Edvo-Kit #191

Forensics Blood Typing

Experiment Objective:
In this experiment students become detectives, learn about techniques forensics scientists use to analyze blood, and solve a classroom crime. The students first check for the presence of blood using a phenolphthalein test. They then confirm the presence of blood and narrow down suspects using blood typing.

See page 3 for storage instructions.
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## Experiment Components

### MODULE I

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage</th>
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<tbody>
<tr>
<td>A Simulated Blood Solution</td>
<td>Refrigerator</td>
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<tr>
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<tr>
<td>C Phenolphthalein Stock Solution</td>
<td>Refrigerator</td>
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</tr>
<tr>
<td>D Hydrogen Peroxide Solution</td>
<td>Refrigerator</td>
<td></td>
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</table>

- Evidence Bag
- Cotton Swabs
- Transfer pipets

### MODULE II

- Control ABO simulated blood samples (A, B, AB, and O) Refrigerator
- Simulated blood sample from Crime Scene (CS) Refrigerator
- Simulated blood samples from three Suspects (S1, S2, and S3) 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.

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### Experiment Requirements *(NOT included with this experiment)*

- 95-100% Ethanol
- Optional: Automatic micropipette (5 – 50 µL)

**NOTE:** All Control blood samples (A, B, AB & O), Simulated Crime Scene (CS) and Simulated Suspect Blood Samples (S1, S2 and S3) will be prepared by instructor just prior to use.

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

Today’s detectives work closely with forensic scientists. The success or failure of a criminal investigation begins with the identification and proper collection of samples from a crime scene. Any sample contamination can lead to false negatives or false positives which compromises the investigation. Detectives must make careful observations and identify any material 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 can use many different assays to quickly and 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, and rule out possible suspects.

**BLOOD IDENTIFICATION**

When detectives encounter a stain or liquid they presume to be blood at a crime scene it must be tested. There are many different blood identification tests that can be used, but most rely on similar unique 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.

**Figure 1: Features of a Red Blood Cell**
Blood identification has at least two steps: presumptive and confirmatory testing (Figure 3). 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. 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.

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**Box 1: Chemistry of the Kastle-Meyer Test**

The phenolphthalein \((C_{20}H_{14}O_4)\) used in the Kastle-Meyer test has been reduced, i.e. it has gained electrons, and is actually called phenolphthalin \((C_{20}H_{16}O_4)\). 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.

\[
Fe^{4+} + C_{20}H_{16}O_4 + H_2O_2 \rightarrow C_{20}H_{14}O_4 + H_2O + Fe^{3+}
\]

The most common presumptive forensic blood test is the Kastle-Meyer test. The Kastle-Meyer test uses a compound known as phenolphthalein (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 (lysed) by applying a few drops of 95% ethanol. Phenolphthalein 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.

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**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).
Another confirmatory test for blood is 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.

**BLOOD TYPING**

How is blood typing performed? Precipitation reactions between antigens (on red blood cells) and antibodies (normally in the blood) can produce visible reactions if both components are in equivalence. In an equivalent state, neither the antibody nor the antigen is in excess, and antigen-antibody complex bind to form large networks that precipitate out of solution (Figure 3).

When an antibody attaches to a red blood cell’s antigen, the reaction is called an agglutination, and the precipitate that is formed is called an agglutinate. Agglutination is a routine and cost-effective procedure because the agglutinate is easily detectable by eye. Blood typing is an example of a clinical agglutination assay. Blood typing is immensely important in blood transfusions and surgery, and is also important in forensic science.

There are 4 possible blood types that a person can have (Figure 4): A, B, AB, and O. These letters refer to groups of proteins, or antigens, on the surface of red blood cells. The two antigens are A and B. These antigens are co-dominant, so a person can have both A and B antigens on their red blood cells, leading to an AB blood type. Someone with only A antigens will have type A blood, and someone with only B will have type B blood (Figure 1B). If a person 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 recipient’s immune system as “non-self”, and the immune system will mount an attack. Antibodies towards the B antigen (anti-B antibodies) will bind to the B antigen on the red blood cells and agglutinate the transfused red blood cells. In many cases, this severe immune response can be deadly. Therefore, it is very important for hospitals and clinics to maintain records of patients blood types. For blood typing experiments, this means that B blood can be easily recognized by the agglutination of B antigens with anti-B antibodies.

