EDVOTEK®

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EDVO-Kit

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What Forensics Information Does Blood Typing Provide?

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to some of the techniques used by forensics scientists for analyzing blood. The students first check for the presence of blood using the phenolphthalein test. Then the students will apply the concept of blood type-based screening for potential suspect(s) present at a crime scene.

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Table of Contents

This experiment is designed for 10 student groups.

No actual blood or blood products are used in this experiment.

Experiment Components

Module I

		Storage
A	Simulated Blood Solution	Refrigerator
B	Simulated Blood-free Solution	Refrigerator
C	Phenolphthalein Stock Solution	Refrigerator
D	Hydrogen Peroxide Solution	Refrigerator
•	"Evidence Bag"	Room Temp.
•	Cotton Swabs	Room Temp.
•	Transfer pipets	Room Temp.

Module II

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

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

Requirements

- 95-100% Ethanol
- Optional: Automatic micropipet (5 – 50 µl)

Background Information

FORENSICS BASICS

Securing and Handling the Evidence

Forensic Scientists collect and analyze evidence from a crime scene in order to identify the nature of the evidence and its source. While this collection process takes place, the scientist cannot make any definitive statements about the nature of the evidence. Before making any conclusions, he or she must wait until extensive testing has revealed the nature of the evidence as well as information that can be gleaned from it. One cannot assume that a red stain on the floor or a latent (not currently visible) stain found by another detection method is actually blood. The first step when dealing with any biological evidence is to correctly identify the material. It may seem obvious that the red stain on a knife found lying next to a murder victim is blood, yet it still must be tested to confirm if the stain is indeed blood. Only when you have determined the exact nature of the evidence, can further testing be done to produce additional information.

Determining the nature of the evidence is a complex multi-step process. Forensic scientists use various assays to quickly and accurately determine the identity of a substance that must also satisfy the following criteria: the test must be quick, inexpensive, and most importantly, it must minimally affect the evidence. It is important that the initial testing be performed quickly and inexpensively in order to determine the direction of the investigation. A lengthy, expensive test would waste time and money if the sample being tested turns out to be something other than what it's thought to be. For example, attempting to generate a DNA profile from a potential bloodstain will give you conclusive results as to the nature of the stain but you will have spent several hours and hundreds of dollars doing so.

Maintaining the Integrity of the Evidence

In addition to collecting and testing evidence, the Forensic Scientist is also one of many people responsible for maintaining the integrity of the evidence itself. Steps must be taken to ensure that nothing is done to the evidence that would minimize or diminish it's value, thus making it less useful in an important situation, as in a courtroom/trial setting. When evidence is collected, it is placed into a collection bag that is then sealed and taped shut, with the initials of the collector and collection date written across the tape. This is the first line of defense against any potential evidence tampering. In order to access the evidence, the seal on the bag/container must be broken. The evidence is then brought to a secure evidence room where signed records of it's arrival are documented. Access to the room is restricted to only a few people who keep track of all the items. Any scientist who wishes to perform a test on the evidence must sign for the items they remove, noting the date and time as well. Eventually, these records provide a detailed picture of when the evidence was collected and every time it was moved or accessed for testing.

Testing procedures are affected in a variety of ways. After retrieving the evidence from the storage room a forensic scientist will note the condition of the container is in. Is the container properly sealed? Does the evidence tape sealing the openings look undisturbed? Are there any new openings in the container? All these questions must be answered and the condition of the container must be noted in the scientist's lab notebook before testing can begin. Next, the scientist must open the container without disturbing the prior sealing done by others. If at all possible, new openings should be made. This

Background Information

allows others who have made openings and sealed them to be able to say that their seal was undisturbed afterwards. This is important to show that the evidence wasn't tampered with and to document everyone who has tested the evidence. Often, by the end of its life, the evidence bag will have numerous openings that have been resealed and signed.

