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The Mystery of the Bloody Stain using Neo/BLOOD™

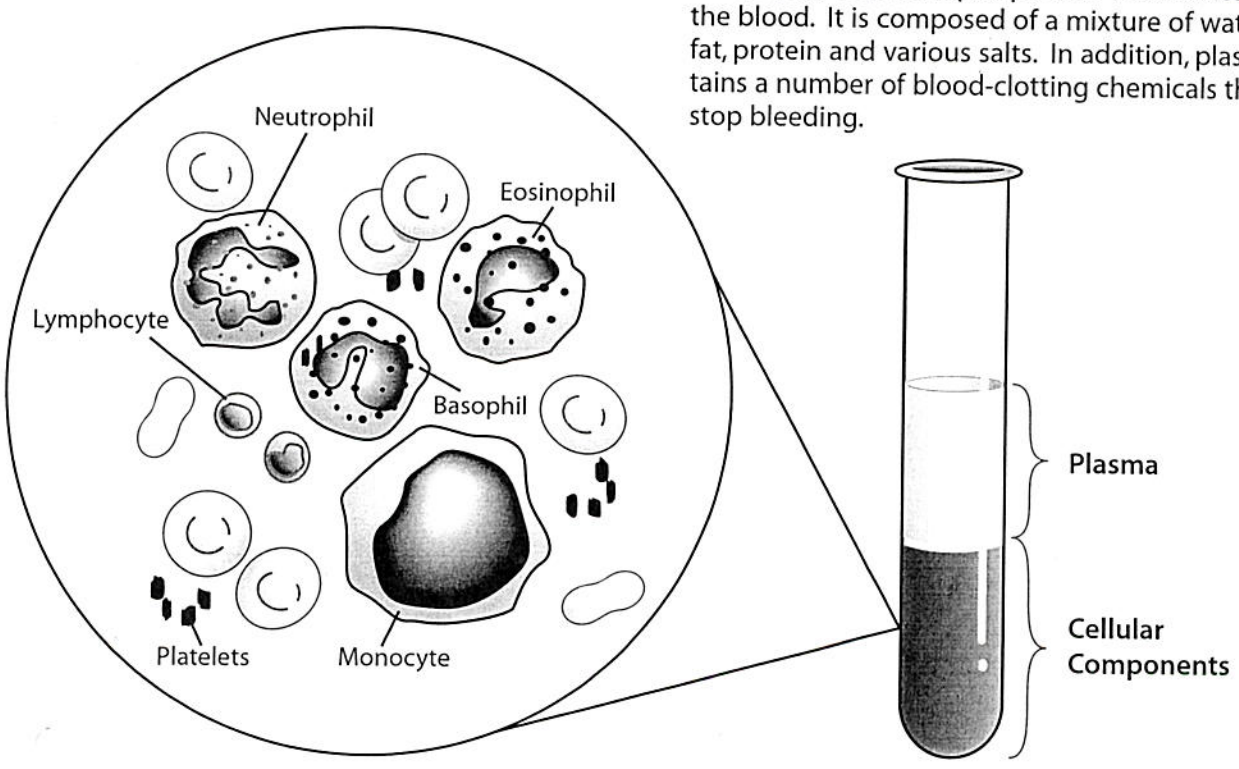
Objectives

- ***Perform*** **a simple test to identify if a red stain is simulated blood**
- ***Determine*** **the ABO and Rh blood type of unknown simulated blood samples**
- ***Identify*** **which of the three suspects is most likely to have committed the crime**
- ***Determine*** **the probability that the same blood type as the suspect's is found in the general population**

Background

Blood and blood stains left behind in a crime scene are important evidence and provide crucial information in a criminal investigation. Blood type evidence is usually used to exclude someone as a suspect, narrowing the list of possible suspects. Forensic scientists look at specific proteins (A, B, and Rh) on the red blood cells and determine the type of an unknown blood sample based upon their presence.

Blood is a tissue comprised of four components: plasma, red and white blood cells and platelets. Plasma is the clear, straw-colored liquid portion that makes up 55% of the blood. It is composed of a mixture of water, sugar, fat, protein and various salts. In addition, plasma contains a number of blood-clotting chemicals that help to stop bleeding.