When something that could be blood is identified at a crime scene, detectives must work quickly and carefully to secure the evidence and send it to a forensic science lab. In that lab, forensic scientists will perform presumptive and confirmatory tests for blood, potentially even recommending additionally testing such as DNA profiling.
**Experiment Overview**

**EXPERIMENT OBJECTIVE**

In this experiment students become detectives, learn about techniques forensics scientists use to analyze blood, and solve a classroom crime. The students first check for the presence of blood using a phenolphthalein test. They then confirm the presence of blood and narrow down suspects using blood typing.

**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.

**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

Test the object collected from the crime scene and control samples to see if they are positive or negative for the presence of blood using the phenolphthalein test. Remember to use a different transfer pipet or pipette tip for each solution.

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

2. Firmly **RUB** the moistened cotton swab against the evidence until the swab absorbs the red stain.

3. Use a new pipet to **ADD** two drops or 40 µL of 95% ethanol to the swab. **NOTE** any color change. There should be no color change.

4. Use a new pipet to **ADD** two drops or 40 µL of the phenolphthalein solution to the swab. **NOTE** any color change. No color change is expected if blood is present.

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.
Module I-B: Analysis

1. Which samples would you recommend for confirmatory testing?

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

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Color Change + / -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
</tr>
<tr>
<td>Crime Scene sample #1</td>
<td></td>
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<tr>
<td>Crime Scene sample #2</td>
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<td>Crime Scene sample #3</td>
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<td>Crime Scene sample #4</td>
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<td>Crime Scene sample #5</td>
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<td>Crime Scene sample #6</td>
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<tr>
<td>Crime Scene sample #7</td>
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<tr>
<td>Crime Scene sample #8</td>
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</tr>
</tbody>
</table>
Module II-A: Confirmatory Test

1. **PLACE** a microtiter plate piece as shown below. Across the top of the plate, **LABEL** the 8 wells A, B, AB, O, C5, S1, S2, and S3 respectively, using a laboratory marking pen. Label the 2 rows Anti-A and Anti-B respectively. The plate should look as pictured below.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
<th>C5</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti A</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<td><img src="image8.png" alt="Image" /></td>
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<tr>
<td>Anti B</td>
<td><img src="image9.png" alt="Image" /></td>
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<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
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</tbody>
</table>

2. **PLATE** 3 drops 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 crime scene collected blood and blood from each of the three suspects. Each well requires 3 drops or 60 µL.

   **IMPORTANT:** Avoid cross-contamination by using a new disposable pipet or pipette tip for each sample.

3. **ADD** one drop or 20 µL of Anti-A serum into each of the wells in row #1.

4. **ADD** one drop or 20 µL of Anti-B serum into each of the wells in row #2.

5. **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 in the Results section.

![Image](image17.png)
Module II-B: Analysis

1. Record your results in the diagram below.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
<th>CS</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti A</td>
<td></td>
<td></td>
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<tr>
<td>Anti B</td>
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</tbody>
</table>

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

3. Based on your observation, which of the three 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?
Study Questions

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

1. What is the composition of blood?
2. What are the basic blood types?
3. Why is the phenolphtalein test a useful, but not definitive confirmatory test, for blood?
NOTES TO THE INSTRUCTOR

Blood typing is an important clinical assay that health care workers use routinely to properly care for their patients. Students should be made aware of the safety concerns when working with human blood products even though all the materials in this EDVOTEK kit are chemicals used to simulate blood.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK website. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