Even when testing the items, the scientist should take absolute care to ensure that evidence is never compromised to a point where contamination can occur. Contamination is the transfer of minute amounts of material from one piece of evidence to another and it can drastically alter the value of the evidence and can result in a complete loss of value. For example, if two articles of clothing are laid on a lab bench together. One is a reference sample collected from a suspect. The other was found on the victim of a crime. By placing the items in the open air in the same room at the same time, you run the risk of material from one article transferring over to the other. This could result in an innocent suspect being punished for a crime he or she did not commit. When working on a crime case, only one piece of evidence should be unsealed at a time. With all items sealed in their respective bags, contamination cannot occur. After testing is complete, all items are then signed back into the evidence room by the scientist who removed them. This procedure is used to document all of those who had access to the evidence and what was done to the evidence.

Presumptive and Confirmatory Tests

Since the amount of evidence collected at a crime scene is often very small, it is imperative that a forensic scientist preserve as much of the evidence as possible. Simple chemical tests are often used for the initial testing of possible biological evidence. There are situations where the amount of evidence is so small that the entire sample would be consumed in a single test. In these cases only, the evidence is saved for the most conclusive tests.

Presumptive Test

The initial tests are called "presumptive" because of their potential results. Presumptive tests can, at best, only strongly indicate that the tested substance is correctly assumed because other substances can also give a positive result. These are called "false positives". Forensic scientists rely on their knowledge and experience to discern between a true positive and a false positive.

Confirmatory Test

Once a presumptive test has indicated that a substance is likely what you assume it to be, the next step is to perform a test that conclusively proves what the material is. As with the presumptive test, it is the results of a confirmatory test that place it in this category. Confirmatory tests do not usually give false positives. A positive result from a confirmatory test for blood confirms that the substance you tested is blood. It is only after you have a positive result from a confirmatory test that you can make a positive identification of the substance.

Background Information

A Presumptive Test: The Kastle-Meyer Test

The Kastle -Meyer test was first described in the early 1900s by two independent doctors for whom the test was named. The chemical Phenolphthalein has a variety of uses, most commonly as an acid/base indicator outside the forensic community. It was Doctors Kastle and Meyer who found that it could be used as an indicator for the presence of blood. Phenolphthalein is colorless in solution between pH values of 0 and 8. Above pH 8, phenolphthalein is characterized by a bright pink color. Phenolphthalein is useful forensically because a color change can be induced in the presence of an oxidizing agent. Additionally, if another molecule that can catalyze this oxidation reaction is present in solution, the color change will occur very quickly, thus making Phenolphthalein very useful as a presumptive test for blood. One of the subunits of the hemoglobin in red blood cells is the heme group. The heme group contains an Iron ion contained in a heterocyclic ring called a porphyrin. This heme group has a peroxidase-like activity. Peroxidases are a family of enzymes that catalyze a reaction that breaks a peroxide bond.

ROOR' + 2e- +2H+ -> ROH +HOR'

In the presence of heme in red blood cells, hydrogen peroxide is catalyzed into water and free oxygen molecules. The free oxygen from the catalyzed peroxide reacts with the reduced phenolphthalein, resulting in a quick, strong change from a colorless liquid to bright pink. As an added first step, ethanol is added to the bloodstain to lyse the cells thus exposing the heme groups, making the test more sensitive. This very simple and consists of 4 easy steps.

1. Wet a cotton swab with deionized water and swab a portion of the suspected bloodstain
2. Squeeze 1-2 drops of Ethanol onto the swab
3. Add 1-2 drops of the prepared phenolphthalein reagent
4. Add 1-2 drops of 3% hydrogen peroxide

Phenolphthalein also has the added forensic benefits of being very specific for blood. There are only a few chemicals that can produce a false positive, mostly iron and copper oxides. However, an experienced examiner can distinguish between these results and true positive results from blood. One can distinguish between true and false positives by studying the color and timing of the reaction. A true positive result has a bright pink color change within 10 seconds of the addition of the hydrogen peroxide, and only once the hydrogen peroxide has been added. If a different color change is observed, the sample is a false positive. If the pink color change occurs before the addition of the hydrogen peroxide, then it is a false positive.

Certain foods, like citrus fruits or beets, can produce a clear false positive: a yellow color change before the addition of the hydrogen peroxide. Some chemicals, such as cobalt acetate, will produce a pink color change at the proper time but its color change is not immediate nor strong as with blood.

Background Information

Phenolphthalein is also a sensitive test. Research has indicated that it can reliably detect bloodstains in up to a 1:1,000,000 dilutions. This puts the sensitivity of the KM test on par with or better than several other presumptive tests.

