EDVOTEK®

PLTW

STUDENT VERSION

EDVOTEK & PLTW Experiment #491

Blood Evidence

Experiment Objective:

In this experiment students become crime scene investigators. They will learn about some of the techniques forensics scientists use to analyze blood and apply them to solve a classroom crime. The students first check a crime scene for the presence of blood using the Kastle-Meyer test. They then confirm the presence of blood and narrow down suspects using blood typing and blood spatter analysis.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

Experiment Components

Component		Storage	Check √
MODULE I - Identifying Blood at the Crime Scene			
A	Simulated Blood Solution	Refrigerator	❑
B	Simulated Blood-free Solution	Refrigerator	❑
C	Phenolphthalin Stock Solution	Refrigerator	❑
D	Hydrogen Peroxide Solution	Refrigerator	❑
•	Evidence Bag	Room Temp.	❑
•	Cotton Swabs	Room Temp.	❑
•	Transfer pipets	Room Temp.	❑
MODULE II - Blood Typing Analysis			
•	Control ABO simulated blood samples (A, B, AB, and O)	Refrigerator	❑
•	Simulated blood samples from Crime Scene (CS1, CS2 and Anna Garcia)	Refrigerator	❑
•	Simulated blood samples from five Suspects	Refrigerator	❑
•	Anti-A and Anti-B serum	Refrigerator	❑
•	Red dye concentrate (for coloring)	Room Temp.	❑
•	Transfer pipets	Room Temp.	❑
•	Microtiter plates	Room Temp.	❑
•	Microcentrifuge tubes	Room Temp.	❑
MODULE III - Blood Spatter Analysis			
•	Simulated blood-spatter solution	Refrigerator	❑

There is enough of each sample for 10 groups.

Experiment Requirements *(NOT included with this experiment)*

- 95-100% Ethanol
- Optional: Automatic micropipette (10 – 100 µL)
- Optional: Microcentrifuge tube racks

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals. No actual blood or blood products are used in this experiment. None of the experiment components are derived from human sources.

Background Information

Modern biotechnology has revolutionized criminal investigations. The success or failure of a criminal investigation begins with the identification and proper collection of samples from a crime scene. Therefore, today's detectives work closely with forensic scientists. Any sample contamination can lead to false negatives or false positives which may compromise the investigation. Detectives must make careful observations and identify any evidence left at the scene.

The materials left behind at a crime scene can be a stain of blood, fingerprints, a few cells caught under the victim's fingernails, a piece of human hair, and many more. However, a red stain on the floor cannot be immediately assumed to be blood, and a piece of hair may not have necessarily been the criminal's. Before making any conclusions about a crime, detectives must wait until extensive forensic testing has been done on each piece of evidence. The first step when dealing with any biological evidence is correctly identifying the material. Detectives must then take the information given to them by forensic scientists and piece together information about motive, ability, and alibis to determine the criminal.

Determining the nature of evidence is a complex and multi-step process. Forensic scientists use many different assays to accurately determine the identity of a substance, however all tests performed should be quick, inexpensive, and minimally affect the evidence. Each of these factors are important because before performing additional tests scientists must understand what they are dealing with. Trying to extract DNA and run forensic analysis from a sample that was never confirmed to be blood could lead to many wasted hours!

Depending on the sample collected, different tests can be used to point investigators towards the criminal. For example, blood is one of the most common forensic samples found at a crime scene. Detectives can perform forensic analysis to detect blood that may not be visible to the naked eye, determine if the blood is from a human or animal, rule out possible suspects, and understand how the crime was committed.

