EDVOTEK® • The Biotechnology Education Company®

Edvo-Kit # 194

Edvo-Kit #194

Forensic Enhancement Techniques

Experiment Objective:

The objective of this experiment is to introduce students to forensic techniques that identify blood at a crime scene. Students will use Luminol and Leucocrystal Violet to enhance material found at the scene of a crime.

See page 3 for storage instructions.

Version 194.190730

EDVOTEK®

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures	
Experiment Overview	7
Module I: Testing Crime Scene Objects Using Luminol	8
Module II: Testing Crime Scene Objects Using Leucocrystal Violet (LCV)	9
Study Questions	10
Instructor's Guidelines	
Notes to the Instructor	11
Pre-Lab Preparations	12
Expected Results	14
Answers to Study Questions	15
Appendices	16
Appendix A: Background Information - The Crime	17
Appendix B: Guide to Implementing a Forensics Unit in the Classroom	18

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

Experiment Components

COMPONENTS		Storage	Check (✓)
A	Simulated blood solution	Refrigerator	❑
B	Simulated blood-free solution	Refrigerator	❑
C	Luminol Stock solution	Refrigerator, in dark	❑
D	Hydrogen Peroxide Stock solution	Refrigerator, in dark	❑
E	Leucocrystal Violet solution	Refrigerator	❑

Experiment #194 is designed for 10 groups.

REAGENTS & SUPPLIES

- "Evidence Bags" containing various samples for blood identification: cardboard, paper bag, and clot ❑
- Spray bottles ❑
- Calibrated transfer pipets ❑
- 50 mL Conical tubes ❑

Requirements *(NOT included with this kit)*

- Distilled water
- Disposable vinyl or latex laboratory gloves

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.

Forensic Enhancement Techniques

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, false positives, and compromise 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 SPATTER

At crime scenes often there is spattering of blood close to a dead victim. Blood takes different shapes based on where it came from or what instrument was used on the victim. Because of the density, surface tension, and composition of blood, it usually forms in droplets. For example, if a murder was caused by the use of a knife, the victim would likely be laying in a pool of blood. If the murderer walked away with the knife, blood droplets may drip off of the knife. How can these droplets inform us of what happened?

Blood is a liquid, and its viscosity causes it to form a perfect sphere if dropped from 90 degrees. Therefore, if the droplet is moving when it is dropped, it will form more of an elongated shape. If the attacker mentioned above walked away slowly, the blood droplets would be more circular. However, if they walked away rapidly the droplets would be elongated with a tail-like shape where the tail points in the direction of the movement.

Blood spatter at the scene of a crime can be useful in constructing what and how events took place. By determining the location of the blood and the shape and size of the droplets, investigators can identify murder weapons (guns leave a very distinct blood spatter) and even the sequence of events. There is a classification system of spatter patterns, which is based upon the velocity of the object causing the impact, the size of the resulting spatter, and the direction of the spatter.

When identifying blood spatter, a detective or science professional will first perform a visual analysis for basic information. Blood spatter analysis comes in many forms. The most basic is the size and shape of the spatter. These can give information about what kind of weapon was used and from what direction the weapon came from.

Low velocity blood spatter comes from dripping blood. Examples of this are when a bleeding victim moves to another area, or an attacker walks away with a dripping weapon. Low velocity blood spatters are typically caused by an impact of less than 5 feet/second. Low velocity blood spots are primarily large and circular (Figure 1A).

Medium velocity blood spatter is caused by the impact of an object between 5-100 feet/second. Examples of this include being hit with a blunt object. Medium velocity blood spots are smaller in diameter and look like they may have come out of a spray bottle (Figure 1B).

Gunshots, and other objects with a force of over 100 feet/second, result in high-velocity blood spatter. High velocity blood spatter looks like a fine spray and the droplets have a diameter of less than 1 mm (Figure 1C).

Between the size of droplets, the direction they're pointing, and other stains, forensic blood analysts can determine the scenario by which violence had occurred.