A Confirmatory Test: Blood Group Typing

Although blood evidence is used primarily for DNA profiling, simpler blood tests are still widely used in the modern forensic laboratory. Blood group testing is one such test. Testing for blood groups relies on the possible precipitation of an antigen-antibody complex from mixing two blood samples together. This phenomenon, called agglutination is a confirmatory test for blood. Only blood will produce this agglutination, which is why it is classified as a confirmatory test.

Testing a sample for its blood group is a useful forensic test in a several ways. In addition to being a confirmatory test for the presence of blood, by knowing the blood group of a sample, we can use the test to screen potential suspects against an unknown sample. In this lab, students will simulate the detection of a suspect's ABO blood type. The ABO system of blood grouping is arguably the most famous and most important of all systems. If you've ever donated blood or had blood drawn at a hospital, you've probably heard of this group. What you may not know, is that the ABO group is just one of many blood groups that have been identified in the last century. In fact, the International Society of Blood Transfusions (ISBT) recognizes 30 blood groups systems!

While we may not be able to use a particular piece of evidence, say a bloodstain for example, to say a unique person was the source of the evidence, we can say that it came from a single group of people, sharing the same blood type. How does this apply to blood? Take a look at the following table showing how many Americans have each type.

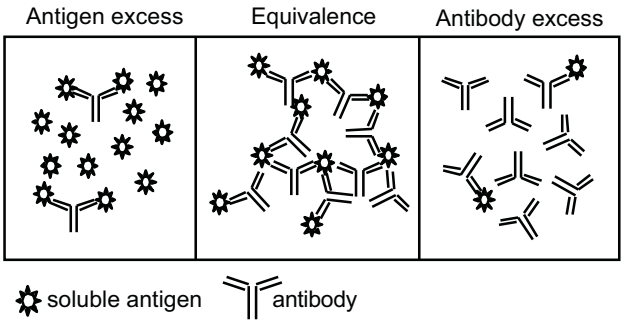
Group	%	Actual # in millions
A	42	127.7
B	10	30.3
AB	4	12.2
O	44	133.8
Total	100	304.0

Let's pretend that we found blood on a broken window at a crime scene. We test it and find that it is Type O. Who left it there? It's pretty easy to see from that table that just because a suspect has the same blood type as the blood found at the scene, he is not necessarily the person who left the blood. Because so many people have each blood type, it is easier to use the information to rule out a suspect. Using just the ABO group information, we can't say for sure that the person with Type O is also the one who left that bloodstain, but we can absolutely say that the person with Type AB did not leave the bloodstain on the broken window.

This use of evidence is very important in Forensic Science. Individual pieces of evidence that may not identify someone on their own can be used to draw a much more specific picture when used together. Using serological evidence in conjunction with physical evidence and eyewitness accounts will always help narrow down the possibilities in a case.

Background Information

Precipitation reactions between soluble antigens and antibodies can be visible reactions if both components are in equivalence. Under this condition neither the antigen nor the antibody is in excess and antigen-antibody complexes form large networks that precipitate out of solution as diagrammed below.



When an antigen is attached to a red blood cell, the reaction is called an agglutination and the lattice of antigen and antibody that is visible at equivalence is called an agglutinate. Agglutination is a routine and cost-effective serological procedure because the agglutinate is very easily detectable.

Blood typing is an example of a clinical agglutination assay that is familiar to all of us. Since the specific blood antigens are on the surface of red blood cells (RBCs) they are termed hemagglutination reactions. Blood typing has various important forensic science applications and remains a powerful forensic tool in linking someone to a crime or accident scene.

The antigenic determinants on the surfaces of red blood cells (RBCs) are the A, B, and O blood group proteins, which are for convenience called A, B, and O antigens.

The two antigens provide for four possible types of blood; type A (only A antigen is on the surface of all RBCs from that person), type B (only B antigen is present); type AB (both A and B antigens are on each RBC); and O (neither A or B antigens are present). Based on the antigens on the surface of RBCs, there are four possible blood types in the ABO blood group system as listed below in Table A.

