

WARD'S

SIMULATED ABO AND RH BLOOD TYPING KIT

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INTRODUCTION

Around 1900, Karl Landsteiner discovered that there are at least four different kinds of human blood based on 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 antibody levels peak at about eight to ten years of age, and the antibodies remain present in declining amounts throughout the rest 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 by other means. A person normally produces antibodies against those antigens that are not on his erythrocytes but does not produce antibodies against those that are present on his erythrocytes. Thus a person with antigen A has anti-B antibodies; a person with B antigens has anti-A antibodies; a person with neither antigen A or B (blood type O) has both anti-A and anti-B antibodies; and a person with both antigens A and B has neither anti-A nor anti-B antibodies. The individual's blood type is based on the antigens, not the antibodies, he has.

The four blood groups are known as 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 (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.

TABLE 1
ABO SYSTEM

Blood Type	(Agglutinogens) Antigens on Erythrocytes	(Agglutinins) Antibodies in Plasma	Can Give Blood to Groups:	Can Receive Blood from Groups:
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

Blood typing is performed with antisera containing high levels of anti-A and anti-B agglutinins. The simple test is performed as follows:

Several drops of each kind of antiserum are added to separate samples of blood. If agglutination (clumping) occurs only in the suspension to which only 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 indicates that the blood is Type O.

TABLE 2
AGGLUTINATION REACTIONS 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

Early attempts to transfer blood from one person to another produced varied results. 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 antigenic characteristics of red blood cells are often used in medico-legal situations involving identification or disputed paternity. In paternity cases a comparison of the blood groups of mother, child and alleged father may exclude the man as a possible parent of the child. For example, a child of blood type AB whose mother is Type A could not have as a father a man whose blood group is O. 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.

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. This antigen in humans, designated as the Rh factor with due respect to the Rhesus monkey, was later found to exist as six antigens, which were given the letters C, c, D, d, E and e by Fischer and Race. Of these six antigens, the D factor is responsible for the Rh+ condition and is found in

Rh System (Continued)

85% of Caucasians, 94% of Blacks and 99% of Orientals. An individual who possesses these antigens is designated Rh+; an individual who lacks them is designated Rh-. 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 of the anti-Rh antibodies the first Rh+ child carried by a previously unsensitized mother is usually unaffected. However, if an Rh-mother or previously sensitized mother (by a blood transfusion or by a previous Rh+ pregnancy) later carries an Rh+ fetus, maternal anti-Rh antibodies may enter the fetal circulation, causing the agglutination and hemolysis of fetal erythrocytes. This would result 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. The replacement of the infant's blood reduces the levels of the anti-Rh antibodies.

The genetics of the Rh blood group system is complicated by the fact that more than one antigen can be identified as the result of 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. For the purpose of simplicity, consider here one allele: Rh+ is dominant over Rh-. Thus a person with Rh+/Rh- genotypes or Rh+/Rh+ has Rh+ blood.

The Genetics of Blood Types

The human blood types A, B, AB and O are inherited by multiple alleles. Multiple alleles refers to 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^O. 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^O; the genotype of an individual with blood type O must be i^Oi^O.

TABLE 3

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

Use I^A for antigen A; I^B for antigen B; i or I^O for no antigens present
 Gene I^A is dominant over i (I^O)
 Gene I^B is dominant over i (I^O)
 AB blood type results when both genes I^AI^B are present

Artificial Blood

Consider the fate of an accident victim in a rural area in need of blood. The nearest hospital or blood bank may be hundreds of miles away. Without the needed blood, such a patient may not survive. What if it were possible to have an artificial blood or blood substitute available that did not need to be matched to the patient's own blood? A readily available blood substitute has a potential of saving thousands of lives each year.

Although research on a blood substitute has been ongoing for decades, a blood substitute for general use is still in the future. Research is progressing in a number of directions. In 1966, Dr. Leland C. Clark of the University of Cincinnati's College of Medicine developed the first prototype of an artificial blood. It is a milky white solution that can carry twice as much oxygen as blood does. The solution is a fluorocarbon emulsion called Fluosol. It contains two fluorocarbons, a number of salts, water and fine particles that are one-seventieth the size of erythrocytes. Because these particles are so small, they can pass through narrowed arteries not possible by erythrocytes. Victims of heart attack and stroke may benefit from this blood substitute with faster recoveries and less tissue damage. Fluosol has been approved for use in Canada, Holland and Italy. The US Food and Drug Administration is reviewing the use of Fluosol for use in the United States.

In another direction, 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 substitute for erythrocytes. As with the Fluosol, their small size allows them to pass through restricted vessels that might not allow the passage of erythrocytes. Victims of heart attack and stroke may therefore recover faster and have less tissue damage using this blood substitute.

ACTIVITY 1

Student Note: This alternative blood typing activity does NOT use real blood or blood sera. You will follow the exact procedure used to type actual human blood and obtain results that closely approximate real blood typing.

ABO and Rh Blood Typing

OBJECTIVES:

1. To determine the ABO blood type of four unknown simulated blood samples.
2. To determine the Rh of four unknown simulated blood samples.

