BACKGROUND

ABO/Rh Blood System

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 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.

In 1940, Landsteiner and Wiener discovered another group of antigens on the surface of red blood cells called Rh factors. They are called Rh factors because they were first found in Rhesus monkeys. An individual who possesses these antigens is designated Rh+ and an individual who lacks them is designated Rh-. Unlike the ABO system, antibodies to the Rh factors are not normally present in the plasma, but are produced upon exposure to Rh factors. Exposure to Rh factors can occur during blood transfusions (if Rh+ blood is transfused to an Rh- recipient), or when an Rh- mother carries an Rh+ fetus.
IYVVY: pan of a

Figure 1

<table>
<thead>
<tr>
<th>Blood Type</th>
<th>Antigens on Erythrocytes (Agglutinogens)</th>
<th>Antibodies in Plasma (Agglutinins)</th>
<th>Can Give Blood To</th>
<th>Can Receive Blood From</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
<td>A, AB</td>
<td>O, A</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
<td>B, AB</td>
<td>O, B</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>Neither Anti-A nor Anti-B</td>
<td>AB</td>
<td>O, A, B, AB</td>
</tr>
<tr>
<td>O</td>
<td>Neither A nor B</td>
<td>Both Anti-A and Anti-B</td>
<td>O, A, B, AB</td>
<td>O</td>
</tr>
</tbody>
</table>

Process of Agglutination

There is a simple test to determine blood type, performed with antisera containing high levels of anti-A, anti-B and anti-Rh 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. Any sample that agglutinates in the presence of anti-Rh serum is considered Rh+.}

DID YOU KNOW?
The average life span of a red blood cell is about 120 days.
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. This problem may not be serious, unless a large amount of blood is transfused.

Blood type, an inherited characteristic, may also be 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.

Whenever blood has been shed in a criminal act, the identification and typing of the blood stains are of primary importance to the crime scene investigator. The ABO blood groups are used to screen out possible suspects involved in the crime. The first step in the investigation is to distinguish the bloodstains from other similar looking compounds such as fruit juices, jam, chemicals, paint, etc. Secondly, along with a number of sophisticated tests to determine the sex of the individual from which the blood came, and the age of the blood stain, a simple blood typing test is also performed. Although a positive match of the suspect’s blood type is not sufficient to convict someone of a crime, it is one type of evidence that is often obtained during a crime investigation.
OBJECTIVES

- Assume the role of a forensics lab technician
- Examine suspected blood evidence found at a crime scene
- Confirm that the evidence is real blood
- Perform the ABO/Rh procedure to determine the blood type
- Relate the evidence to four possible suspects

MATERIALS

MATERIALS NEEDED PER GROUP

- 4 Pieces of stained cloth squares
- 6 Blood typing trays
- 18 Toothpicks
- 1 Microscope slide
- 1 Compound microscope

SHARED MATERIALS

- 1 Vial, WARD'S Simulated Blood - Victim's blood
- 1 Vial, WARD'S Simulated Blood - Suspect #1
- 1 Vial, WARD'S Simulated Blood - Suspect #2
- 1 Vial, WARD'S Simulated Blood - Suspect #3
- 1 Vial, WARD'S Simulated Blood - Suspect #4
- 1 Vial, anti-A serum
- 1 Vial, anti-B serum
- 1 Vial, 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.*

**Scenario:**

Crime investigators were called to the scene of a burglary. Mr. Smith had come home, only to find someone robbing his apartment. As the criminal rushed to leave the apartment, he ran into a glass door cutting his arm and tearing his shirt. The crime investigators were able to remove small pieces of clothing that appeared to be blood stained from the broken glass door. The blood samples from the crime scene, along with the victim's blood, were sent to the forensic lab to be analyzed. After the crime investigators carefully reviewed all of the available evidence, they apprehended four suspects. The last remaining piece of evidence needed to solve the crime is to match the blood type found at the scene of the crime with one of the suspects. You, along with your classmates, have been chosen to provide this last piece of evidence and determine which of the suspects is the burglar.

**Part A. Microscopic Investigation**

The first step in the investigation is to distinguish the blood stains from other similar looking compounds such as fruit juices, foods, chemicals, paint, etc.

1. Use the provided piece of stained cloth found at the scene of the crime. Place the stained cloth flat on a microscope slide and place one drop of water on it.

2. View the cloth under low and high power (400X) for any clues that would lead you to prove that the stain on the cloth is indeed blood.

3. Describe your observations and state your conclusion in Table 1 in the Analysis section. State whether or not you believe the sample is stained with blood.
Part B. ABO/Rh Blood Typing

You will now determine the blood type of the victim, the four suspects, and the blood found at the scene of the crime.

