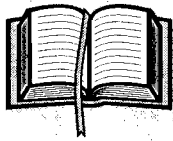


# WARD'S

## Simulated ABO & Rh Blood Typing Lab Activity Student Study Guide

### BACKGROUND



**Agglutinogens (Antigens):**

Agglutinogens are substances found on the surface of erythrocytes.

**Agglutinins (Antibodies):**

Agglutinins are antibodies found in plasma.

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 (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 ten years of age, and the antibodies remain, in declining amounts, throughout the rest of a person's of life. The stimulus for antibody production is not clear; however, it has 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 nor 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 (Figure 1). 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 and B agglutinogens, is the most common in the United States and is found in 45% of the population. Type A is next in frequency, and is found in 39% of the population. The frequencies at which types B and AB occur are 12% and 4% respectively.

**Figure 1**

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

## ABO System Process of Agglutination



### DID YOU KNOW?

The average life span of a red blood cell is about 120 days.

There is a simple test performed with antisera containing high levels of anti-A and anti-B agglutinins to determine blood type. 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 (Figure 2).

**Figure 2**

**Agglutination Reaction of ABO Blood-Typing Sera**

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

## Importance of Blood Typing



### DID YOU KNOW?

Donor blood contains only packed red blood cells. There is no plasma in donor blood, thus there are no antibodies present.

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 cannot prove that an individual is the father of a child; it merely indicates whether or not he possibly could be. For example, a child with a blood type of AB, whose mother is type A, could not have a man whose blood type is O as a father.

## The Genetics of Blood Types



### DID YOU KNOW?

Camels and their relatives are the only mammals having oval red blood cells.

The human blood types (A, B, AB, and O) are inherited by multiple alleles, which occurs when three or more genes occupy a single locus on a chromosome. Gene  $I^A$  codes for the synthesis of antigen (agglutinin) A, gene  $I^B$  codes for the production of antigen B on the red blood cells, and gene  $i$  does not produce any antigens. The phenotypes listed in the table below are produced by the combinations of the three different alleles:  $I^A$ ,  $I^B$ , and  $i$ . 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  $i$  so the genotype of an individual with blood type O must be  $ii$  (Figure 3).

Figure 3

Phenotype	Possible Genotypes
A	$I^A I^A$ $I^A i$
B	$I^B I^B$ $I^B i$
AB	$I^A I^B$
O	$ii$

Use  $I^A$  for antigen A,  $I^B$  for antigen B, and  $i$  for no antigens present. Genes  $I^A$  and  $I^B$  are dominant over  $i$ . AB blood type results when both genes  $I^A$  and  $I^B$  are present.

## Rh System



### DID YOU KNOW?

Rh is so named because the initial study was done with Rhesus monkeys.

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 Caucasians. These antigens, six in all, were designated as the Rh (Rhesus) factor, and 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 an  $Rh^+/Rh^-$  or  $Rh^+/Rh^+$  genotype 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. Sensitization can occur when Rh<sup>+</sup> blood is transfused into an Rh<sup>-</sup> recipient, or when an Rh<sup>-</sup> mother carries a fetus who is Rh<sup>+</sup>. 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<sup>+</sup> child carried by a previously unsensitized mother is usually unaffected. However, if an Rh<sup>-</sup> mother, or a mother previously sensitized by a blood transfusion or a previous Rh<sup>+</sup> pregnancy, carries an Rh<sup>+</sup> 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<sup>+</sup> blood is removed and replaced with Rh<sup>-</sup> blood from an unsensitized donor to reduce the level of anti-Rh antibodies.

## Blood Components



### DID YOU KNOW?

Leukocytes are capable of amoeboid movement and are sometimes called amoebocytes.

The formed elements in blood include erythrocytes, or red blood cells (RBCs); various types of leukocytes, or white blood cells (WBCs); and platelets.

Erythrocytes are circular, biconcave disks of 5 to 8 micrometers. Their chief function is to transport oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>). The transport of O<sub>2</sub> and CO<sub>2</sub> depends largely on the hemoglobin present in the erythrocytes. The biconcave shape is also related to the erythrocytes function of transporting gases, in that it provides an increased surface area through which gases can diffuse.

The number of circulating RBCs is closely related to the blood's oxygen-carrying capacity. Any changes in the RBC count may be significant. RBC counts are routinely made to diagnose and evaluate the course of various diseases.

