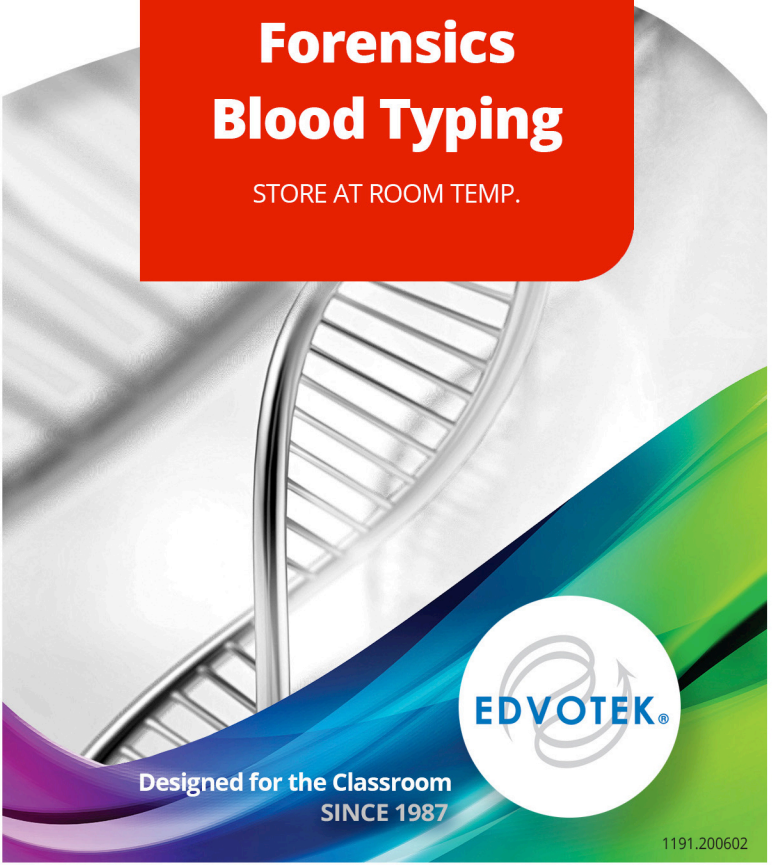
EDVOTEK® MyLab™ #1191

Forensics Blood Typing

STORE AT ROOM TEMP.



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SINCE 1987

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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.

PRE-LAB PREP COMPONENTS

- Graduated Transfer Pipette
- Microcentrifuge tube for AB blood type

MODULE I - COMPONENTS

- Phenolphthalein Stock Solution
- Hydrogen Peroxide Solution
- Evidence Bag containing positive control, negative control, and Simulated Crime Scene samples
- Cotton Swabs
- Transfer pipets

MODULE II - COMPONENTS

- Control ABO "blood samples"
- "Blood sample" from Crime Scene (CS)
- "Blood samples" from two Suspects
- Anti-A and Anti-B serum
- Red dye concentrate (for coloring)
- Transfer pipets
- Microtiter strip

REQUIREMENTS

- Concentrated Ethanol or Isopropanol (50% or higher)
- Optional: Automatic micropipette (5 – 50 µL) with tips

PRELAB PREPARATIONS

(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) , add 1 drop 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, use the graduated transfer pipettes to combine approx. 100 µL of Control blood sample A and approx. 100 µL of Control blood sample B (prepared in step 1) in a labeled microcentrifuge tube. Cap and mix well.

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 info about motive, ability, and alibis to determine the criminal.

BLOOD IDENTIFICATION

Blood identification has at least two steps: presumptive and confirmatory testing. 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, the carrier of oxygen in red blood cells, 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.

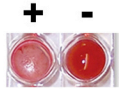
Step 1: Presumptive Tests

Hemoglobin can be detected by forensic analysis even if it is not visible to the naked eye. To 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. 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 swab turns pink, it means that there was likely hemoglobin in the sample.

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.



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.

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: 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

BACKGROUND INFORMATION, CONTINUED

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 with have type B blood. 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.

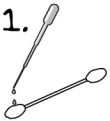
Blood Type	Antigen on Red Blood Cells	Antibody in Blood	Percentage of Population
A	A	anti-B	42%
B	B	anti-A	10%
AB	A & B	none	4%
O	O	anti-A & anti-B	44%

Figure 1: Types of Blood in the Population

MODULE I: PRESUMPTIVE BLOOD TEST

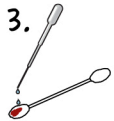
Test the object collected from the crime scene and control samples to see if they are positive or negative for the presence of blood using the phenolphthalein test. Remember to use a different transfer pipet or pipette tip for each solution.

