**BIOL 104 Forensic Biology**

**Lab 6 Blood**

1. **Introduction**

from Edvotek #191 What Forensic Information Does Blood Typing

Provide?

**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 con- sumed 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 posi- tive 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.

1. **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.

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 is it strong as with blood.

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.

1. **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 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 group systems!

While we may not be able to use a particular piece of evidence, 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

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.

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 antibody 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 nor 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**

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**

from Edvotek #194 Forensics Enhancement Techniques: Show Me the Blood I Cannot See

1. **Using Luminol to Visualize Blood**

Often trace amounts of blood cannot be detected by the naked eye, however it can easily be enhanced and made visible by spraying the area with certain chemical enhancers such as Luminol. Traces of blood can be detected when Luminol is prepared with hydrogen peroxide as a basic solution. The reaction requires a small amount of iron (that is a component of blood) to initiate the reaction. When a catalytic amount of iron is provided from hemoglobin it will generate the blue chemical glow that is described as chemiluminescence. The glow will last for a short amount of time 10-20 sec., enough time to take a photograph.

1. **Materials & Methods**

**Wash your hands but DO NOT wipe down your lab bench. Be sure to wear your safety glasses and gloves.**

1. **A Presumptive Test: The Kastle-Meyer Test**
2. Work with only one sample at a time, starting with the positive and negative controls and then testing the crime scene samples.
3. Lightly moisten a cotton swab with deionized water and firmly rub the swab against the sample.
4. Use a new pipette to add 1-2 drops of 95% ethanol onto the swab.
5. Use a new pipette to add 1-2 drops of the phenolphthalein reagent onto the swab.
6. Use a new pipette to add 1-2 drops of 3% hydrogen peroxide onto the swab.
7. Record the color and indicate the time it took any color to appear on the table in the Results section.
8. **A Confirmatory Test: Blood Group Typing**
9. Place a microtiter plate 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.
10. Using a different pipet for each sample, plate 2-3 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.
11. Use a new pipet to add one drop of Anti-A serum into each of the wells in row #1.
12. Use a new pipet to add one drop of Anti-B serum into each of the wells in row #2.
13. Let the plate sit undisturbed on the lab bench for 5-10 minutes.
14. 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.
15. **Using Luminol to Visualize Blood**
16. Turn off the lights and make sure you are wearing your safety glasses and gloves.
17. Spray your lab bench with Luminol and use a long-wave UV light to determine if and where blood is present.
18. Clean your lab bench and repeat the procedure. Can you still detect the blood?

**Return all materials to the bins. Wipe down your lab bench and wash your hands.**

Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Score:

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **Results**
2. **A Presumptive Test: The Kastle-Meyer Test**

|  |  |
| --- | --- |
| **Sample** | **Result** |
| Positive Control |  |
| Negative Control |  |
| Crime Scene 1 |  |
| Crime Scene 2 |  |

1. **A Confirmatory Test: Blood Group Typing**



1. **Conclusions**
2. What is the difference between a presumptive and a confirmatory test?
3. What are “false positives”?
4. What does the ethanol do in the Kastle-Meyer Test? What is the purpose of using it?
5. How does phenolphthalein indicate the presence of blood when used with hydrogen peroxide in the Kastle-Meyer Test?
6. Why should you work with only one sample at a time and be sure to use a new pipette with each reagent?
7. What is the purpose of the positive and negative controls?
8. Which crime scene sample(s) was positive for blood?
9. Why is blood typing considered to be a confirmatory test?
10. What is agglutination and when does it occur?
11. Which suspect(s) matched the type of blood found at the crime scene? How do you know?
12. How does Luminol indicate the presence of blood? What is chemiluminescence?