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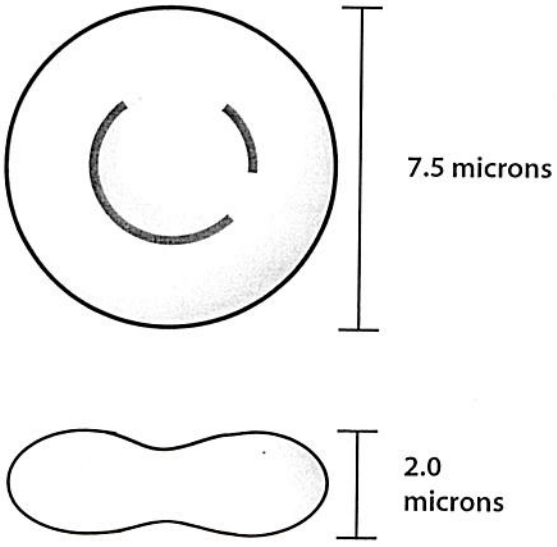
Red and white blood cells and platelets make up the remaining 45% of the blood. Red blood cells, or "erythrocytes," are tiny biconcave disks. Each red blood cell contains the oxygen-binding protein, hemoglobin. Hemoglobin contains four iron ions which bind with oxygen (O_2) and carbon dioxide (CO_2).



The shape of a red blood cell provides a greater surface area through which gases can diffuse and bind to the iron groups. The average normal red blood cell is approximately 7.5µm in diameter and 2µm in thickness.

Blood functions principally as a vehicle which transports gases, metabolic waste products and hormones throughout the body. As blood passes through the lungs, oxygen molecules attach to the hemoglobin. As blood passes through the body's tissues in capillary beds, the hemoglobin releases the oxygen. Carbon dioxide and other waste gases are, in turn, transported by the hemoglobin back to the lungs. Thereafter, the process is repeated.

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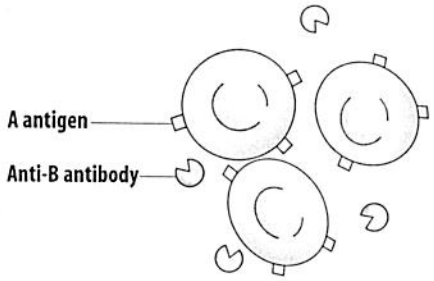
Determining the ABO-Rh Blood Type of Simulated Blood Samples

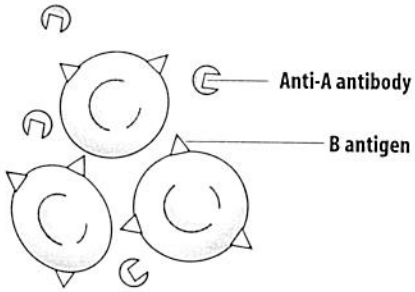
Surface proteins on red blood cells determine an individual's blood type. These surface proteins are called "antigens."

The system used to classify human blood is called the "ABO system." Dr. Karl Landsteiner, an Austrian physician, received the Nobel Prize in physiology for this discovery in 1930. With the ABO system, the kinds of antigens present on red blood cells determines the blood type. An individual with A antigens has "Blood Type A", one with B antigens has "Blood Type B", one with both A and B antigens has "Blood Type AB", and one with no antigens on the surface of his/her red blood cells has "Blood Type O."

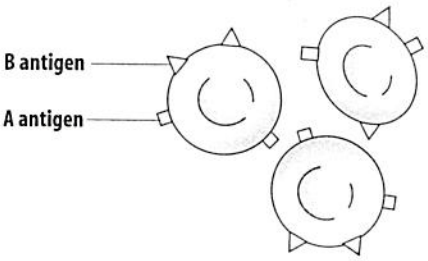
Blood plasma has circulating proteins called "antibodies". For example, individuals with A surface antigen have anti-B antibodies; those with B surface antigen have anti-A antibodies. Those with both A and B surface antigens have no antibodies. Individuals with no surface antigens have both anti-A and anti-B antibodies.