1. Use a wax pencil to label each of your six blood typing trays as follows:

   Tray #1: Crime scene  
   Tray #2: Victim  
   Tray #3: Suspect #1  
   Tray #4: Suspect #2  
   Tray #5: Suspect #3  
   Tray #6: Suspect #4

2. To determine the type of blood found at the crime scene, place a piece of the blood stained cloth, provided by your teacher, in each of the A, B, and Rh wells of blood typing Tray #1 labeled “Crime scene”.

3. Place 3-4 drops of the simulated anti-A serum on the blood stained cloth in the A well of the tray.

4. Place 3-4 drops of the simulated anti-B serum on the blood stained cloth in each B well of the tray.

5. Place 3-4 drops of the simulated anti-Rh serum on the blood stained cloth in each Rh well of the tray.

6. Obtain three toothpicks. Stir each sample of anti-serum and blood stained cloth with a separate clean toothpick for 30 seconds. To avoid splattering the simulated blood, do not press too hard on the typing tray.

7. Observe the slide and record your observations in Table 2 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.

<table>
<thead>
<tr>
<th>Agglutination</th>
<th>No Agglutination</th>
</tr>
</thead>
</table>

Once you have determined the type of blood found at the scene of the crime, you will then type the blood of the victim and four suspects.

8. Place 3-4 drops of the victim’s blood in each of the A, B, and Rh wells of Tray #2: Victim.

9. Place 3-4 drops of Suspect #1’s blood in each of the A, B, and Rh wells of Tray #3: Suspect #1.

10. Place 3-4 drops of Suspect #2’s blood in each of the A, B, and Rh wells of Tray #4: Suspect #2.

11. Place 3-4 drops of Suspect #3’s blood in each of the A, B, and Rh wells of Tray #5: Suspect #3.

12. Place 3-4 drops of Suspect #4’s blood in each of the A, B, and Rh wells of Tray #6: Suspect #4.

13. Place 3-4 drops of the simulated anti-A serum in each A well on the five trays.

14. Place 3-4 drops of the simulated anti-B serum in each B well on the five trays.

15. Place 3-4 drops of the simulated anti-Rh serum in each Rh well on the five trays.

16. Stir each sample with a separate clean toothpick for 30 seconds. To avoid splattering the simulated blood, do not press too hard on the typing tray.

17. Observe the slide and record your observations in Table 2. To confirm agglutination try reading text through the mixed sample. If you cannot read the text, assume you have a positive agglutination reaction.

**DID YOU KNOW?**

Luminol is a chemical that is used as a forensic tool for the detection of trace blood patterns at crime scenes.

WARD’S Simulated blood is non-biological and non-toxic and may be flushed down the drain.
WARD'S
Simulated Blood Typing
Whodunit Lab Activity

ANALYSIS

Table 1

<table>
<thead>
<tr>
<th>Observations</th>
<th>Conclusion</th>
</tr>
</thead>
</table>

Table 2

<table>
<thead>
<tr>
<th>Blood Source</th>
<th>Anti-A Serum</th>
<th>Anti-B Serum</th>
<th>Anti-Rh Serum</th>
<th>Blood Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crime Scene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Victim</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspect 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. As a forensics technician, the courts have asked you to summarize your findings to the jury. Explain what you would say in the space below (remember, it is your job to report the facts, not decide who is guilty).

2. How would you respond to the accused suspect's denial of having the blood type you identified, since he has proof that he had just received a blood transfusion with type O blood?

3. Why is it necessary to type the victim's blood?

4. a. Pick one of the following suspects:
   Suspect #1       Suspect #2
   Suspect #3       Suspect #4
   Suspect #:_______

   b. Using the information from Figure 1 and the data from your blood type analysis, what ABO agglutinogens are present on the suspect's red blood cells?____________________

   c. What ABO agglutinins are found in the suspect's blood plasma?______________

   d. What is the suspect's blood type?________________

   e. If the suspect needed a blood transfusion, what blood types could he/she receive?__________

   f. What blood types could safely receive this suspect's blood?____________________
5. Create a graph or chart that depicts the percentages of each ABO blood type in the United States.

6. Using the following graphic organizer, fill in the properties which are unique to agglutinins in the bubbles on the right, properties common to both agglutinins and agglutinogens in the center bubbles and properties unique to agglutinogens in the bubbles on the left.
7. **How** are agglutinins like security guards?

8. You are a defense attorney representing the accused. Your client has been shown to have the same blood type as that found at the scene of the crime. Explain to the jury why this information alone is not enough to convict your client.

9. As the client's attorney, you have also been provided with police videotape of the crime scene analysis. In the video, you notice that one of the investigating officers has a bandage on his hand. How could you use this evidence to your advantage?

10. Blood typing is one method of gathering evidence at the scene of a crime. Describe at least two other methods that can be used in the attempted identification of a guilty suspect.

11. Many times, a suspect may attempt to clean the area containing a blood stain at the scene of a crime. One tool utilized by forensic investigators is a substance called luminol. Research luminol on the internet or at the library and explain how it may be used to aid investigators at a crime scene.