Blood Stain Analysis

Part One

Investigators often find blood stains during their examination of a crime scene. They also find stains that could be either blood or some other similar substance, like reddish-brown paint. What other things can you think of that might look like blood? How would you test a stain to see if it is blood?

Have you ever used hydrogen peroxide to clean a cut or a scrape? What happened when the hydrogen peroxide came in contact with the blood from the wound?

Blood contains an enzyme called catalase, which breaks down hydrogen peroxide into water and oxygen gas.

$$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2$$

When this reaction occurs, the oxygen gas is released as bubbles. The catalase enzyme performs an important function to living organisms because hydrogen peroxide is very toxic to living cells. Other organisms, including plants and some bacteria, also make catalase.

If you place a few drops of hydrogen peroxide on a substance that contains catalase, it will bubble profusely. These substances that bubble with the addition of hydrogen peroxide are said to test positive for catalase.

Criminal investigators do not typically use the catalase test at crime scenes. Other simple tests are better at detecting very dilute concentrations of blood –

sometimes so dilute the human eye can longer see the stain. These tests (listed below), while more reliable, require more expensive chemicals.

Benzidine
Leucomalachite green
Phenolphthalein
Takayama test
Tetra-methyl bezidine
Luminol
and Spectrophotometric tests.

Most of these tests rely on the activity of peroxidase enzymes in blood to react with a chemical stain causing it to change color, or in the case of luminol, glow in the dark.

In this activity, you will be comparing the results of the **catalase test** using hydrogen peroxide with the **phenolphthalein** test, to see how each reacts with blood and other substances.

Which of the following substances do you think would test positive for catalase? Make a prediction for each, and explain your reasoning. Make sure you make a prediction for each substance before conducting your test.

Substance	**Do you predict it will be catalase positive or negative?**	**Explain your prediction.**	**Result: Catalase positive or negative?**
red paint			
fresh tomato (smashed)			
cooked tomato sauce			
red food coloring			
fresh, raw beet			
blood (chicken or cow)			

Test each of these substances to see if it is catalase positive or negative by placing a few drops of hydrogen peroxide on a small amount of each. Record results in the table above. **SAFETY NOTE:** ***Even though you will not be using any real human blood in this activity, you should wear appropriate protection such as gloves.***

Which of the following substances do you think would test positive for blood using the phenolphthalein test? Make a prediction for each, and explain your reasoning. Make sure you make a prediction for each substance before conducting your test.

Substance	**Do you predict it will be positive or negative? (phenolph-thalein)**	**Explain your prediction.**	**Result: positive or negative? (phenolph-thalein)**
red paint			
fresh tomato (smashed)			
cooked tomato sauce			
red food coloring			
fresh, raw beet			
blood (chicken or cow)			

Test each of these substances to see if it is positive or negative for the presence of blood using the phenolphthalein test. Place a few drops of phenolphthalein working solution and a drop or two of hydrogen peroxide on a piece of cloth stained with a small amount of each substance. Record results in the table above. **SAFETY NOTE:** ***The phenolphthalein solution may burn or irritate skin. WEAR GLOVES.***

Analysis of evidence from the crime scene:

Test any stains from the crime scene that you suspect may be blood stains. You should only test part of each sample and not the whole sample. Why?

Record your results below.

stain	Catalase +/-	Phenolphthalein +/-
A		
B		

Which of these stains is probably blood? Could it be anything else other than blood? Check your answers against the key provided.

Blood Stain Analysis

Part Two

Once you know that a stain is blood, what else would you do as a forensic scientist? There is a lot of potential information in a blood stain.

Pattern and shape: The shape and pattern of blood drops can reveal important information about the nature of the wound from which the blood came. Was the bleeding person standing still or walking? What distance did the blood drop fall? Did the blood spatter in all directions? A good investigator would carefully photograph all blood stains from different angles both so that a forensic scientist could examine the pattern and to be able to present the evidence to a jury.

DNA: Blood contains DNA, and depending on the size of the stain and its condition (old, new, dry, etc.), a forensic scientist may be able to get enough information to obtain a highly probable match of a suspect with the evidence. Two techniques are heavily used by forensic scientists in evaluating DNA evidence from blood or other body tissues – polymerase chain reaction (PCR) and variable number tandem repeats (VNTR's).

Type: Blood typing can be used as an initial test to exclude some suspected sources of a bloodstain. For example, if a blood stain at the crime scene contains Type A blood, but the key suspect has Type O blood, the suspect could be excluded as a source of the blood stain – meaning he or she definitely did not leave the blood stain. However, blood type alone usually cannot positively identify a suspect because many people share the same blood type.

Investigators have collected blood samples from each of the suspects in the case. The samples and the evidence are labeled A-D. It will be your job to type each sample. You will determine both the ABO blood type of each sample as well as the Rh factor type.

ABO blood group:
There are three alleles at the locus that determines an individual's ABO blood type, and there are four possible types -- A, B, AB, and O. Type A individuals

have "A" antigens in their blood. Antigens are proteins that the body's immune system recognizes and either mounts an immune response to, if the antigen is from a foreign source, or ignores, if the antigen is part of the body itself. Type A individuals do not mount an immune response against A antigens. If they did, the immune system would produce A antibodies that would bind to the A antigens and cause the blood to thicken and clot. Individuals who are type B don't produce antibodies against B antigens, but they do produce antibodies against A antigens. Individuals who are type O have neither A antigens or B antigens, so they have antibodies to both types. Individuals who are type AB, have both antigens and do not have antibodies to either A or B. There are no O antigens. Type O individuals simply do not produce any antigens in this blood type group.

	Type A	Type B	Type AB	Type O
antigens	A	B	A and B	neither A nor B
antibodies	B	A	neither A nor B	A and B

Rh factor:

Another commonly tested blood antigen group is the Rh factor. Individuals who produce Rh antigens are referred to as Rh positive. Individuals who do not produce Rh antigens are referred to as Rh negative.

Follow the directions provided with your blood typing kit and determine the blood types of the samples labeled A, B, C, and D. ***Remember to wear gloves while handling the blood samples.***

Record the blood types of each individual below. Consult the key to the labels and write in the identity of each sample.

Label	Blood Type	Identity
evidence		
suspect 1		
suspect 2		
suspect 3		

Answer the following questions regarding your results:

1.) Based on the results of the blood type analysis, can you exclude any of the suspects as having left the blood stain found at the crime scene?

2.) Based on the results of the blood type analysis, which suspect(s) could have left the blood stain at the crime scene?

3.) If you were allowed to perform additional tests using this blood stain from the crime scene, what would you recommend?