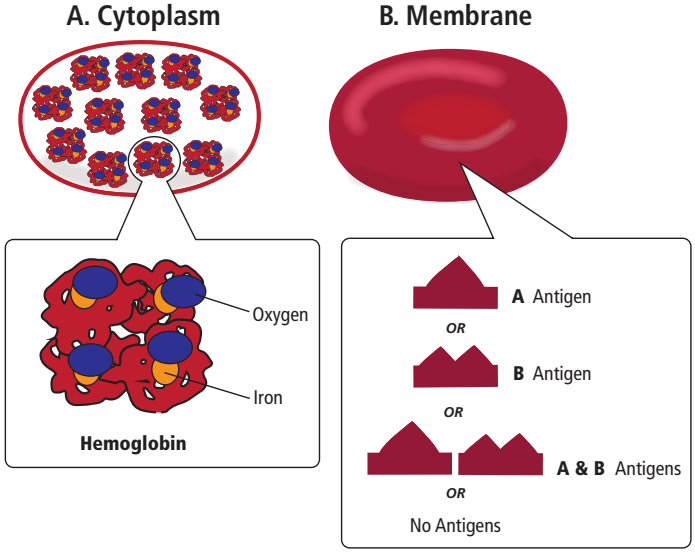


Figure 1: Features of a Red Blood Cell

BLOOD IDENTIFICATION

When a detective encounters a suspicious stain or liquid at a crime scene, it must first be analyzed. Not every red stain is blood, therefore a forensic scientist must confirm the identity. There are many different blood identification tests that can be used, but most rely on similar qualities of blood. Blood is composed of many different cell types suspended in plasma. The major cell types in the blood are white blood cells, platelets, and red blood cells. White blood cells play a large role in the immune system, platelets are responsible for clotting blood during bleeding, and red blood cells are the major carriers of both iron and oxygen in the body.

Red blood cells are anucleate, meaning that they lack a cell nucleus. Being anucleate, they contain much more cytoplasm than most other cells. In red blood cells, this cytoplasm is largely filled with a molecule called hemoglobin (Figure 1A). Hemoglobin carries iron, storing it when levels are high and releasing it when levels are low. Hemoglobin can also bind to oxygen molecules. When air fills the lungs, oxygen is transported into the pulmonary capillaries and is taken in by red blood cells. Hemoglobin binds to the oxygen molecules, and later releases them to various tissues in the body. Given the abundance of hemoglobin in blood, and it's very unique characteristics, it is often the protein used to identify blood at the scene of a crime.

Blood identification has at least two steps: presumptive and confirmatory testing (Figure 2). Presumptive testing is the initial testing that takes place which suggests that a sample may be blood. These tests are typically based on the properties of hemoglobin, however they can produce false positives to substances that have similar properties. Confirmatory testing relies on other unique properties of blood, such as the proteins present on the surface of red blood cells (Figure 1B).

Step 1: Presumptive Tests

Hemoglobin can be detected by forensic analysis even if it is not visible to the naked eye. Even if a large blood spill was cleaned up from a carpet, leucocrystal violet (LCV) can be used to detect remaining hemoglobin molecules and identify the blood. Similarly, if blood was cleaned up from tile with cleaner, luminol can be used to fluorescently detect remaining hemoglobin molecules. However, both of these tests can yield false negatives for blood. To more accurately identify blood, detectives swab areas detected by LCV or luminol testing as presumptive blood areas and bring the evidence back to the forensic science lab for additional testing.

The most common presumptive forensic blood test is the Kastle-Meyer test (Box 1). The Kastle-Meyer test uses a compound known as phenolphthalin (pr. fee-nawl-thal-een), which reacts with the iron carried by hemoglobin. First, presumptive blood is gathered on a cotton-tipped swab. The cellular membranes of cells on the swab are then broken open (lysed) by applying a few drops of 95% ethanol. Phenolphthalin solution is then applied, followed quickly by hydrogen peroxide. If the cotton swab turns pink, it means that there was likely hemoglobin in the sample.

Box 1: Chemistry of the Kastle-Meyer Test

The phenolphthalein ($C_20H_{16}O_4$) used in the Kastle-Meyer test has been reduced, i.e. it has gained electrons, and is actually called phenolphthalin ($C_20H_{14}O_4$). The reaction in the Kastle-Meyer test is based on the reaction between the iron in hemoglobin and hydrogen peroxide (H_2O_2). The iron in hemoglobin reduces (supplies electrons to) the H_2O_2, creating water (H_2O). This reaction depletes the hemoglobin of electrons, which are in turn supplied by phenolphthalin. The oxidation, i.e. the release of electrons, of phenolphthalin turns it back into phenolphthalein, which has a characteristic pink color.

$$Fe^{4+} + C_20H_{14}O_4 + H_2O_2 \rightarrow C_20H_{16}O_4 + H_2O + Fe^{3+}$$

Step 2: Confirmatory Tests

Presumptive tests, such as the Kastle-Meyer, must be confirmed using a test that definitively detects blood. These are known as confirmatory tests. Confirmatory tests are often much more expensive and can take more time than presumptive tests. The most common confirmatory test for blood is the Rapid Stain Identification of Human Blood (RSID). The RSID works similarly to a pregnancy test. The sample is applied to the device, and antibodies that recognize blood proteins specifically bind to the sample. If the antibodies bind and the sample is positive for blood, a visible line is shown in the viewing window (Figure 2).