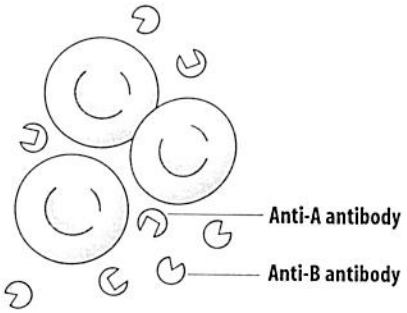
Blood Type A



Blood Type B



Blood Type AB



Blood Type O

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Data Table 1
ABO Blood Types Summary

Blood Type	Antigen on Red Blood Cells	Antibodies in Plasma	Can Recieve Blood From ...	Can Donate Blood To ...
A	A	B	O, A	A, AB
B	B	A	O, B	B, AB
AB	A and B	None	O, AB, A, B	AB
O	None	A and B	O	O, A, B, AB

Data Table 2
Agglutination Reactions in the ABO System

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

Blood typing is performed using "antiserum" - blood that contains specific antibodies. "Anti-A Serum," which contains anti-A antibodies, and "Anti-B Serum," which contains Anti-B antibodies, are used in ABO blood typing.

To perform a blood typing test, Anti-A and Anti-B sera are each separately mixed with a drop of sample blood and observed for "agglutination" or clumping.

Another important antigen on the surface of red blood cells is the Rh protein, named for the rhesus monkey in which it was first studied.

People who have this protein are "Rh-positive," and those who lack it are "Rh-negative."

Rh-negative individuals who have been transfused with Rh-positive blood can produce Rh antibodies. They may develop a transfusion reaction, during which agglutination may occur, if they are transfused again with Rh-positive blood. Usually Rh compatibility is tested when the ABO blood type is determined.

Data Table 3
Rh Agglutination Reactions

Rh Agglutination Reaction	Rh Factor
Agglutination	+
No agglutination	—

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Data Table 4
Frequency of ABO Blood Types in the U.S.

Blood Type	Frequency Percentage
A	42
B	10
AB	4
O	44

Data Table 5
Frequency of individuals with Rh+ and Rh- blood in the US

Blood Type	Frequency Percentage
Rh +	85
Rh -	15

Another, less-known and used system is the "MNS System." In addition to the A and B antigens, individuals carry one of the antigens, M, N or S on their red blood cells. This system is used to further exclude any potential suspects or clarify conflicts arising when two suspects may have the same ABO blood group.

Data Table 6
Frequency of individuals with M, N and S antigens in the US

Blood Type	Frequency Percentage
M	30
N	22
S	48

Using the frequencies of the specific antigens listed in Tables 4, 5 and 6, one can calculate the probability that an individual in a given population would have a particular combination of blood types. For example, to determine the likelihood that an individual has blood type combination of B, M and Rh+, first look up the percentages for each blood type in the tables above. "B" occurs in 10% of the US population or in 1 out of 10 people which is a ratio of 10, "M" occurs in 22 percent or a ratio of 4.55 and "Rh+" occurs in 85% of the population or a ratio of 1.18. Multiply these three ratio values, (10) x (4.55) x (1.18) to obtain a value of 54.

B		M		Rh+	
$\frac{100}{10}$	X	$\frac{100}{22}$	X	$\frac{100}{85}$	=
(10)	x	(4.55)	x	(1.18)	= 54

This means that 1 out of every 54 people in the US has blood types B, M, and Rh+.

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Safety & Disposal

The simulated Neo/BLOOD and sera samples provided in this kit contain no biological components and are therefore safe from any potential biological hazards. However, you should always wear safety goggles, gloves and a lab apron to protect the eyes and clothing when working with any chemicals. The dye in the simulated Neo/BLOOD solution will stain your skin and clothing. Be sure that you wash your hands before leaving the laboratory.

Any simulated Neo/BLOOD waste from this lab may be disposed of by pouring it down the drain with copious amounts of water.

Crime Scenario

A team of crime investigators were called to the scene of a burglary. Late last night someone broke into a jewelry store. Upon arrival, the crime investigators noticed that several of the glass display cases were smashed, scattering glass throughout the store. A closer investigation of the glass fragments revealed that small pieces of what appeared to be blood-stained clothing were left behind. The pieces of the clothing material were carefully collected and sent to the forensic lab for analysis. After the crime investigators carefully reviewed all of the available evidence, they apprehended three suspects.

Since select red colored stains may look like blood, forensic scientists first need to determine if the red stain found at a crime scene is actual blood. A test is performed to confirm the presence of one of blood's components, such as hemoglobin. If the stain is indeed blood, it is then collected and taken to the forensic lab for further testing, such as blood typing.

