Name:

No Trace Left Behind

A Forensic Serology Laboratory Activity

Written by Tyiesha Moore for use on the MdBioLab

In the late hours of April 4, 2008, officers discover the body of Leif Steele, 36, having received a phone call reporting a disturbance at the Steele home.

Steele was found dead with multiple wounds in the kitchen. The refrigerator door was open, and there was a broken bottle of what appeared to be hot sauce. There were red splatter patterns inside the refrigerator. Mr. Steele's pants pocket was turned inside out, but there was no evidence that anything had been stolen from the house. Examination of Mr. Steele's hands revealed cuts on the knuckles of his right hand and a brown substance under his nails. The door leading from the kitchen to the garage was open. The family cat was found hiding in the garage.

Crime scene investigators meticulously processed the murder scene. Leif Steele, a Maryland native, was last seen at Mr. Crab's Crab Shack four hours before his body was discovered. He left the restaurant in his 2006 black Mustang. The vehicle has not been recovered. He made several calls on his cell phone. Investigators have submitted evidence for forensic examination. Because of the violent nature of the crime, the suspect may have left blood. Samples that test positive for blood will be used for DNA analysis.

www.bloodsplatter.com

IDENTIFY THE PROBLEM: What is the problem you are trying to solve?

Forensic serology - Forensic serology is the analysis of trace evidence and the identification of the evidence as possible body fluids (blood, saliva, urine etc) or hair. Trace evidence is the material present at crime scenes.

Blood - Blood is a red semi-solid substance that transports oxygen from the lungs and delivers it to body tissues and organs. The blood also removes the metabolic waste carbon dioxide from these tissues and organs where it is transported to the lungs and exhaled. The major components of blood are Red Blood Cells (RBC), white blood cells (WBC) and platelets. The non-cellular portion is called plasma. The red blood cells make-up 45% of blood volume and are the cells that transport oxygen. It is the hemoglobin protein that binds the oxygen and gives red blood cells the red color.

Leuco-malachite green - The leuco-malachite green test, also known as LMG, is used by forensic scientists to presumptively indicate whether blood is present on an object. LMG is normally clear and has a very faint green tint, but in the presence of hemoglobin and hydrogen peroxide, it becomes dark green/blue. This color test relies on the hemoglobin present in blood. Hemoglobin is a protein found in red blood cells. It is the hemoglobin that catalyzes the reaction of LMG with hydrogen peroxide.

Hemoglobin + LMG (**clear**) + H_2O_2 → oxidized LMG (**dark green/blue**) + H_2O + Hemoglobin

***Hemoglobin is a catalyst and is unchanged

Tests using LMG are presumptive because the test may produce a false positive result. Certain plants are oxidants (reducing agents), and will give a false positive result. Assume that no oxidants were present at the crime scene.

QUICK CHECK: What is a catalyst?

Laboratory Activity

The following is a list of the materials you will be using for the experiment:

Evidence Samples	Micropipette and tips	LMG (leuco-malachite green)
Bovine Hemoglobin	6-well plate	Hydrogen Peroxide
Marker	Gloves	Water

PART ONE: PREPARE THE CONTROLS

You and your partner will test evidentiary materials for blood. Your test results could be key in solving this case. You and your partner need to figure out if any of the evidence has blood. How will you know if evidence is positive for blood? Forensic Serologists use controls to check the results of their experiments. You and your partner will use a positive and a negative control for this experiment.

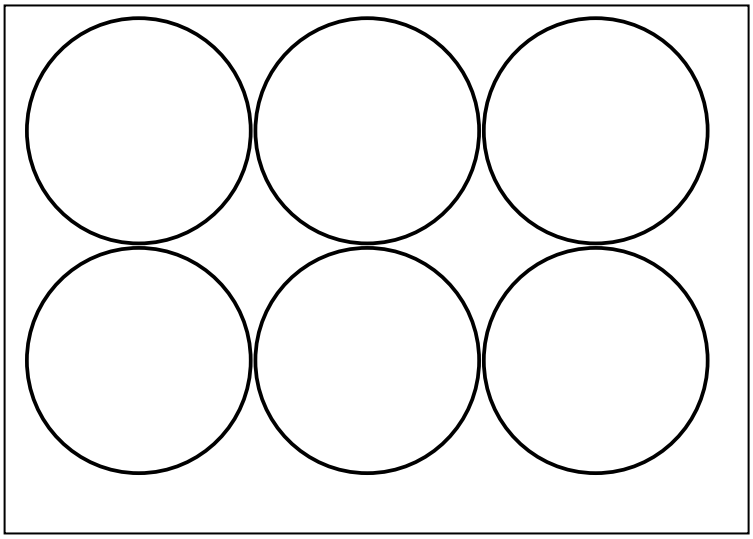
What are some things you could use for a positive control?

What are some things you could use for a negative control?

As a positive control, you will be using hemoglobin taken from the blood of a cow. As your negative control, you will be using water, something known to not contain hemoglobin.

The following steps are your protocol for performing a presumptive test for blood. Work with your partner and follow the directions carefully – be sure to check off each step as you complete it.

☐ 1. Locate the 6-well plate. Label the diagram below according to the well you will use for your controls and evidentiary samples.



Positive control: _____
We know this will test positive for blood

Negative control: _____
We know this will test negative for blood

Evidentiary sample # 1: _____
You will test this evidence to determine if blood is present

Evidentiary sample # 2: _____
You will test this evidence to determine if blood is present

Evidentiary sample # 3: _____

You will test this evidence to determine if blood is present

- ☐ 2. Put 50 uL of the positive control to the center of a well. Dispose of tip.
- ☐ 3. Put 50 uL of the negative control to the center of a well. Dispose of tip.
- ☐ 4. Add 50 uL of hydrogen peroxide directly to controls. Is there any color change?
- ☐ 5. Add 50 uL of LMG directly to controls.
- ☐ 6. Record your observations.

What happened to the positive control when you added the LMG? What does this indicate?

What happened to the negative control when you added the LMG? What does this indicate?

PART TWO: TEST THE CRIME SCENE EVIDENCE

- ☐ 7. Put your evidence sample in the appropriate well.
- ☐ 8. Add 50 uL of hydrogen peroxide directly to the evidence. Dispose of tip.
- ☐ 9. Add 50 uL of LMG.
- ☐ 10. Record your observations.

What happened to evidentiary sample #1 when you added the LMG?

What happened to evidentiary sample #2 when you added the LMG?

What happened to evidentiary sample #3 when you added the LMG?

DATA ANALYSIS

Analyze the results of your experiment. Compare the evidence samples to the controls. Which evidence, if any, tested positive for the presence of blood (circle your answers)?

Positive control?	YES	NO
Negative control?	YES	NO
Evidentiary sample #1?	YES	NO
Evidentiary sample #2?	YES	NO
Evidentiary sample #3?	YES	NO

CONCLUSION

Interpret the results of your experiment. Be sure to use the positive and negative controls to make a determination about the evidentiary samples. When making a conclusion, forensic serologists have to make up their mind about proceeding with additional confirmatory tests. Should you proceed with confirmatory tests for blood? Is it human blood? Should you eventually perform DNA analysis?

What conclusions can you make from the experiment?