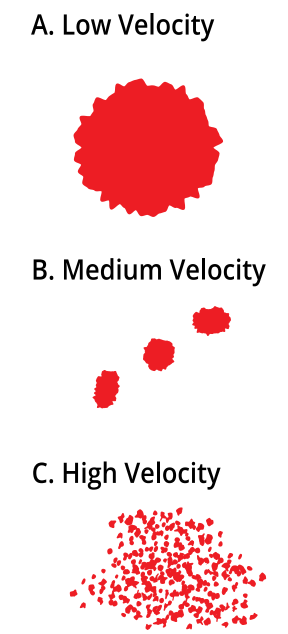


Figure 1: Blood Spatter Velocity

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 other cells. In red blood cells, the cytoplasm is largely filled with a molecule called hemoglobin (Figure 2A). 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 molecule, and later releases it 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.

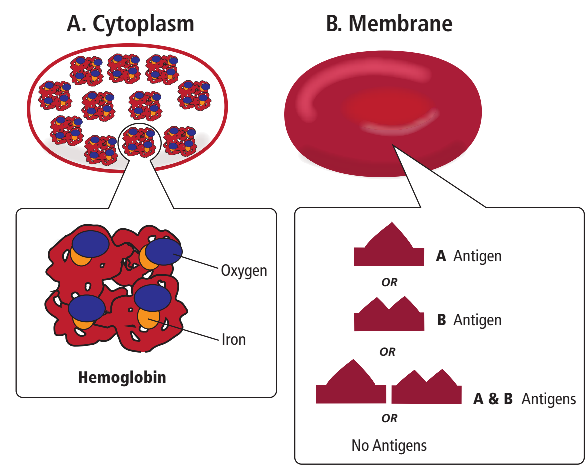


Figure 2: Features of a Red Blood Cell

Blood identification has at least 2 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 2B).

Step 1: Presumptive Tests

Hemoglobin can be detected by forensic analysis even if it is not visible to the naked eye. The properties in hemoglobin, such as its ability to bind iron, can be utilized with chemical reactions to detect its presence. Two of these tests are using the chemicals luminol and leucocrystal violet (LCV). Luminol reacts with hemoglobin to produce a glowing light which can be seen when the lights are turned off. LCV reacts with hemoglobin to create a purple reaction. These are both very handy. If there was a large blood spill that was cleaned up on a carpet, LCV could be sprayed to detect remaining hemoglobin. Similarly, if blood was cleaned up from tile with a 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.

Step 2: Confirmatory Tests

Presumptive tests, such as luminol and LCV, must be confirmed using a test that definitively detects blood, or 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 3).

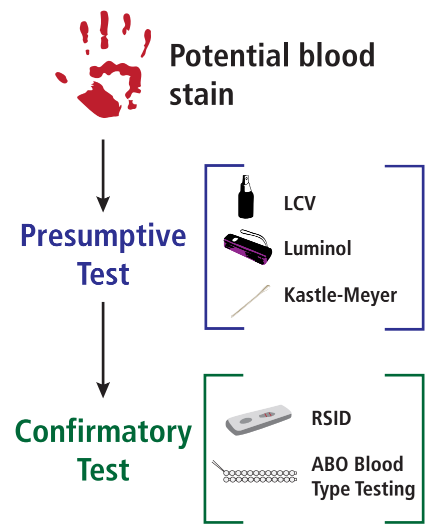


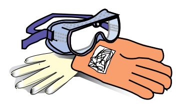
Figure 3: Blood Identification Steps

Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to forensic techniques that identify blood at a crime scene. Students will use Luminol and Leucocrystal Violet to enhance material found at the scene of a crime.

LABORATORY SAFETY



Wear gloves and safety goggles

1. No human blood or other materials are used in this experiment.
2. Handle all stained samples being processed with gloves.
3. Remember to change gloves as needed to avoid cross-contaminating samples and items being examined.
4. Lab coat, gloves and safety goggles should be worn as good laboratory practice.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: Testing Crime Scene Objects Using Luminol

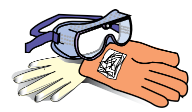
Blood spatter at the crime scene can be useful in reconstructing what and how events may have taken place by determining the original location of the blood source and the direction of movement.

Test the objects collected from the crime scene and the control samples to see if they are positive or negative for the presence of blood using the Luminol test.

1. Work with one item at a time to avoid cross contamination or sample mix-up. Examine the object for the visible red-brown staining and general characteristics.

2. Place the item on a flat, clean surface. Describe visible stains in Table 1 below or in a lab notebook.

When ready for testing the stains, darken the room! Turn off lights and, if possible, darken windows!