Gather the positive control, negative control, and two crime scene samples from the evidence bag and place on a paper towel.

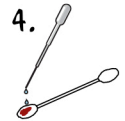


Distilled water

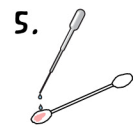
RUB



95% ethanol



Phenolphthalein solution



Hydrogen peroxide

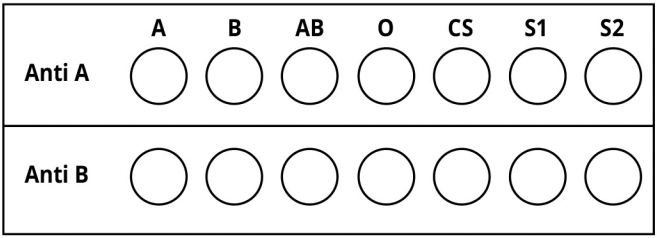
1. Working with only one item at a time to avoid cross contamination, lightly **MOISTEN** a cotton swab with distilled water.
2. Firmly **RUB** the moistened cotton swab against the evidence until the swab absorbs the red stain.
3. Use a new pipet to **ADD** two drops of alcohol to the swab. **NOTE** any color change. There should be no color change.
4. Use a new pipet to **ADD** two drops 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 of hydrogen peroxide to the swab. **NOTE** any color change. An immediate pink color is expected if blood is present. **RECORD** your results.

MODULE I: ANALYSIS

1. Which samples would you recommend for confirmatory testing?
2. What is the purpose of the positive and negative control?

MODULE II: CONFIRMATORY BLOOD TEST

1. **PLACE** a microtiter plate piece as shown below. Across the top of the plate, **LABEL** the 7 wells A, B, AB, O, CS, S1, and S2 respectively, using a laboratory marking pen. Label the 2 rows Anti-A and Anti-B respectively (as shown below).
2. Using a different pipet or pipette 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 suspects. Each well requires 3 drops. ***IMPORTANT: Avoid cross-contamination by using a new disposable pipet or pipette tip for each sample.***
3. Use a new pipet to **ADD** one drop of Anti-A serum into each of the wells in the Anti-A row (row #1).
4. Use a new pipet to **ADD** one drop of Anti-B serum into each of the wells in the Anti-B row (row #2).
5. Let the plate **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 below.



MODULE II: ANALYSIS

1. What are the ABO blood types of the crime scene collected blood and the suspects' blood?
2. Based on your observation, which of the suspects would you conclude might have left the blood stain at the crime scene?
3. What next steps would you take to confirm the suspect's identity?

STUDY QUESTIONS

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

GENERAL SAFETY PRECAUTIONS

Parental or adult supervision required.

1. Designate a clean and uncluttered area for performing experiments.
2. Read all instructions before you begin.
3. Do not eat, drink, smoke, apply make-up or contact lenses during experiment.
4. Wash your hands before and after performing the experiment.
5. Gloves and goggles should be worn routinely as good laboratory practice.
6. Disinfect the counter top or bench with 70% isopropyl alcohol (rubbing alcohol, or place clean newspaper over the area to be used.

MODULE I ANALYSIS

1. The samples recommended for confirmatory testing should be the ones that provided a positive reaction.
2. 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.

MODULE II ANALYSIS

1. CS is type A blood. S1 has type O blood and S2 has type A blood.
2. Based on the results of the blood typing test, it would seem that suspect 2 left the blood stain at the crime scene.
3. DNA testing would have to be performed to conclusively say that the blood belongs to suspect 2.

ANSWERS TO STUDY QUESTIONS

1. Blood is composed of both fluid and cells. The fluid portion, called plasma is 90% aqueous and is approximately 55% of normal total blood volume. Biological components in plasma amount to 10% and include different proteins and dissolved substances such as electrolytes and nutrients. The main cells in the blood are white blood cells, platelets, and red blood cells.

Based on the antigens on the surface of red blood cells, there are four possible blood types in the ABO blood group. They are (1) A antigen (2) B antigen (3) AB (both A and B) antigens and (4) O (neither A or B antigens).

2. The phenolphthalein test has the same reaction with human blood as it does with any other hemoglobin-based blood (i.e., animal blood.) Therefore, further testing would be required to determine from which species the blood originated. Additionally, phenolphthalein can give positive results if it reacts with other iron-containing compounds.