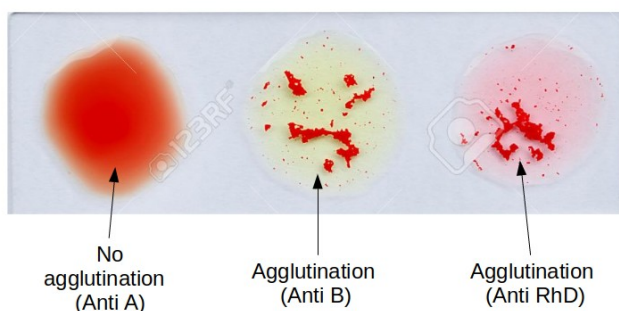
Note: Proper care should be taken while disposing the lancets.

Observation: (write the appropriate observation for your own blood group)

- If agglutination is observed when blood is mixed with Anti A reagent – individual has blood group “A”
- If agglutination is observed when blood is mixed with Anti B reagent – individual has blood group “B”
- If agglutination is observed when blood is mixed with Anti A as well as Anti B reagents – individual has blood group “AB”
- If no agglutination is observed when blood is mixed with Anti A and Anti B reagents – individual has blood group “O”
- If agglutination is observed when blood is mixed with Anti RhD reagent – individual has “+ve” Rh factor
- If no agglutination is observed when blood is mixed with Anti RhD reagent – individual has “-ve” Rh factor

(draw the appropriate diagram for your own blood group)

Example:



In the given example, blood group of the person tested is B +

Troubleshooting:

Sr. No.	Problem	Possible Cause	Solution
1	False positive result	The antisera reagents mix with each other	Ensure that the antisera reagents are added properly onto the respective cavity without spilling to the sides
		Incubated for a long time	The results should be read with the prescribed time period (30 sec to 1 min)
2	No agglutination observed	Antisera not stored under proper conditions	Ensure that the antisera are stored in a refrigerator (2-8°C)