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

<table>
<thead>
<tr>
<th>Preparation For:</th>
<th>What to do:</th>
<th>When:</th>
<th>Time Required:</th>
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<tbody>
<tr>
<td>Module I: Presumptive Test</td>
<td>Preparation of samples.</td>
<td>Anytime before performing the experiment.</td>
<td>15 min.</td>
</tr>
<tr>
<td></td>
<td>Aliquot test solutions.</td>
<td>Anytime before performing the experiment.</td>
<td>10 min.</td>
</tr>
<tr>
<td>Module II: Confirmatory Test</td>
<td>Prepare blood samples.</td>
<td>No more than 24 hours before the lab.</td>
<td>15 min.</td>
</tr>
</tbody>
</table>

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

EXPERIMENTAL GUIDE

The background beginning on Page 4 is designed for classrooms that want to implement their own forensic scenario. In Appendix A, we also provide a background linked to a specific crime scene scenario where a fictional teacher’s laptop has been stolen.

For more information on creating a Forensics Unit for your class, see Appendix B.
Pre-Lab Preparations

MODULE I

Preparation of Control and "Blood Stained" Samples

There are 10 squares of various materials to be prepared as evidence. One item is to be designated as Positive Control, one item is to be designated as Negative Control. The remaining 8 items are to be designated as crime scene samples, which can yield either positive or negative results, depending on the teacher’s preference and preparation.

It is elective which items are Positive Control, Negative Control, or Crime Scene. Remove the samples from the Evidence Bag, label them Positive Control, Negative Control and Crime Scene samples 1 - 8. Keep record accordingly. **It is recommended that the teachers work with only one item at a time to avoid cross contamination.**

1. Treat the Positive Control and positive Crime Scene samples with Simulated Blood solution (Component A) as follows:
   a. Place the item on a flat, clean surface.
   b. Use a transfer pipet to draw some of the blood from the Simulated Blood solution tube (Component A). If using an automatic micropipette, measure 50 µL.
   c. Drop the blood onto the evidence from a distance of about 5 inches.
   d. Allow the evidence to soak for approximately 1 minute.
   e. Repeat steps (a) – (d) for the remaining samples.

2. Treat the Negative Control and negative Crime Scene samples with Simulated Blood-free solution (Component B) as follows:
   a. Place the item on a flat, clean surface.
   b. Use a transfer pipet to draw some of the blood from the Simulated Blood-free solution (Component B). If using an automatic micropipette, measure 50 µL.
   c. Drop the blood onto the evidence from a distance of about 5 inches.
   d. Allow the evidence to soak for approximately 1 minute.
   e. Repeat steps (a) – (d) for the remaining samples.

3. Either distribute one item per group and have the class share results, or disperse around a "crime scene" and allow students to walk around sampling evidence. Evidence can be tested multiple times as long as it is still absorbing onto cotton swabs.

Aliquot Test Solutions

1. Label 10 microtest tubes "Phenolphthalein" and aliquot 50 µL of Phenolphthalein solution (Component C) to each tube. Distribute one tube per student group.

2. Label 10 microtest tubes "Hydrogen Peroxide" and aliquot 50 µL of Hydrogen Peroxide solution (Component D) per tube. Distribute one tube per student group.

3. Label 10 microcentrifuge tubes "Ethanol" and aliquot 50 µL 95-100% Ethanol to each tube. Distribute one tube per student group. If 95-100% Ethanol is not available, we recommend using Isopropanol.

4. Label 10 small beakers or cups as "water" and dispense 5 mL of distilled water into each. Distribute one per student group.
Pre-Lab Preparations

MODULE II

Preparing the Microtiter Plate

Each group will require one microtiter plate piece (2 rows of 8 wells).

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</table>

Preparation of Control and Patient Blood Samples

*(Prepare no more than 24 hours before starting the experiment.)*

1. To prepare the Control blood samples (A, B, & O), Simulated Crime Scene (CS) and Simulated Suspect blood samples (S1, S2 & S3), add 4 drops or 50 µL of Red dye concentrate to the appropriate blood samples provided in the kit. Cap tubes and mix well.

2. To prepare Control blood sample AB, combine 1 mL of Control blood sample A and 1 mL of Control blood sample B (prepared in step 1) in a labeled microcentrifuge tube. Cap and mix well.

