

WARD'STM

Simulated ABO and Rh Blood Typing

Student Study and Analysis Sheets

36 W 0019

Note: In response to the concerns of many science educators, WARD'S has developed an alternative blood typing activity that does not use real blood. Students will follow the same procedure used to type actual human blood and obtain results that closely approximate those obtained by real blood typing.

Introduction

Around 1900, Karl Landsteiner discovered that there are at least four different kinds of human blood, determined by the presence or absence of specific agglutinogens (agglutinating antigens) on the surface of red blood cells (erythrocytes). These antigens have been designated as A and B. Antibodies against antigens A or B begin to build up in the blood plasma shortly after birth, the levels peak at about eight to 10 years of age, and the antibodies remain, in declining amounts, throughout the rest a person's of life. The stimulus for antibody production is not clear; however, it had been proposed that antibody production is initiated by minute amounts of A and B antigens that may enter the body through food, bacteria, or other means. Humans normally produce antibodies against those antigens that are not on their erythrocytes: A person with A antigens has anti-B antibodies; a person with B antigens has anti-A antibodies; a person with neither A or B antigens has both anti-A and anti-B antibodies; and a person with both A and B antigens has neither anti-A nor anti-B antibodies. Blood type is based on the antigens, not the antibodies, a person possesses.

The four blood groups are types A, B, AB, and O. Blood type O, characterized by the absence of A or B agglutinogens, is the most common in the United States, in 45% of the population. Type A is next in frequency, found in 39% of the population. The incidences of types B and AB are 12% and 4% respectively.

ABO System

Blood Type	Antigens on Erythrocytes (Agglutinogens)	Antibodies in Plasma (Agglutinins)	Can Give Blood To	Can Receive Blood From
A	A	Anti-B	A, AB	O, A
B	B	Anti-A	B, AB	O, B
AB	A and B	Neither Anti-A nor Anti-B	AB	O, A, B, AB
O	Neither A nor B	Both Anti-A and Anti-B	O, A, B, AB	O

Process of Agglutination

There is a simple test to determine blood type, performed with antisera containing high levels of anti-A and anti-B agglutinins. Several drops of each kind of antiserum are added to separate samples of blood. If agglutination (clumping) occurs only in the suspension to which the anti-A serum was added, the blood type is A. If agglutination occurs only in the anti-B mixture, the blood type is B. Agglutination in both samples indicates that the blood type is AB. The absence of agglutination in any sample indicates that the blood type is O.

Agglutination Reaction of ABO Blood-Typing Sera

Reaction		Blood Type
Anti-A Serum	Anti-B Serum	
Agglutination	No Agglutination	Type A
No Agglutination	Agglutination	Type B
Agglutination	Agglutination	Type AB
No Agglutination	No Agglutination	Type O

Importance of Blood Typing

As noted in the table above, people can receive transfusions of only certain blood types, depending on the type of blood they have. If incompatible blood types are mixed, erythrocyte destruction, agglutination and other problems can occur. For instance, if a person with Type B blood is transfused with blood type A, the recipient's anti-A antibodies will attack the incoming type A erythrocytes. The type A erythrocytes will be agglutinated, and hemoglobin will be released into the plasma. In addition, incoming anti-B antibodies of the type A blood may also attack the type B erythrocytes of the recipient, with similar results. This problem may not be serious, unless a large amount of blood is transfused.

The ABO blood groups and other inherited antigen characteristics of red blood cells are often used in medico-legal situations involving identification of disputed paternity. A comparison of the blood groups of mother, child, and alleged father may exclude the man as a possible parent. Blood typing does not prove that an individual is the father of a child; it merely indicates whether or not he is a possible parent. For example, a child with a blood type of AB, whose mother is type A, could not have as a father a man whose blood type is O.

