Presumptive Blood Test

IS 9002

INTRODUCTION

In 1910, the French professor Dr. Edmond Locard started the first dedicated forensic science laboratory in Lyon, France. While certainly not the first to employ forensic science in criminal investigations, as fingerprints, fibers, blood, footprints, etc. had already been used (both successfully and unsuccessfully) in prior court cases, Locard was perhaps the first to see the full potential and value of these types of evidence.

It was Locard's theory that in any contact between two items, there will be some sort of exchange. As applied to criminal investigations, Locard believed any perpetrator(s), no matter how careful, will either leave something, take something, or both. In Locard's own words:

"Wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as a silent witness against him. Not only his fingerprints or his footprints, but his hair, the fibers from his clothes, the glass he breaks, the tool mark he leaves, the paint he scratches, the blood or semen he deposits or collects. All of these and more, bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are. It is factual evidence. Physical evidence cannot be wrong, it cannot perjure itself, it cannot be wholly absent. Only human failure to find it, study and understand it, can diminish its value."

-Dr. Edmond Locard

This concept in many ways revolutionized police work and criminal investigations, and would eventually become known as Locard's Exchange Principle. Again, as mentioned above, Locard was certainly not the first to use forensic science, nor did he create the concept. Rather, the value of Locard's approach was in demonstrating not only the importance of, but also the depth of, the power in these traces of evidence. Whereas previously investigators may have inadvertently stumbled across or collected only the most "obvious" evidence, Locard showed the importance of taking nothing for granted, helping train investigators to look closer and deeper, as well as developing and refining analytical techniques in his own laboratory to further extract value from the evidence collected from the scene.

Presumptive Blood Testing

Blood evidence found at a crime scene can provide a wealth of information. Since the discovery of different blood types in the early 20th century, blood evidence has proven a potent investigative aid. Analysis of blood and blood types can not only help investigators decide whether or not to pursue certain suspects but also in court cases where it may be used as supporting evidence to help either incriminate or exonerate a suspect. With the advent of DNA fingerprinting later in the 20th century, blood evidence became an even more powerful tool.

Each blood cell contains a person's entire genetic code. Much like fingerprints, each person's genetic code is unique to that individual (with the exception of identical twins). While all DNA is constructed from the basic components, it is the order in which these components are put together that makes DNA so unique. By examining several regions of DNA that are known to show great variance between individuals, a statistical probability can be determined as to the likelihood that the DNA is from a certain person, the more regions examined, the higher the probability. One drawback of blood analysis, DNA fingerprinting included, is that it is both expensive and time-consuming. For this reason, investigators want to be sure (or almost sure) that a sample collected at a crime scene for blood analysis actually is blood. To waste time and money, as well as slow down an investigation waiting for results, only to discover a suspected blood sample wasn't even really blood serves no benefit. For this reason, an investigator that suspects a sample found at a crime scene is blood will usually perform a presumptive test prior to collecting the sample and sending it for analysis.

Crime scene investigators are trained to be objective, to make no assumptions upon examining a scene. Upon walking into the scene of a violent crime and seeing spattered drops of a red substance covering a surface, it would be easy to jump to the conclusion that the red substance must be blood. While that may or may not be true, there are several other red liquids that might cause such a display (red paint for example) and therefore the possibility cannot be ruled out based on physical observation alone.

A presumptive blood test will not prove that a sample is definitely blood. It simply supports the idea that the sample could be blood (or might not be blood). There are several types of presumptive blood tests, based on different methodologies, which can be employed. One of the most common ones used by investigators is the Kastle-Meyer test.

Kastle-Meyer Blood Test

The Kastle-Meyer test is inexpensive, easy to perform, and provides quick results, making it ideal for use at a scene. It also does not compromise the integrity of the sample. Using this test does not destroy any part of the sample, such as DNA, which may be necessary for further analysis. The Kastle-Meyer test is also very sensitive, with a sensitivity of 1:10,000. In other words, one drop of blood diluted in 10,000 drops of water can still be detected by the Kastle-Meyer test.

A positive test results in a color change. It works due to the presence of the blood compound hemoglobin. Hemoglobin is an iron-containing protein that carries oxygen through the blood stream and is the reason blood appears red. Hemoglobin also oxidizes hydrogen peroxide, one of the three components in the Kastle-Meyer test. The other two components are alcohol and phenolphthalein. In the test, a small amount of the sample is removed, most often with a cotton swab or small piece of filter paper. It is then exposed to each reagent in a specific order, the purpose (and order) of each reagent being:

Alcohol – cleans the sample and exposes more hemoglobin. This increases the sensitivity of the test.