3. Label microcentrifuge tubes:
   - 10 tubes "A"
   - 10 tubes "B"
   - 10 tubes "AB"
   - 10 tubes "O"
   - 10 tubes "CS"
   - 10 tubes "S1"
   - 10 tubes "S2"
   - 10 tubes "S3"

4. Aliquot 100 µL of each Control and Patient blood samples (prepared in steps 1 and 2) to the appropriately labeled tubes. Use a new pipet or pipette tip for each sample.

5. Label 10 tubes "anti-A". Aliquot 180 µL of Anti-A serum to each. Distribute one tube per student group.

6. Label 10 tubes "anti-B". Aliquot 180 µL of Anti-B serum to each. Distribute one tube per student group.

7. Students will also require automatic micropipets and tips or 10 transfer pipets for dispensing the samples.
Expected Results

MODULE I

The crime scene samples yield either positive or negative result, depending on the teacher’s preparation. Consult with your teacher for expected results.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Color Change + / -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>+</td>
</tr>
<tr>
<td>Negative Control</td>
<td>-</td>
</tr>
<tr>
<td>Crime Scene sample #1</td>
<td></td>
</tr>
<tr>
<td>Crime Scene sample #2</td>
<td></td>
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<tr>
<td>Crime Scene sample #3</td>
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<td>Crime Scene sample #4</td>
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<td>Crime Scene sample #5</td>
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<td>Crime Scene sample #7</td>
<td></td>
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<tr>
<td>Crime Scene sample #8</td>
<td></td>
</tr>
</tbody>
</table>

1. Which samples would you recommend for confirmatory testing?

The samples recommended for confirmatory testing should be the ones that provided a positive phenolphthalein reaction.

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

The purpose of the positive control is to have a reference for what a positive test result looks like. The purpose of the negative control is to have a reference for what a negative result looks like.
2. **What are the ABO blood types of the crime scene collected blood and the three suspects’ blood?**

   The crime scene sample is type A blood. Suspect 1 has type O blood, suspect 2 has type A blood, and suspect 3 has type B blood.

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

   Based on the results of the blood typing test, it would seem that suspect 2 left the blood stain at the crime scene.

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

   While blood typing can narrow down suspects, DNA testing would have to be performed to conclusively say that the blood belongs to suspect 2.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A  Background Information: The Crime
B  Guide to Implementing a Forensics Unit in the Classroom

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

Background Information - The Crime

On the next page is a crime scene scenario for your students. In it, someone has broken into a classroom to steal the teacher’s laptop. Presumptive blood left behind must be tested.

In Module I, students will identify which samples may be blood. Below, we have outlined 3 potential suspects for the crime. Their blood will be typed against the blood found at the scene. If using this scenario, you may either label the simulated blood samples “suspect 1”, “suspect 2”, and “suspect 3” or label them using the suspect’s name.

Enough blood was collected to be analyzed for blood typing analysis, and Ms. Penelope has identified 3 potential suspects based on their lack of alibi and motive.

• **Suspect 1** is Alice. Alice is a straight A student, however she did not complete last week’s project and got a zero. Ms. Penelope believes that she may have stolen the computer to change her grade.

• **Suspect 2** is the principal, Mr. Smith. He had been asking teachers to evaluate him, and got upset when he learned that Ms. Penelope was going to give him a poor evaluation. He may have broken in and stolen her laptop to be sure she could not complete the evaluation.

• **Suspect 3** is Mr. Eric, Ms. Penelope’s co-teacher. Mr. Eric is jealous that Ms. Penelope’s class has been doing so well, and she believes he may have broken in to steal her lesson plans.

Blood samples have been collected from each of the three suspects and will be analyzed alongside the crime scene DNA. Should one match, they must then be sent for DNA typing to provide a 100% match.
**SCENARIO**

Your teacher, Ms. Penelope, enters the classroom to a crazy scene: the window is broken, there seems to be blood everywhere, and her laptop has been stolen! However, Ms. Penelope is a notoriously messy eater, and some of the stains on her desk and floor could be old ketchup stains. Ms. Penelope calls on your class to investigate - you are now a detective! There were no fingerprints left at the scene, and the security cameras had been disabled. However, there are potential blood samples left at the scene all over the office from the broken window.

