Chapter 7.3.8

Blood Group and Rh Factor Determination

Principle

In 1900, Karl Landsteiner grouped human blood into four groups based on presence of two antigens on the surface of the RBCs. These groups are designated as A, B, AB and O. ABO system is an instance of multiple alleles. In this multiple allelic systems two of the alleles are dominant and is symbolized as I^A and I^B respectively. The only recessive allele is symbolized as I or I^O. ABO locus is located in chromosome 9 (9q34.1-9q34.2).An antigen called O or H antigen is produced by all individuals irrespective of their ABO blod group. In the ABO locus, an enzyme is coded which is known as galactosyl transferase that adds further sugar groups on O antigen.The I^A allele codes a specific enzyme that uses a cofactor UDP-N acetyl-galactose which converts O antigen into A type. Whereas I^B allele produce a specific enzyme that uses a cofactor UDP-galactose that converts O antigen into B antigen. The genotype; phenotypes, RBC antigens and compatible donors of various blood groups exhibited in the table (Table 7.3.8.1).

In addition to antigens of ABO system the red cells of 80-85% of the human also have an additional antigen called as Rh antigen or Rh factor.In 1940, Landsteiner and Weiner reported that rabbit sera contains antibodies against RBCs of the Rhesus monkey that agglutinates RBCs of some human being. The antigen was later named as Rh (Rhesus) factor. Later, it was found that D antigen is responsible for the Rh positive

Table 7.3.8.1 : Genotypes, phenotypes, RBC antigens and compatible donors of various blood groups

Phenotypes	Genotypes	RBC antigens (agglutinogens)	Plasma antibodies (agglutinins)	Compatible donor
A	$I^A\ I^A$ or $I^A I^O$	A	Anti-B	O, A
B	$I^B\ I^B$ or $I^B I^O$	B	Anti-A	O, B
AB	$I^A\ I^B$	A, B	Nil	A, B, AB, O
O	$I^O I^O$	Nil	Anti-A, Anti-B	O

condition. Person's RBCs whose red cells contain this additional antigen are called "Rh positive" whereas those persons who donot have this antigen is called as "Rh negative". There are several varieties of Rh antigen – C, D, E, c, d and e, but it is seen that D antigen is most common. Therefore Rh +ve persons are also referred as D +ve in contrary Rh –ve persons are referred as D –ve. There is no naturally occurring antibodies against Rh(D) antigen. Rh(D) antigen is absent in body fluids and tissues, but only present on RBCs (See Table 7.3.5).

Such an opposing property of Anti-A and Anti-B antibodies and antigens (A and B) are applied for the purpose of determination of ABO blood groups in man.

Again, during Rh determination blood Anti-D serum is mixed with blood and if there is agglutination then the blood has Rh(D) antigen and if it doesnot agglutinate then the blood does not have Rh(D) antigen.

Materials required

1. Human candidate.
2. Saline solution.
3. Absorbent cotton.
4. Disposable sterile needle.
5. Glass slide.
6. (i) Antiserum-A (contains monoclonal anti-A antibodies against human or α-agglutinins), (ii) Antiserum-B (contains monoclonal anti-B antibodies against human or β-agglutinins) (iii) Antiserum-D (contains monoclonal anti-Rh (D) antibodies against human called anti-D agglutinins.
7. Microscope.
8. 90% ethyl alcohol.
9. 3.8% sodium citrate solution.
10. Toothpick.

Procedure

1. Blood samples are collected by scrubbing the middle finger with a piece of cotton saturated with 90% ethyl alcohol and then pricking with a disposable sterile needle.

2. A drop of blood is placed on three points of a grease free slide (one on left side, one on right side and other on the centre of the slide) to each drop of blood added one drop of 3.8% sodium citrate solution, mixed thoroughly and to each added a drop of Antiserum-A (on left side), Antiserum-B (on right side) and Antiserum-D (the centre).