The Genetics of Blood Types

The human blood types (A, B, AB, and O) are inherited by multiple alleles—three or more genes that occupy a single locus on a chromosome. Gene I^A codes for the synthesis of antigen (agglutinogen) A, gene I^B codes for the production of antigen B on the red blood cells, and gene i (I^O) does not produce any antigens. The phenotypes listed in the table below are produced by the combinations of the three different alleles: I^A , I^B , i . When genes I^B and I^A are present in an individual, both are fully expressed. Both I^A and I^B are dominant over i ; the genotype of an individual with blood type O must be $i i$.

Phenotypes	Possible Genotypes
A	$I^A I^A$ $I^A i$ (or I^O)
B	$I^B I^B$ $I^B i$ (I^O)
O	$i i$ ($I^O I^O$)

Use I^A for antigen A, I^B for antigen B, i or I^O for no antigens present

Genes I^A and I^B are dominant over i (I^O)

AB blood type results when both genes I^A and I^B are present

Rh System

In the period between 1900 and 1940, a great deal of research was done to discover the presence of other antigens in human red blood cells. In 1940, Landsteiner and Wiener reported that rabbit sera containing antibodies for the red blood cells of the Rhesus monkey would agglutinate the red blood cells of 5% of white humans. These antigens, six in all, were designated as the Rh (Rhesus) factor; they were given the letters C, c, D, d, E, and e by Fischer and Race. Of these six antigens, the D factor is found in 85% of Caucasians, 94% of African Americans, and 99% of Asians. An individual who possesses these antigens is designated Rh+; an individual who lacks them is designated Rh-.

The genetics of the Rh blood group system is complicated by the fact that more than one antigen can be identified by the presence of a given Rh gene. Initially, the Rh phenotype was thought to be determined by a single pair of alleles. However, there are at least eight alleles for the Rh factor. To simplify matters, consider one allele: Rh+ is dominant over Rh-; therefore, a person with Rh+/Rh- or Rh+/Rh+ genotypes has Rh+ blood.

The anti-Rh antibodies of the system are not normally present in the plasma, but anti-Rh antibodies can be produced upon exposure and sensitization to Rh antigens. There are several ways sensitization can occur—for example, if Rh+ blood is transfused into an Rh- recipient, or when an Rh- mother carries a fetus who is Rh+. In the latter case, some of the fetal Rh antigens may enter the mother's circulation and sensitize her so that she begins to produce anti-Rh antibodies against the fetal antigens. In most cases, sensitization to the Rh antigens takes place toward the end of pregnancy, but because it takes some time to build up the anti-Rh antibodies, the first Rh+ child carried by a previously unsensitized mother is usually unaffected. However, if an Rh- mother, or a mother previously sensitized by a blood transfusion or a previous Rh+ pregnancy, carries an Rh+ fetus, maternal anti-Rh antibodies may enter the fetus' circulation, causing the agglutination and hemolysis of fetal erythrocytes and resulting in a condition known as erythroblastosis fetalis (hemolytic disease of the newborn). To treat an infant in a severe case, the infant's Rh+ blood is removed and replaced with Rh- blood from an unsensitized donor to reduce the level of anti-Rh antibodies.

Artificial Blood

At times it is difficult to find a correct match for a blood type of a person requiring a transfusion. It would be ideal to have some type of artificial blood or blood substitute that wouldn't need to be matched to a patient's blood type; it could save thousands of lives each year. Although the research for artificial blood and blood substitutes continues, it may take years before one is available.

In 1966, Dr. Leland C. Clark, of the University of Cincinnati's College of Medicine, developed the first artificial blood prototype. This milky white solution, which can carry twice as much oxygen as blood does, is a fluorocarbon emulsion called Fluosol. It is made up of two fluorocarbons, a number of salts, water, and fine particles that are $\frac{1}{70}$ the size of erythrocytes. Because these particles are so small, they can pass through narrowed arteries that the larger erythrocytes can't get through, making it an ideal blood substitute for heart attack and stroke victims; they would recover faster and have less tissue damage. Fluosol has been approved for use in Canada, Holland, and Italy. The US Food and Drug Administration is reviewing Fluosol for use in the United States.