Phenolphthalein – a solution that changes color upon oxidation (exposure to oxygen). If this reaction occurs, the solution changes from clear to pink.

Hydrogen peroxide – a chemical with the formula H_2O_2 (like water, H_2O, with one extra oxygen atom attached). When exposed to hemoglobin, the extra oxygen is released.

In the test, a small portion of the sample is swabbed. Sometimes, especially in the case of what might be a dried blood sample, water may be added to the swab to facilitate transfer of the sample. A couple of drops of alcohol are placed on the sample-containing swab, followed by a couple of drops of phenolphthalein. At this point, the material on the end swab should be observed for a couple of seconds. The sample should remain colorless and if any color development is seen after the addition of the phenolphthalein, the results of the test should not be considered reliable. If no color develops, the final solution, hydrogen peroxide is added to the swab. If any hemoglobin is present, it will release the extra oxygen atoms from the hydrogen peroxide. This oxygen will then react with the phenolphthalein and show a pink color on the end of the swab.

It should be noted that the phenolphthalein solution in the Kastle-Meyer test is specially-prepared. Phenolphthalein is a very common indicator found in many labs and is often used as a pH indicator, changing color when a certain pH is reached. For this test, a standard lab solution of phenolphthalein will not suffice. The phenolphthalein in the Kastle-Meyer test is first prepared as a solution and then boiled while exposed to a substance, such as zinc, which removes oxygen. It is this “oxygen-starved” form of phenolphthalein that allows the test to work.

One of the drawbacks of the Kastle-Meyer test is the fact that other substances besides hemoglobin are capable of undergoing reaction with hydrogen peroxide. If any of these substances are in the area swabbed, they will show positive results for the test even if blood is not present. It is important to remember that it is a presumptive blood test, simply used to decide if further analysis should be pursued. Lastly, the test is used to confirm the presence of blood, not the source of the blood. It will not distinguish between human and animal blood, simply indicate that the sample might be some type of blood.

Materials Included in the Kit

2 btl. Phenolphthalein, 25ml
2 btl. Ethyl alcohol (95%), 25ml
1 btl. Hydrogen peroxide (3%), 50ml
5 Positive control blood strips (non-human blood source)
50 Cotton swabs

Materials Needed but not Supplied

Distilled water (for any dried blood samples, including the positive controls included in the kit)

Safety

Gloves
Safety goggles
Lab apron

Notes to Instructor:

Students should first perform the procedure using the positive control samples (included in the kit) to learn the procedure.

The kit contains enough materials for a large number of tests. Using the remaining materials, you may incorporate the presumptive blood test into any further forensics investigations.

- Drops of animal blood (such as that found in the bottom of trays containing meat purchased from the grocery store) may be placed around a mock "crime scene" to represent human blood evidence for the students to test.
- A simulated blood mixture may be prepared from water, corn syrup, red food coloring, and an inorganic catalyst or the enzyme catalase. Mix some corn syrup with water until a blood-like consistency is achieved, add a small amount of red food coloring and the oxidizing agent. Use this as blood evidence in a simulated crime investigation.

Possible inorganic catalysts:

Manganese dioxide, potassium iodide, copper nitrate, ferric oxide

Possible sources of catalase:

Ground beef liver, ground potato, ground horseradish

Note: *Be sure to test the simulated blood with added catalyst before performing the test with students. Some catalysts are more effective than others.*

Procedure

1. Moisten the end of a cotton swab with a small amount of distilled water.

2. Wearing gloves, gently swab a small area of one of the included positive control strips. Rub the swab back and forth a couple of times over a small area, being sure not to swab so hard as to tear the control strip.

3. Add one or two drops of ethyl alcohol to the end of the swab containing the positive control.

4. Add one or two drops of phenolphthalein to the end of the swab containing the positive control.

5. Observe the end of the swab for a few seconds before proceeding. If any color development occurs it is a false positive. Assume the test is compromised and begin again with a fresh swab and sample. If no color development occurs, continue to the next step.

6. Add one or two drops of hydrogen peroxide to the end of the swab. Observe for the development of pink color indicating the test was successful. If no color develops, something is interfering with the test and it is a false negative. Repeat using fresh material.