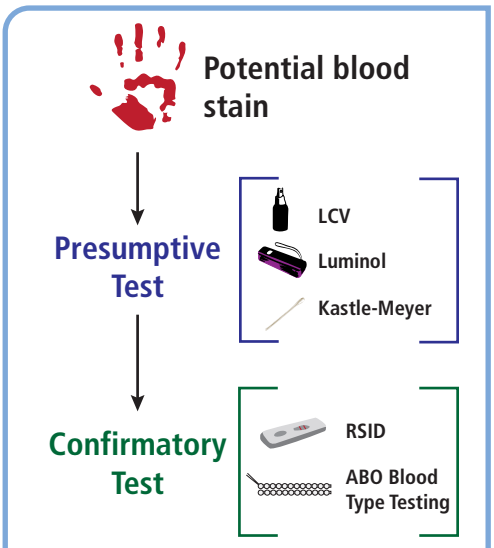


Figure 2: Blood Identification Steps

Another confirmatory test for blood is ABO blood type testing. Testing for blood groups relies on the precipitation of an antigen-antibody complex, called agglutination. Only blood will produce this agglutination, which is why it is classified as a confirmatory blood test.

In addition to being a confirmatory test, ABO blood typing is also a faster and more affordable identity test than other analysis techniques such as DNA fingerprinting. Indeed, forensic blood typing serves both as a confirmatory test and provides information about the suspect in the form of their blood type. Even though blood typing cannot point to a specific person as the criminal, it can point to a group of people that share the same blood type or eliminate suspects whose blood type does not match.

BLOOD TYPING

Blood typing is an immensely important clinical procedure. It is one of the first procedures performed during blood transfusions and surgery, and is also important in forensic science. Blood typing is an example of an agglutination assay, the precipitation of an antigen on red blood cells and antibodies in the blood. When both components are present at a similar concentration they are in a state known as equivalence (Figure 3). In an equivalent state, neither the antibody nor the antigen is in excess, and the antigen-antibody complex forms large networks that precipitate out of solution. Importantly, the precipitate is easy to detect by eye, making agglutination assays both easy and cost-effective to perform.

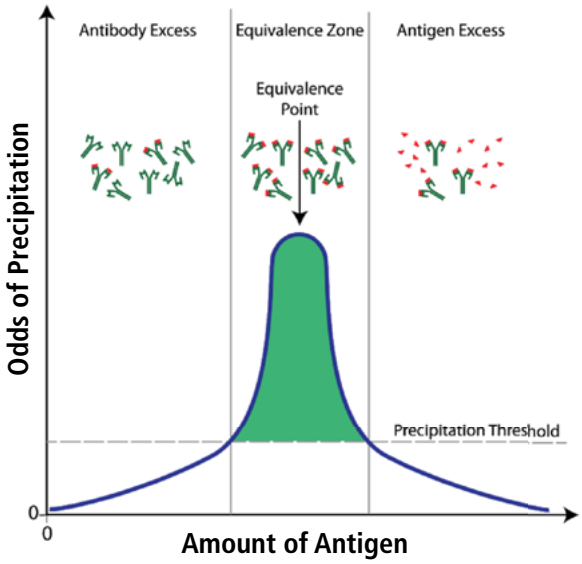


Figure 3: Equivalence Zone

The most common blood typing system relates to the presence of the A and B antigens on the red blood cells. This system, known as the ABO blood types, produces four possible blood types: A, B, AB, and O (Figure 4). Individuals with only A antigens will have type A blood, while someone with B antigens has type B blood. The A and B antigens are co-dominant, so a person can have both antigens on their red blood cells, leading to the AB blood type. If an individual has neither A nor B antigens they have type O blood.

A person with type A blood will recognize red blood cells with the A antigen as "self". However, if that person gets a blood transfusion with type B blood, the new red blood cells will be recognized by the immune system as foreign and will cause an immune response. Antibodies targeting the B antigens (anti-B antibodies) will bind to the B antigen on the transfused cells and agglutinate. In many cases, this severe immune response can be deadly. Therefore, it is important for hospitals and clinics to maintain records of patient blood types.