Blood Type	Antigen on Red Blood Cells
A	A
B	B
AB	both A and B
O	neither A nor B

TABLE A

Background Information

Blood A and B antigens are common in the human population, as well as in nature, including bacteria to which we are exposed. When exposed to bacteria with the same blood group antigen, the immune system of the individual will recognize that antigen as "self" and no immune response will be mounted against it. By contrast, when exposed to bacteria with different blood group antigens, the human immune system will see that antigen as foreign and produce antibodies against it. These serum antibodies can then agglutinate RBCs from individuals with a different blood type. For example, anti-A antibodies from one individual's serum will agglutinate another person's RBCs that have the A antigen on their surface. Anti-B antibodies will agglutinate RBCs that have the B antigen on their surface as demonstrated below in Table B.

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	both A and B	neither anti-A nor anti-B
O	neither A nor B	both anti-A and anti-B

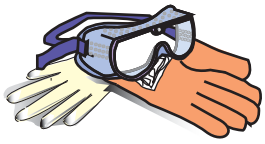
TABLE B

Experiment Overview and General Instructions

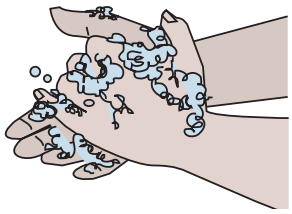
EXPERIMENT OBJECTIVE:

Experiment Objective: The objective of this experiment is to introduce students to some of the techniques used by forensics scientists for analyzing blood. The students first check for the presence of blood using the phenolphthalein test. Then the students will apply the concept of blood type-based screening for potential suspect(s) present at a crime scene.

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. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Experiment Procedure

Student Experimental Procedures

MODULE I: PRESUMPTIVE TEST FOR TESTING STAIN FROM THE CRIME SCENE THAT MIGHT BE BLOOD

The phenolphthalein solution may burn or irritate skin. WEAR GLOVES & GOGGLES!

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 pipet 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. Firmly rub the moistened cotton swab against the item until the swab absorbs the red stain.

2. 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.

3. 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.

4. 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 below:

Sample ID	Phenolphthalein + / -
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	

Which crime scene samples from the crime scene are believed to stain with real blood? Which samples gave negative result?

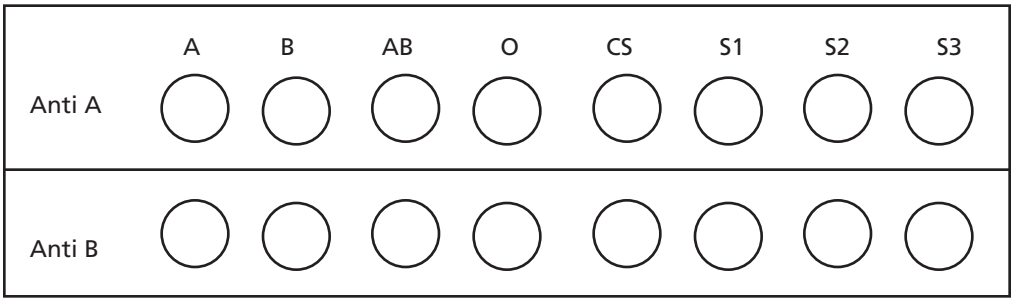
Student Experimental Procedures

IMPORTANT: This is a simulation blood exercise. No actual blood or blood products are used in this experiment.

Experiment Procedure

MODULE II: CONFIRMATORY TEST FOR SCREENING CRIME SCENE COLLECTED BLOOD AND SUSPECTS' BLOOD

1. Place a microtiter plate piece as shown below. Across the top of the plate, label the 8 wells A, B, AB, O, CS, 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.



IMPORTANT: Avoid cross-contamination by using a new disposable pipet or pipet tip when using an automatic micropipet for each blood sample.

PUT ON YOUR GLOVES NOW.

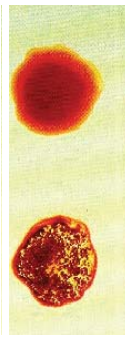
2. Using a different pipet or pipet tip for each sample, plate 3 drops of each control blood type sample into each of the two corresponding wells. For example, control A blood type goes into the two wells under the letter A. Repeat the same procedure for crime scene collected blood and blood from each of the three suspects. Each well requires 3 drops or 50 µl.