Ms. Penelope turns to your forensic class to try to determine if any of the potential blood samples could actually be blood. First, you will test all of the pieces of evidence for blood using the Kastle-Meyer Test. Then, you will work as a class to determine the blood type of the suspect.
Appendix B
Guide to Implementing a Forensics Unit in the Classroom

Forensic science is the application of scientific knowledge to answer questions of interest within the legal system. Forensics incorporates diverse fields such as biotechnology, toxicology, chemistry, and physics to characterize physical evidence found at the scene of a crime. Given forensics' widespread reach in popular culture and mainstream media, it's a great way to introduce the applications of biotechnology to your class. However, putting together a standalone forensics unit can be a lot of work, and there are a lot of options for activities. Here, we outline a basic forensic investigation and the different experiments that your class could use to solve a classroom crime.

Where to Start

The first step in incorporating forensics into your classroom is coming up with a crime scene scenario. Many teachers will use suspects from their school or community to fabricate a crime. Should you not want to come up with your own scenario, a scenario for this kit and background on the characters involved is provided in Appendix A.

The next step is to create the evidence! Forensic experiments from Edvotek include both physical evidence (fingerprints, ransom notes, etc) and simulated biological evidence (blood, saliva, etc).

Below is an image of a (staged) murder in an alley. Each potential piece of evidence is marked with an Edvotek kit’s catalog number.

- Could someone have been drugged?
  - Cat. #195
  - Cat. #193

- Can we identify this handwriting?
  - Cat. #196

- Whose blood is this?
  - Cat. #130
  - Cat. #5-51
  - Cat. #225
  - Cat. #109
  - Cat. #371

- Is that blood?
  - Cat. #191
  - Cat. #194

- What kind of blood is that?
  - Cat. #192
  - Cat. #140

- Are there fingerprints? Do they match a criminal’s?
  - Cat. #5-91
Student Investigation

As forensic investigators, students will collect the evidence and determine whether it is physical or biological. Once they have confirmed the presence of a biological sample (blood, saliva, etc.), the next step is to perform DNA analysis on it to rule out or implicate suspects. Analyzing several polymorphisms within a person’s genome generates a unique DNA “fingerprint”. DNA fingerprints can allow us to distinguish one individual from another and match crime scene DNA to a suspect.

In every Edvotek® forensic DNA kit, you will be provided with crime scene and suspect DNA. DNA is analyzed by first amplifying it using the polymerase chain reaction (PCR), and then visualized using agarose gel electrophoresis. Depending on the skill level of your class, Edvotek® offers many different options for DNA fingerprinting exercises.

**Level 1: Easy - Edvo-Kit #S-51**
This experiment includes simulated pre-amplified DNA which is packaged in Ready-to-Load™ QuickStrips™. Students simply puncture through the aluminum foil and load their samples directly into the DNA gel. The DNA is simulated using dyes, so no post-electrophoresis staining is necessary.

**Level 2: Intermediate - Edvo-Kit #130 and Edvo-Kit #109**
These kits include simulated crime scene and suspect DNA which is packaged into Ready-to-Load™ QuickStrips™. The samples have already been subject to PCR amplification alone (Edvo-kit #130) or with additional restriction enzyme analysis (Edvo-Kit #109). The samples contain DNA and require post-electrophoresis staining using the FlashBlue™ provided in the kit.

**Level 3: Advanced - Edvo-Kit #225 and Edvo-Kit #371**
Students perform the DNA analysis themselves in these kits! In Edvo-Kit #225, crime scene and suspect DNA is provided, along with restriction enzymes. Students digest the DNA with restriction enzymes and analyze the banding patterns using agarose gel electrophoresis. In Edvo-Kit #371, the crime scene and suspect DNA has not been amplified by PCR. Template DNA and primers are provided, along with a PCR EdvoBead™. Students combine the reagents and perform PCR. The PCR products are then analyzed using agarose gel electrophoresis.

No matter the level your students are at, Edvotek® can help you bring the exciting world of forensic DNA fingerprinting directly into your classroom.