Blood Type	Antigen on Red Blood Cells	Antibody in Blood	Percentage of Population
A	A	anti-B	42%
B	B	anti-A	10%
AB	A & B	none	4%
O	O	anti-A & anti-B	44%

Figure 4: Types of Blood in the Population

The same reaction that can lead to severe immune responses in a patient is used for clinical and forensic blood typing experiments. For example, type B blood can be easily recognized by the agglutination between the anti-B antibodies and the B antigen. When something that is suspected to be blood is found at a crime scene, detectives will work quickly to secure the evidence and send it to a forensic lab for testing. In the lab, forensic scientists will perform presumptive and confirmatory tests for blood, potentially recommending additional testing such as DNA profiling.

BLOODSTAIN PATTERN ANALYSIS

Bloodstain pattern analysis (BPA) is the identification and interpretation of bloodstains. BPA attempts to reconstruct the events that resulted in bloodshed to help investigators better understand the crime scene, identify or exclude potential suspects, and potentially solve the crime. Forensic scientists must use multiple scientific disciplines while examining blood patterns, including biology (principles of blood, anatomy, and physiology), physics (velocity, adhesion/cohesion), and mathematics (angle, volume and distance). Through careful examination of the physical evidence BPA can potentially determine what caused a wound, the positioning of the victim and perpetrator(s), and what occurred before, during, and after an altercation.

BPA specialists are frequently used as expert witnesses by both the prosecution and defense teams during criminal trials. However, it is important for investigators to understand both the benefits and the potential shortcomings of BPA before incorporating it into a forensic workflow. For example, there are many aspects that can complicate analysis, including temperature and humidity, the surface that the blood lands on, and the complexity of the crime scene. Because of this,

BPA is typically used to confirm or refute other evidence, such as witness or suspect testimony, rather than to solve a crime independently.

One common form of blood evidence involves the blood drip patterns that result when drops of blood fall from a victim, weapon, or other surface due to gravity. This type of blood spatter can reveal details regarding the positioning of the victim and the direction or speed of individuals and objects. For example, drops of blood will spread and spatter further when dropped from greater heights, potentially providing evidence of the position of the victim at the time of the attack.

In this experiment you will use multiple aspects of forensic blood analysis to help uncover the truth behind a crime. First, you will use the Kastle-Meyer test to identify samples found at the crime scene. Samples testing positive will be further analyzed to identify the bloodtype, which can be compared to the victim and potential suspects. Finally, you will design an experiment using simulated blood to determine the position of the victim at the time of the attack. This evidence will be collected, cataloged, and stored for later use in identifying the perpetrator of the crime.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment students become crime scene investigators. They will learn about some of the techniques forensics scientists use to analyze blood and apply them to solve a classroom crime. The students first check a crime scene for the presence of blood using the Kastle-Meyer test. They then confirm the presence of blood and narrow down suspects using blood typing and blood spatter analysis.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

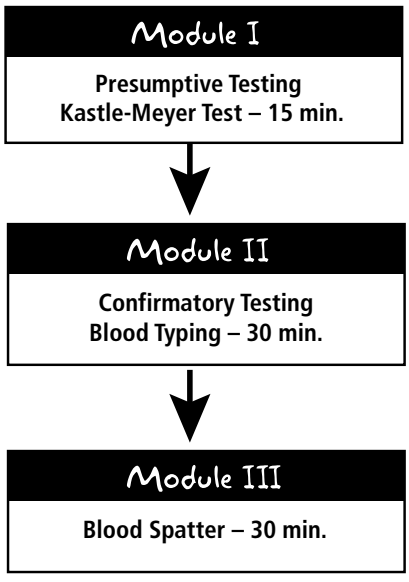
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

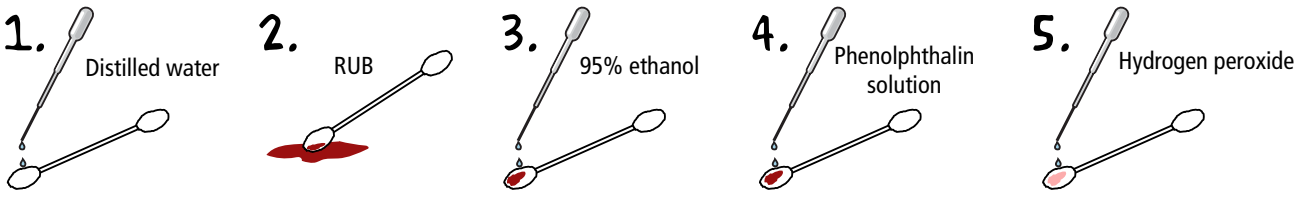
After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.