Leukocytes range in size from approximately 9 to 25 micrometers and function primarily to control various disease conditions. Leukocytes can move against the current of the bloodstream through amoeboid movement, and pass through the blood vessel walls to enter the tissues. The total WBC count normally varies from 5,000 to 10,000/mm<sup>3</sup>. Certain infectious diseases are accompanied by an increase in WBCs. If the number exceeds 10,000/mm<sup>3</sup>, the person has an acute infection. If it drops below 5,000/mm<sup>3</sup>, the person may have a condition such as measles or chicken pox. The percentage of the different types of leukocytes present in the blood may also change in particular diseases, this number is important for diagnostic purposes and is called a differential count.

## OBJECTIVES

- Define agglutinin and agglutinin
- Perform an actual blood typing procedure
- Observe the antigen/antibody reaction in simulated blood
- Determine the ABO and Rh blood type of four unknown samples
- Prepare a wet mount of simulated blood
- Estimate the number of erythrocytes and leukocytes in normal blood
- Understand requirements for blood transfusions

## MATERIALS

### MATERIALS NEEDED PER GROUP

- 4 Blood typing slides
- 12 Toothpicks
- 1 Microscope slide
- 1 Coverslip
- Compound microscope (400X magnification)
- Marker

### SHARED MATERIALS

- 4 Unknown blood samples:
  - Mr. Smith
  - Mr. Jones
  - Mr. Green
  - Ms. Brown
- Simulated Anti-A Serum
- Simulated Anti-B Serum
- Simulated Anti-Rh Serum

## PROCEDURE



*Although WARD'S Simulated Blood is completely safe, non-biological, and non-toxic, you should wear the proper personal protective equipment to mimic the experience of an actual hematology laboratory.*

### PART A: ABO and Rh BLOOD TYPING

1. Label each blood typing slide:
  - Slide #1: Mr. Smith
  - Slide #2: Mr. Jones
  - Slide #3: Mr. Green
  - Slide #4: Ms. Brown



#### DID YOU KNOW?

Prior to the mid-1500s, no one had any conception of blood circulating through the organs in the body.



#### DID YOU KNOW?

WARD'S Simulated Blood contains components that simulate red and white blood cells; they are similar in proportion and size to those found in real human blood and can be seen under the microscope without staining.

2. Place three to four drops of Mr. Smith's blood in each of the A, B, and Rh wells of Slide #1.
3. Place three to four drops of Mr. Jones's blood in each of the A, B, and Rh wells of Slide #2.
4. Place three to four drops of Mr. Green's blood in each of the A, B, and Rh wells of Slide #3.
5. Place three to four drops of Ms. Brown's blood in each of the A, B, and Rh wells of Slide #4.
6. Place three to four drops of the simulated anti-A serum in each A well on the four slides.
7. Place three to four drops of the simulated anti-B serum in each B well on the four slides.
8. Place three to four drops of the simulated anti-Rh serum in each Rh well on the four slides.
9. 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.
10. 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

11. Dispose of all materials according to your teacher's instructions.



WARD'S Simulated blood is non-biological and nontoxic and may be flushed down the drain.

Be sure to wash and save the blood typing trays and toothpicks for future use.



**Phlebotomists** collect blood samples and body fluids from patients for laboratory testing.

## PART B: Blood Cell Count

1. Thoroughly shake one of the vials of WARD'S Simulated Blood. Add one drop of simulated blood to a microscope slide, and cover with a coverslip. Lower the coverslip slowly to avoid trapping air bubbles on the slide.
2. Examine the slide with the low power (10X). Find an area of the slide with an even distribution of cells.
3. Switch to high power (40X). Refocus and count the number of simulated red blood cells (red spheres) in the field of view. Count the cells in any clump separately. Record the number in Table 2.
4. Count the number of simulated white blood cells (blue spheres). Record the number in Table 2.
5. Repeat the counting procedure with two other fields of view. Record these counts in Table 2.
6. Calculate the average of the three red blood cell counts and the three white blood cell counts. Record the results in Table 2.
7. Multiply the average number of red and white blood cells by the dilution factor to determine the number of red and white blood cells per cubic millimeter. Record each value in Table 2.
8. Dispose of all materials according to your teacher's instructions.



*WARD'S Simulated blood is non-biological and non-toxic and may be flushed down the drain.*

**WARD'S**  
**Simulated ABO and Rh**  
**Blood Typing Lab Activity**

Name: \_\_\_\_\_  
 Group: \_\_\_\_\_  
 Date: \_\_\_\_\_

**ANALYSIS**

**Table 1**

	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				

**Table 2**

Blood Cell Type	Cell Count			Total # of Cells	Avg. # Cells or Total/3	Dilution Factor	Total # Blood Cells per mm <sup>3</sup> or Avg. # Cells x Dilution Factor
	1	2	3				
Red (Red)						150,000	
White (Blue)						5,000	