Wear gloves and safety glasses.

3. Wear laboratory goggles and use the fine-mist sprayer provided to test the stains by gently spraying the targeted area on the object with the Luminol solution from a distance of about 2-3 inches.

4. The luminescence should appear immediately in the dark. Luminol will generate a bright blue color when it makes contact with blood.

5. The development of a bright blue color within 5 – 10 seconds is indicative of a positive reaction. Photograph can be taken during that time.

6. Share your results with the class and collect data for the other evidence.

7. Record your sample ID and observations for your sample and the samples tested by the rest of the class in Table 1 below or in a lab notebook:

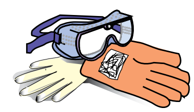
Sample ID	**ID & Description of Stains**	**Luminol + / -**
Positive Control		
Negative Control		
Crime Scene Sample #1		
Crime Scene Sample #2		
Crime Scene Sample #3		
Crime Scene Sample #4		
Crime Scene Sample #5		
Crime Scene Sample #6		
Crime Scene Sample #7		
Crime Scene Sample #8		

Table 1: Effect of Luminol on objects collected from the crime scene.

Module II: Testing Crime Scene Objects Using LCV

Test the objects collected from the crime scene and control samples to see if they are positive or negative for the presence of blood using the Leucocrystal Violet (LCV) test. ***NOTE: This test does not have to be done in the dark.***

1. Work with one item at a time to avoid cross contamination or sample mix-up. Examine the object for the visible red-brown staining and general characteristics.
2. Place the item on a flat, clean surface. Record your data about the stains in Table 2 below or in a lab notebook.
3. Wear laboratory goggles and use the fine-mist sprayer provided to test the stains by gently spraying the targeted area on the object with the Leucocrystal Violet (LCV) solution from a distance of about 2-3 inches.



Wear gloves and safety glasses.

4. In a short period of time, the reaction will provide purple/violet coloration will appear.
5. LCV generates a purple/violet color and indicates the presence of blood stain on certain specimen collected at the crime scene.
6. Share your results with the class and collect data for the other evidence.
7. Record your sample ID and observations for your sample and the samples tested by the rest of the class in Table 2 below or in a lab notebook:

Sample ID	ID & Description of Stains	LCV + / -
Positive Control		
Negative Control		
Crime Scene Sample #1		
Crime Scene Sample #2		
Crime Scene Sample #3		
Crime Scene Sample #4		
Crime Scene Sample #5		
Crime Scene Sample #6		
Crime Scene Sample #7		
Crime Scene Sample #8		

Table 2: Effect of Leucocrystal Violet on objects collected from the crime scene.

Study Questions

1. What is chemiluminescence?

2. How is Luminol used to detect blood?

3. What molecular biology discoveries helped forensics investigations?

4. What do you think happened at the scene of the crime?

Instructor's Guide

NOTES TO THE INSTRUCTOR

Class size, length of laboratory sessions, and availability of equipment are factors, which must be considered in planning and implementing this experiment with your students.

While the background introduction is generic, if you would like a pre-written scenario for this kit, see Appendix A. For more information on creating a Forensics unit in your classroom, see Appendix B.

These guidelines can be adapted to fit your specific set of circumstances. 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. Technical Service is available from 8:00 am to 5:30 pm, ET zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Information

All the materials in this EDVOTEK experiment are simulated, however the protein hemoglobin is used. No human or animal blood, or live cells, are included in this experiment. Students should be made aware of the safety precautions when working with human blood products in real life since various infectious agents may be present in blood samples obtained from a donor.

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

Pre-Lab Preparations

PREPARATION OF CONTROL AND "BLOOD STAINED" SAMPLES FOR MODULES I AND II

There are 2 Evidence Bags provided in this experiment. Each of the 2 bags contains 10 pieces of evidence collected from the crime scene.

NOTE: Luminol and LCV cannot both be performed on the same sample as they deplete the iron from hemoglobin. We recommend using one evidence bag for luminol and one evidence bag for LCV.

The classroom teacher needs to designate an item to be 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 to 8. The "crime scene" samples can yield either positive or negative results, depending on the teacher's preference and preparation.

It is recommended that the teachers work with only one set at a time (negative or positive samples) to avoid cross contamination.