PERFORMING THE EXPERIMENT

Materials: (per team of two students)

- 4 Blood Typing Slides
- 8 Toothpicks

Helpful Hint! →

Shared Materials:

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

Obtain three toothpicks per blood typing slide. Stir each well with a separate clean toothpick for 30 seconds. To avoid splattering the simulated blood, do not press too hard on the typing tray.

Observe each slide and record your observations in Table 1 of the Analysis section. To confirm agglutination try reading text through the mixed sample. If you cannot read the text, assume you have a positive agglutination reaction.



Agglutination



No Agglutination

Procedure:

Each team will determine the blood type of each of the four unknown blood samples.

1. Pre-label each of your four blood typing slides as follows:
 - Slide #1: Mr. Smith
 - Slide #2: Ms. Jones
 - Slide #3: Mr. Green
 - Slide #4: Ms. Brown
2. Place 3-4 drops of Mr. Smith's blood in each of the A, B and Rh₀ wells of Slide #1.
3. Place 3-4 drops of Ms. Jones's blood in each of the A, B and Rh₀ wells of Slide #2.
4. Place 3-4 drops of Mr. Green's blood in each of the A, B and Rh₀ wells of Slide #3.
5. Place 3-4 drops of Ms. Brown's blood in each of the A, B and Rh₀ wells of Slide #4.
6. Add 3-4 drops of the simulated anti-A serum in each A well on the four slides.
7. Add 3-4 drops of the simulated anti-B serum in each B well on the four slides.
8. Add 3-4 drops of the simulated anti-Rh₀ serum in each Rh₀ well on the four slides.
9. Use separate toothpicks to stir each sample of serum and blood. Record your observations and results in the table below.

DATA TABLE 1
Agglutination Reactions

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

Note: A positive test is indicated by a strong agglutination reaction. See Table 2 for aid in interpreting the test results.

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ACTIVITY 2

Blood Cell Count

WARD'S Simulated Blood contains microcomponents that simulate red and white blood cells. These microcomponents are similar in proportion and size to those found in real human blood and can be seen under the microscope without staining. The red stained microcomponents simulate red blood cells, whereas the blue stained microcomponents simulate white blood cells.

OBJECTIVES:

1. To count the number of simulated red and white blood cells under the microscope, using a simplified counting method.
2. To observe agglutinated "red and white blood cells" under the microscope.

PERFORMING THE EXPERIMENT

Materials: (per team of two students)

- 1 Microscope Slide
- 1 Cover Slip
- WARD'S Simulated Blood*
- Compound Microscope (400X magnification)

Procedure:

1. Thoroughly shake one of the vials of simulated blood. Add a minute drop of blood to a glass slide.
2. Cover the simulated blood sample with a cover slip. Try to avoid trapping bubbles of air beneath the cover slip by lowering it slowly onto the drop of water.
3. Remove any excess sample from the edge of the cover slip using an absorbent piece of paper.
4. Place the slide under the slide clips on the microscope stage and scan it with the low power objective to find that area of the slide with the best distribution of cells. To count the cells switch to 400X magnification.
5. Count the number of red blood cells under a field of view that has a minimum of 20 cells. Record the number counted in Data Table 2. Count the number of white blood cells and record the number counted in Data Table 2 as well.

Note: Some cells tend to clump together, similar to real blood cells when stored at room temperature for long periods of time. Count each cell in any clump separately.

6. Repeat Step 5 using two different fields of view. Record these counts in Data Table 2 and calculate the average of the three counts.
7. Multiply the average number of cells by the dilution factor to determine the number of "red" and "white blood cells" per mm^3 .

Note: The average count is multiplied by the factor indicated in the Data Table for "red" and "white blood cells" to correct for dilution and for the fact that only a small volume of blood was observed.

DATA TABLE 2

Blood Cell Type	Cell Count #1	Cell Count #2	Cell Count #3	Average Number of Cells	Dilution Factor	Total * Number of Blood Cells per mm ³ (Avg. # of Cells x Dilution Factor)
Red Blood cells (RED)					150,000	
White Blood Cells (BLUE)					5,000	

* Normal human blood counts.

Note: Class counts may be combined for greater accuracy.

ANALYSIS OF RESULTS:

ABO Blood Group

1. What ABO agglutinogens are present on the red blood cells of Mr. Green's blood?
2. What ABO agglutinins are present in the plasma of Mr. Green's blood?
3. If Ms. Jones needed a transfusion, what ABO type(s) of blood could she safely receive?
4. If Ms. Brown were serving as a donor, what ABO blood type(s) could receive her blood safely?
5. Why is it necessary to match the donor's and the recipient's blood before a transfusion is given?
6. What happens to red blood cells that are agglutinated?
7. What is the difference between agglutininogen and agglutinin?
8. Explain the basis of ABO blood types.
9. Could a man with an AB blood type be the father of an O child? *Explain.*

10. Could a man with an O blood type be the father of an AB child? *Explain.*
11. Could a Type B child with a Type A mother have a Type A father? *Explain.*
12. What are the possible genetic combinations of an offspring when the blood types of the parents 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. Explain how erythroblastosis fetalis may develop.
3. Under what conditions might a person with Rh- blood develop Rh agglutinins?
4. Why can Rh+ blood be given only 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?