3. Use a new pipet to add one drop or 20 µl of Anti-A serum into each of the wells in row #1.

4. Use a new pipet to add one drop or 20 µl of Anti-B serum into each of the wells in row #2.

5. Let the plate sit 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 rather than smooth. Record your results in the diagram in the Results section.



- (no agglutination)

+ (agglutination)

Student Experimental Results

1. Record your results in the diagram below.

	A	B	AB	O	CS	S1	S2	S3
Anti A	◯	◯	◯	◯	◯	◯	◯	◯
Anti B	◯	◯	◯	◯	◯	◯	◯	◯

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?

Study Questions

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

1. Why is the phenolphthalein test is useful but not definitive confirmatory test for the detection of a suspect?

2. What are the basic blood types?

2. What is the composition of blood?

Instructor's Guide

GENERAL INFORMATION

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.

Notes to the Instructor & Pre-Lab Preparations

MODULE I

A. Preparation of Control and "Blood Stained" Samples

There are 10 pieces of evidence in the "Evidence Bag" provided. 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 that the teacher designates 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 - 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 micropipet, 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 micropipet, 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. Distribute one item per student group.

Notes to the Instructor & Pre-Lab Preparations

MODULE I, CONTINUED

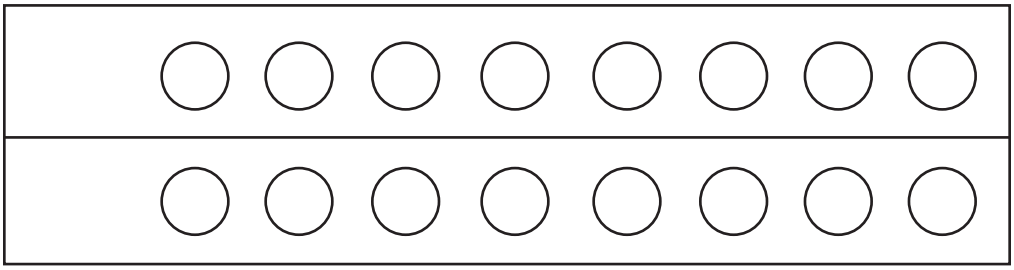
B. Preparation of Phenolphthalein and Hydrogen Peroxide Solutions

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.

MODULE II

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



B. Preparation of Control and Patient Blood Samples
(Prepare no more than 24 hours before starting the experiment.)

1.a. 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 sample provided in the kit. Cap tubes and mix well.

1.b. 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.a.) in a labeled microcentrifuge tube. Cap and mix well.

2. Label microcentrifuge tubes "A", "B", "AB", & "O", "CS", "S1", "S2" and "S3". Aliquot 100 µl of each Control and Patient blood samples (prepared in steps 1.a. & 1.b.) to the appropriately labeled tubes. Use a new pipet or pipet tip for each sample.

3. Label tubes "anti-A" and "anti-B" and aliquot 180 µl of each to the respective tubes. Distribute one tube of each antiserum sample per student group.

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

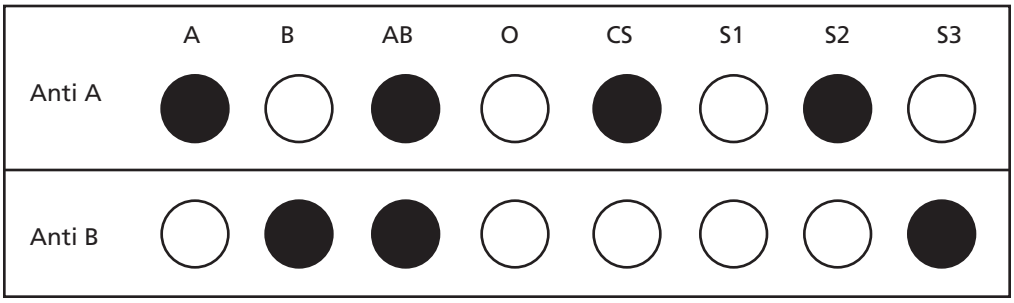
Expected Results

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.

Sample ID	Phenolphthalein + / -
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	

MODULE II



Please refer to the kit insert for the Answers to Study Questions