1. **Positive sample Preparation** - Treat the Positive Control and positive Crime Scene samples with Simulated blood solution (Component A) as follows:

   - Transfer the entire content of the Simulated blood solution (Comp. A) to a 50 mL conical tube (provided).
   - Either (a) or (b):
     (a) Soak the pieces of evidence designated as Positive Control and positive Crime Scene samples in the Simulated blood solution for 15 seconds. Remove all the treated items and place them on a covered lab bench to air-dry for a few minutes.
     (b) Use a transfer pipet to draw some of the blood from the Simulated Blood solution tube (Comp. A). If using an automatic micropipette, measure 50 µL. Drop the blood onto the evidence from a distance of about 5 inches. Allow the evidence to soak for approximately 1 minute. Repeat with remaining evidence samples.

2. **Negative sample Preparation** - Treat the Negative Control and negative Crime Scene samples with Simulated blood-free solution (Component B) as follows:

   - Transfer the entire content of the Simulated blood-free solution (Comp. B) to the other 50 mL conical tube provided.
   - Either (a) or (b):
     (a) Soak the pieces of evidence designated as Negative Control and negative Crime Scene samples in the Simulated blood-free solution for 15 seconds. Remove all the treated items and place them on a covered lab bench to air-dry for a few minutes.
     (b) Use a transfer pipet to draw some of the solution from the Simulated blood-free solution tube (Comp. B). If using an automatic micropipette, measure 50 µL. Drop the solution onto the evidence from a distance of about 5 inches. Allow the evidence to soak for approximately 1 minute. Repeat with remaining evidence samples.

3. Distribute one item per student group per module.

Pre-Lab Preparations, continued

PREPARATION OF LUMINOL SOLUTION FOR MODULE I

Prepare the day of the lab

1. Label two fine-mist spraying bottles provided as “Luminol”.
2. To prepare the “Luminol” solution, using the transfer pipets provided, combine 2.5 mL of Luminol Stock solution (Comp. C) with 2.5 mL of Hydrogen Peroxide Stock solution (Comp. D) in each spraying bottle.
3. Invert the bottles to mix well.

PREPARATION OF LEUCOCRYSTAL VIOLET SOLUTION FOR MODULE II

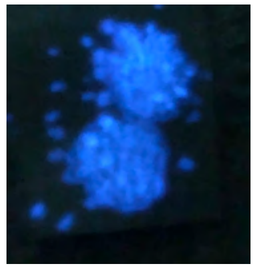
Prepare the day of the lab

1. Label the other fine-mist spraying bottles provided as "LCV".
2. To prepare the "LCV" solution, combine 2.5 mL of Leucocrystal violet solution (Comp. E) with 2.5 mL Hydrogen Peroxide Stock solution (Comp. D) in each spraying bottle.
3. Invert the bottles to mix well.

Expected Results

The crime scene samples will yield either positive or negative when tested with Luminol.

BEFORE AFTER



Sample ID	Luminol + / -
Positive Control	+
Negative Control	-
Crime Scene Sample # 3 to 8	Variable

The crime scene samples will yield either positive or negative results when tested with Leucocrystal Violet.

BEFORE AFTER

Sample ID	Leucocrystal Violet + / -
Positive Control	+
Negative Control	-
Crime Scene Sample # 3 to 8	Variable

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

THE CRIME

You arrive at school early and head to the cafeteria for a hot breakfast. You walk in and immediately notice something is suspicious. The regular lunch lady, Ms. Plum has been replaced with someone new. When you inquire as to her whereabouts, you're told that she would not be returning to the school. You head over to a table where you notice the floor seems to be especially clean, and is that a blood stain on the edge of the table? Was there a fight? Foul play with Ms. Plum?

You alert the principal to your findings, but she doesn't want to cause alarm if the samples turn out to be ketchup stains. She asks you to do a preliminary test on the samples to determine if they could be blood, and if the tests come back positive she will alert the authorities. You are now a detective! You must be very careful; a wrong move in the early steps of a criminal investigation can be disastrous!

Your Next Steps

Enlisting the help of your science teacher, you prepare LCV and Luminol. Multiple different samples were identified, and you will now test each sample to determine if there is potentially blood present. Afterwords, you can make recommendations to the principal on sending the samples out for confirmatory testing.