Module I-A: Presumptive Test

The first steps of your forensic analysis will use the Kastle-Meyer test as a presumptive test for blood. You will test the objects collected from the crime scene and control samples to see if they are positive or negative for the presence of blood. ***Remember to use a different transfer pipet or pipette tip for each solution to prevent cross contamination and incorrect results.***



1. Working with only one item at a time to avoid cross contamination, lightly **MOISTEN** a cotton swab with distilled water.

2. Gently **RUB** the moistened cotton swab against the evidence until the swab absorbs some of the red stain. **NOTE:** The swab does not need to be red, but there should be some color visible.

3. Use a transfer pipet to **ADD** two drops, or 40 µL, of 95% ethanol to the swab. **NOTE** any color change. **PLACE** the pipet and remaining ethanol to the side for testing additional samples.

4. Use a new pipet to **ADD** two drops, or 40 µL, of the phenolphthalin solution to the swab. **NOTE** any color change. No color change is expected even if blood is present. **PLACE** the pipet and remaining phenolphthalin to the side for testing additional samples.

5. Use a new pipet to **ADD** two drops, or 40 µL, of hydrogen peroxide to the swab. **NOTE** any color change. An immediate pink color is expected if blood is present. **RECORD** your results in the chart on the next page.

6. **REPEAT** steps 1-5 for each sample.

Module I-B: Analysis

1. Would you recommend sending any of the samples to the laboratory for confirmatory testing?

2. What is the purpose of the positive and negative control?

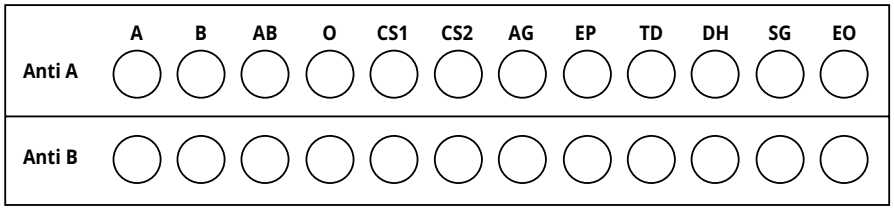
Sample ID	Color Change	Blood? Y/N
Positive control		
Negative Control		
Crime Scene sample #1		
Crime Scene sample #2		

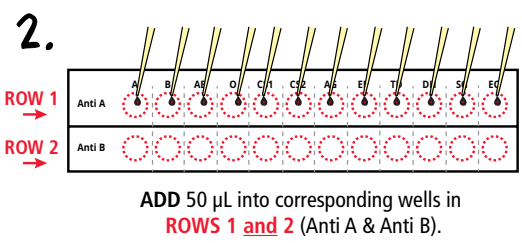
Module II-A: Confirmatory Test

Now that some samples from the crime scene have been identified as likely blood using the Kastle-Meyer test, it is necessary to confirm the identity of the samples and potentially identify suspects. In this Module, you will perform ABO testing on samples from the crime scene and potential suspects.

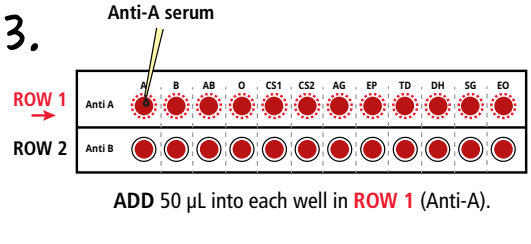
NOTE: This module requires adjustable volume micropipettes or plastic transfer pipets. If using transfer pipets, they can be reused by sucking up distilled water and expelling it into the sink after each new sample. Ensure the pipet is clean to minimize cross-contamination between samples.

1. **PLACE** a microtiter plate piece as shown below. Across the top of the plate, **LABEL** the 12 wells as indicated below using a laboratory marking pen. Label the 2 rows Anti-A and Anti-B respectively. The plate should look as pictured below.

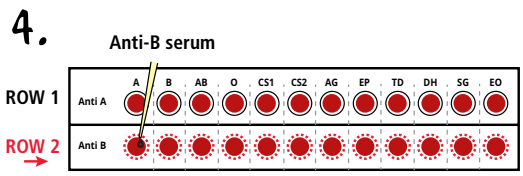




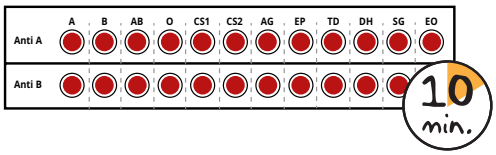
ADD 50 µL into corresponding wells in ROWS 1 and 2 (Anti A & Anti B).