3. Each drop is the thoroughly mixed with a toothpick and then observed under a microscope.

4. One has to wait for 8-10 minutes. Then observations are made of the 3 antisera, first with naked eye to see whether there is any clumping and haemolysis has occurred or not, then it is confirmed under lower magnification (10x) of a microscope.

Observation

If any agglutination occurs it is visible to naked eyes. The haemolysed red blood cells are separated as dark cell masses of different sizes and shapes. There is a brick coloured serum formed through the haemoglobin released from haemolysed red blood cells. Under low power lens the clumps are visible as dark masses where the red cells have lost their definite outline.

(a) If agglutination occurs with Anti-A; then the group is blood group A.

(b) If agglutination occurs with Anti-B; then the group is blood group B.

(c) If agglutination does not occur with Anti-A or with Anti-B then the group is blood group O.

(d) If agglutination occur with Anti-A or with Anti- B then the group is blood group AB.

(e) If agglutination occurs with Anti-D, then the blood is Rh positive, if it does not the blood is Rh negative (refer Figure 7.3.8.1; Table 7.3.8.2).

Table 7.3.8.2 : Determination of Blood groups

(+) Denotes agglutination **(–) Denotes no agglutination**

Agglutination		Candidates Blood group	Candidates RBC contain agglutinogens	Candidates Plasma contains agglutins	Candidates Plasma with agglutinate RBC of group
Anti-A serum	Anti-B serum				
+	–	A	A	Anti-B	B,AB
–	+	B	B	Anti-A	A,AB
+	+	AB	A,B	None	None
–	–	O	O	Both Anti-A, Anti-B	A,B,AB

In case of Rh(D) blood group :

- Agglutination + : When candidates RBCs contain Rh(D) antigen — One is Rh(D) +ve
- No agglutination – : When there is no Rh(D) antigen in the candidate's RBC — One is Rh(D) –ve

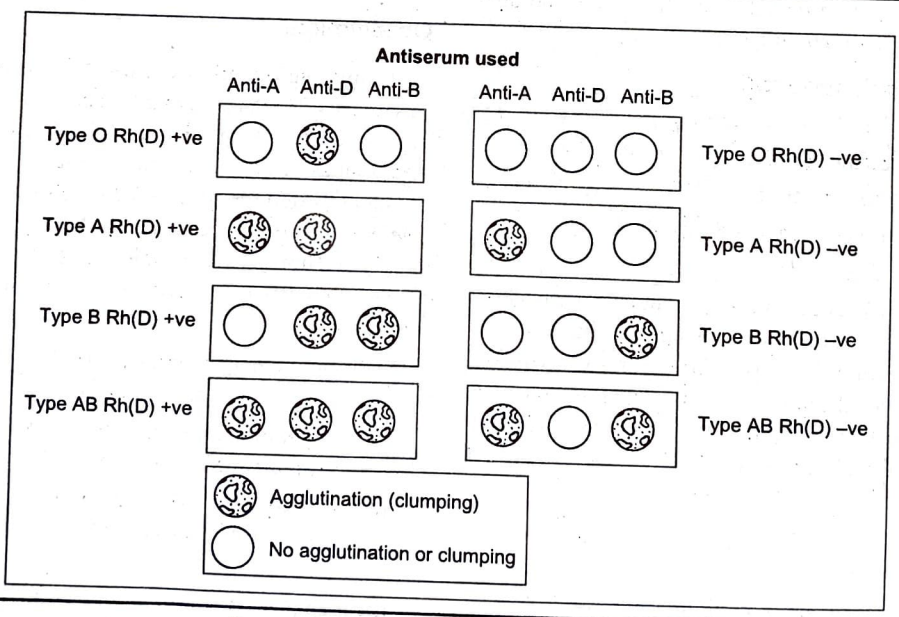

Figure 7.3.8.1 : Human blood group determination.

Note : The slides used should be dust free. The slides must be labelled A,B,D before putting three antisera i.e. Anti-A, Anti-B and Anti-D on the slides. Droppers supplied with the antisera should not be interchanged.