Appendix B

Guide to Implementing a Forensics Unit in the Classroom

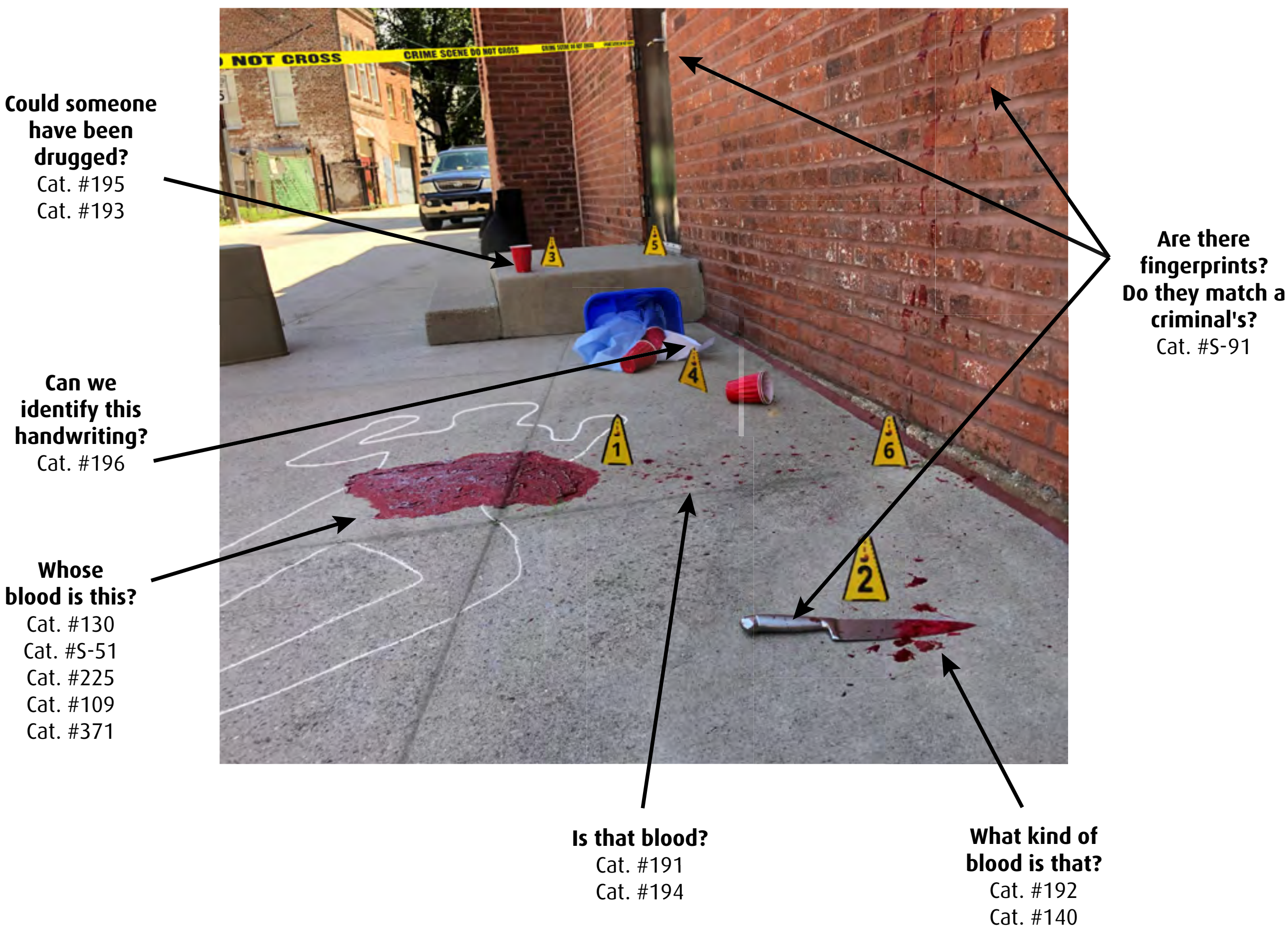
Forensic science is the application of scientific knowledge to answer questions of interest within the legal system. Forensics incorporates diverse fields such as biotechnology, toxicology, chemistry, and physics to characterize physical evidence found at the scene of a crime. Given forensics' widespread 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.



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.