Your job as a forensic scientist is to provide crime investigators with additional proof in order to determine which of the suspects may have committed the burglary. You will first perform a test to verify that the stain is indeed blood and then type the blood sample found at the crime scene to match its type with the blood samples drawn from 3 suspects.

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ACTIVITY 1

Presumptive Test for Blood

One of the preliminary tests that crime investigators perform at the scene of a crime is to determine whether or not a suspicious red stain is blood.

Since the blood you'll be using is simulated, it does not contain hemoglobin, the presence of which confirms that the stain is actual blood. Instead, you'll look for the actual presence of simulated blood cells under a microscope.

What you need

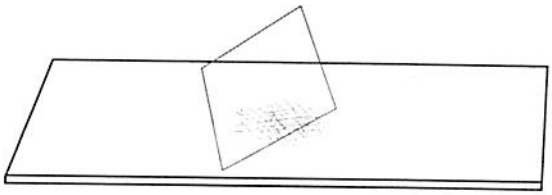
Per Student:

1	Cloth, stained
1	Compound Microscope
1	Coverslip
1	Microscope slide
	Paper towels
	Water

What to do...

Step 1

Your teacher will provide you with a red-stained piece of cloth. Cut out a small portion of the stained cloth or remove individual fibers and place them flat on a microscope slide.



Step 2

Place a drop of water over the stained cloth and place a coverslip over it.

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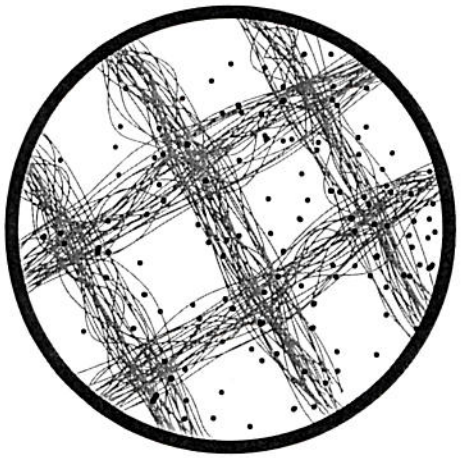
Step 3

Place the prepared slide under a microscope and examine it under 100X magnification. Scan the entire area of the stain and note the presence of any simulated blood cells, either attached to the fibers or nearby. Simulated red blood cells will appear pink, while white blood cells are stained blue.



Questions

1. Did your stained cloth sample contain simulated blood cells? What does the presence of these simulated cells indicate?

2. Why does the presence of hemoglobin provide proof that a suspicious stain is actual blood?

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ACTIVITY 2

ABO and Rh Blood Typing

Once it is verified that the stain is indeed blood, a sample is carefully collected and taken to the forensics lab. The next test is to type the blood sample collected at the crime scene and compare it to the blood types of the three apprehended suspects.

What you need

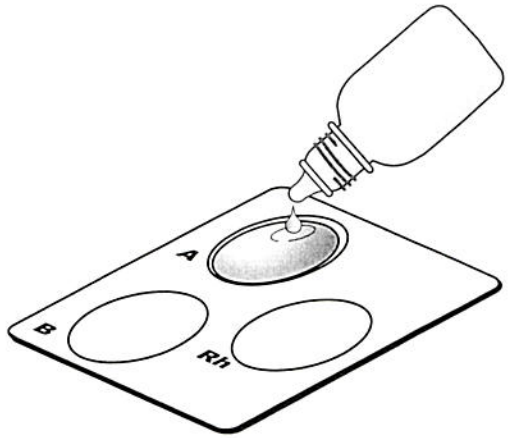
Per Student:

- Anti-A Serum (simulated)
- Anti-B Serum (simulated)
- Anti-Rh Serum (simulated)
- 4 Blood samples (simulated):
  - Crime Scene
  - Suspect 1
  - Suspect 2
  - Suspect 3
- 1 Blood typing tray
- Paper towels
- 1 set Stirring sticks (blue, green and yellow)

What to do...

Step 1

Your teacher will provide you with simulated blood scene and suspect samples, along with the anti-sera. Place 5 drops of the Crime Scene Simulated Blood Sample in each well on your blood typing tray.



Step 2

Place 3 drops of Anti-A Simulated Serum in Well A.

Step 3

Place 3 drops of Anti-B Simulated Serum in Well B.

Step 4

Place 3 drops of Anti-Rh Simulated Serum in Well Rh.