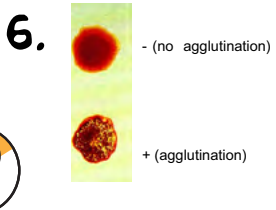


ADD 50 µL into each well in ROW 1 (Anti-A).



ADD 50 µL into each well in ROW 2 (Anti-B).

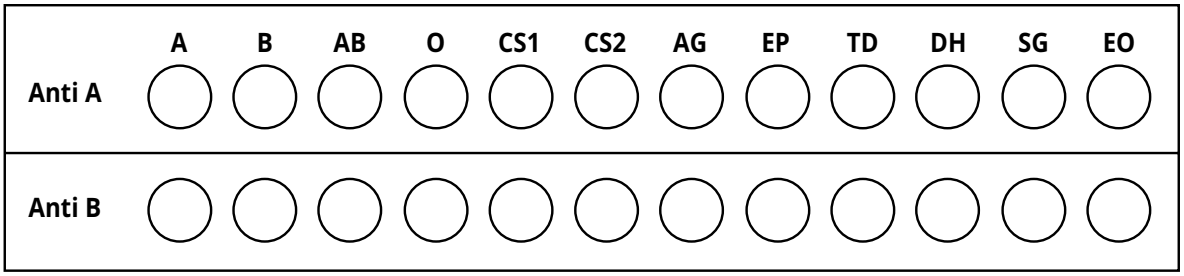




2. **ADD** 50 µL (or two drops from a transfer pipet) of each control blood type sample into each of the two corresponding wells. For example, control A blood type goes into the two wells under the letter "A". Repeat the same procedure for the crime scene collected blood samples (CS1 and CS2), the victim blood sample (AG), and blood from each of the five suspects. Each well requires 50 µL.
3. Use a new pipette tip to **ADD** 50 µL of Anti-A serum into each of the wells in row #1. The same tip or transfer pipet can be used for all samples in row #1.
4. Use a new pipette tip to **ADD** 50 µL of Anti-B serum into each of the wells in row #2. The same tip or transfer pipet can be used for all samples in row #2.
5. Let the samples **INCUBATE** undisturbed on the lab bench for 5-10 minutes.
6. **OBSERVE** the wells for the presence or absence of agglutination. Agglutination has occurred if the mixture appears to be granular and thick rather than smooth and watery. **RECORD** your results in the diagram on page 12.

Module II-B: Analysis

1. **RECORD** your results in the diagram below.



2. What are the ABO blood types of the crime scene collected blood and the suspects' blood samples?

3. Based on your observation, which of the suspects would you conclude might have left the blood stain at the crime scene?

4. What next steps would you take to confirm the suspect's identity?

Module III: Blood Spatter

The final forensic analysis you will perform involves examining the blood drops found at the crime scene. Design an experiment using blood stain pattern analysis to determine the height from which the blood was dropped.

1. **COLLECT** a tube of simulated blood and determine which surfaces you will test (e.g. floor, table top, piece of paper).
2. **RECORD** the surfaces and heights you will be testing in the chart below. We recommend testing a height range between 5 cm and 160 cm.
3. Using a transfer pipet, **REMOVE** some of the simulated blood. **RETURN** at least one drop to the tube to ensure there are no air bubbles in the pipet.
4. Carefully **APPLY** at least 5 drops to your surface from each previously selected height.
5. **RECORD** your observations for each height. Include the diameter of the drops.

Surface	Height (cm)	Observations

6. **GRAPH** the results of your height (x-axis) and diameter (y-axis) to create a standard curve of your data.
7. **COMPARE** your results to the crime scene blood drops, provided by your instructor, to determine the height from which the blood fell.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What is the composition of blood?

2. Describe the four different blood types possible from the ABO blood groups. What distinguishes each blood type?

3. Why is the Kastle-Meyer test useful, but not definitively confirmatory, for detecting blood?

4. What types of information can an investigator learn from bloodstain pattern analysis (BPA) of a crime scene? Are there any limitations to the technique?

5. What information can be obtained from blood drip patterns?