Anthony Hunt and colleagues at the University of California at San Francisco are working with artificial red blood cells called neohemocytes. Neohemocytes, which are microscopic spheres of hemoglobin surrounded by lipids, are capable of carrying oxygen. These microspheres are proving to be a successful substitute for erythrocytes. As with Fluosol, their small size allows them to pass through restricted vessels that might not allow the passage of erythrocytes.

Objective

To use WARD'S Simulated Blood to determine the blood type and Rh factor of four individuals. Also to use a simplified counting technique to estimate the number of red and white blood cells per cubic millimeter.

A. ABO and Rh Blood Typing

Materials Needed per Lab Group

- 4 Blood Typing Slides
- 8 Toothpicks

Shared Materials

- 4 Unknown Blood Samples
 - Mr. Smith
 - Mr. Jones
 - Mr. Green
 - Ms. Brown
- Simulated Anti-A Typing Serum
- Simulated Anti-B Typing Serum
- Simulated Anti-Rh Typing Serum

Procedure

1. Label each blood typing slide:
 - Slide #1: Mr. Smith
 - Slide #2: Mr. Jones
 - Slide #3: Mr. Green
 - Slide #4: Ms. Brown
2. Place three to four drops of Mr. Smith's blood in each of the A, B, and Rh_o wells of Slide #1.
3. Place three to four drops of Mr. Jones's blood in each of the A, B, and Rh_o wells of Slide #2.
4. Place three to four drops of Mr. Green's blood in each of the A, B, and Rh_o wells of Slide #3.
5. Place three to four drops of Ms. Brown's blood in each of the A, B, and Rh_o wells of Slide #4.
6. Add three to four drops of the simulated anti-A serum in each A well on the four slides.
7. Add three to four drops of the simulated anti-B serum in each B well on the four slides.
8. Add three to four drops of the simulated anti-Rh serum in each Rh_o well on the four slides.
9. Stir each mixture with a different clean toothpick. Use only one toothpick per well to avoid cross contamination.
10. Observe and record the results in Table 1.

Analysis

Table 1
Agglutination Reactions

	Anti-A Serum	Anti-B Serum	Anti-Rh Serum	Blood Type
Slide #1: Mr. Smith				
Slide #2: Mr. Jones				
Slide #3: Mr. Green				
Slide #4: Ms. Brown				

Questions

ABO Blood Group

1. What is Mr. Smith's blood type? What ABO agglutinogens are present in his red blood cells?
2. What is Mr. Green's blood type? What ABO agglutinins are present in the plasma of his blood?
3. What is Mr. Jones's blood type? If Mr. Jones needed a transfusion, what blood type(s) could he safely receive?
4. What is Ms. Brown's blood type? What blood type(s) could safely receive her donated blood?
5. Why is it necessary to match the donor's and recipient's blood before a transfusion?
6. What happens to red blood cells that are agglutinated?

7. What is the difference between agglutinogens and agglutinins?

8. How are ABO blood types determined?

9. Could a man with an AB blood type be the father of a child with type O blood?

10. Could a man with an O blood type be the father of a child with type AB blood?

11. Could a type B child with a type A mother have a type A father?

12. What are the possible genetic combinations of a child whose parents' blood types are A and B?

Rh Blood Group

1. Suppose Mr. Smith marries Ms. Brown. What are the chances for an Rh+ child? an Rh- child?
2. How does erythroblastosis fetalis develop?
3. Under what conditions might a person with Rh- blood develop Rh agglutinins?
4. Why can Rh+ blood be given once to a non-sensitized person who is Rh-?
5. What is likely to happen to a donor's cells if an Rh- person who is sensitive to Rh+ blood receives a transfusion of Rh+ blood?