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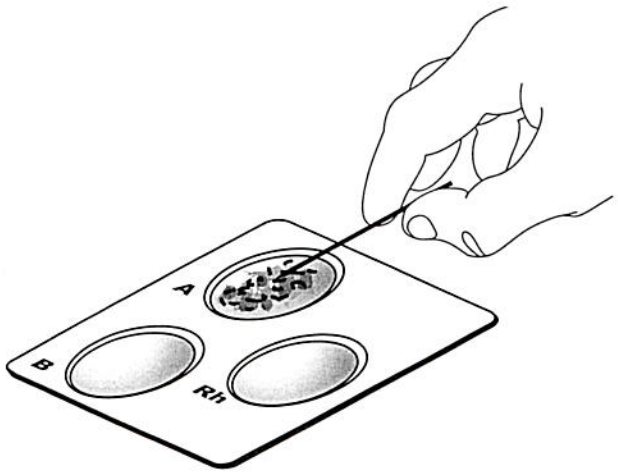
Step 5

Use a separate stirring stick to mix the simulated blood and serum in each well for approximately 10 seconds.



Step 6

Carefully examine each well to determine if the simulated blood in each well has clumped or agglutinated. Record your results and observations in Data Table 1.

Step 7

Thoroughly rinse the tray and stirring sticks and repeat Steps 1-6 to type the simulated blood samples of the three suspects.

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Understanding your results:

Clumping indicates that the simulated blood sample contains antigens that reacted against the antibodies in the typing serum that you mixed it with.

Type A

If the blood in Well A is the only blood that agglutinates or clumps, then the blood sample you tested is Type A blood.

Type B

If the blood in Well B is the only blood that agglutinates or clumps, then the blood sample you tested is Type B blood.

Type AB

If the blood in both Well A and Well B agglutinates or clumps, then the blood sample you tested is type AB blood.

Type O

If the blood in both Well A and Well B does not agglutinate or clump, then the blood sample you tested is Type O blood.

Rh

If the blood in Well Rh agglutinates or clumps, then the blood sample you tested is Rh Positive blood.

Data Table 5

Simulated Blood Sample	Agglutination in Well A (+/-)	Agglutination in Well B (+/-)	Agglutination in Well Rh (+/-)	Blood Type	Observations
Crime Scene					
Suspect 1					
Suspect 2					
Suspect 3					

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Questions

1. Based upon your results, which of the suspect's blood type matches the blood type found at the crime scene?

2. Based upon your blood typing results, do you think there is enough evidence to prove the suspect committed the burglary?

3. How is this simulated blood typing activity similar to actual human blood typing?

4. Calculate the probability that a person would have blood that is types AB, N and Rh-.

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Going Further

Study how blood typing is used to exclude people in paternity cases.

Research further how blood found in crime scenes is collected and taken to a forensics lab.

Research how a blood stain found at a crime scene can be distinguished as human or animal blood.

Since blood typing can only exclude potential suspects, research how a much more powerful technique known as DNA fingerprinting is used to provide more conclusive evidence in solving crimes.

Learn & Read More About It

Joe Nickell and John F. Fischer. *Crime Science: Methods of Forensic Detection*. University Press of Kentucky.

John Houde. *Crime Lab: A Guide for Nonscientists*. Calico Press, 1998.

Barbara J. Bain. *A Beginner's Guide to Blood Cells*. Blackwell Science, 1996.

Jackie Hardie. *Blood and Circulation*. Heinman Library, 1997.

Arthur Selzer. *Heart: Its Function in Health and Disease*. University of California Press, 1996.

Knapp, Loren. *Perspectives in Human Biology*. Wadsworth Publishing, 1998.

Neat Websites

Forensic education and related links
http://www.forensicdna.com

American Academy of Forensic Sciences
http://www.aafs.org/links/links.htm

Blood stain evidence institute
http://myweb.servtech.com/~forensic/index.html

Provides information on blood banking
http://cer.hs.washington.edu/John/blbnk.htm

Blood typing tutorial
http://www.biology.arizona.edu/human_bio/problem_sets/blood_types/Intro.html

Information on blood and blood typing from Red Cross
http://www.redcross.org/oh/northernohio-blood/bloodtype.html

National Heart, Lung and Blood Institute
http://www.nhlbi.nih.gov/nhlbi/nhlbi.htm