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

 **A Presumptive Test for Blood**

Objective: You will use a variety of chemicals to confirm the presence of blood.

Background:The Kastle-Meyer test is often used in television crime dramas to show the presence of blood at a crime scene. Evidence that appears to be blood is tested to determine if it is actually blood, and not something that just looks like blood. A spot that might be blood is wiped with a cotton swab to collect some of the substance. A drop of phenolphthalin reagent is added to the sample, and after a few seconds, a drop of hydrogen peroxide is applied to the swab. Sometimes the swab is first treated with a drop of ethanol in order to break open the cells that are present, resulting in increased sensitivity and specificity. This test is nondestructive to the sample, which can then be kept and used in further tests at the lab, such as in DNA analysis. If the swab turns pink rapidly, it is said to test positive for blood. The test result is actually presumptive positive, meaning it is not a conclusive test for blood, and other analyses would typically be carried out to confirm the presence of blood. Certain chemicals and biological fluids that do not contain blood can also cause the color change. These substances have to be avoided since they produce false positive results; that is, a positive result (color change) in the absence of blood. This test is very sensitive. One drop of blood diluted in 10,000 drops of water can still be detected by the Kastle-Meyer test. The Kastle-Meyer test relies on the iron in hemoglobin, which is the iron-containing portion of a red blood cell, to promote the oxidation of phenolphthalin to phenolphthalein. Phenolphthalin is colorless, but in the presence of blood and hydrogen peroxide, it changes to phenolphthalein, which makes the solution pink.

**Function of Chemicals in the test**

* Ethyl Alcohol: This increases the sensitivity of the Kastle-Meyer test by sterilizing the blood stain of contaminants. It does not affect the test’s reaction to blood.
* Phenolphthalein: This is the color changing agent; it turns bright pink when it encounters blood and oxygen.
* Hydrogen Peroxide: This helps to speed up oxidation and develop the phenolphthalein.
* Distilled Water: Used to wet a dried stain so it can be transferred to the swab.

Part 1 Procedure: Use the Kastle-Meyer test to test samples that could be blood. Record results below.

**Data Table 1: Test Results**

|  |  |  |
| --- | --- | --- |
| **Stains** | **Pink or not?** | **Is it blood or not?** |
| Blood (positive control) |  |  |
| Ketchup (negative control) |  |  |
| Unknown 1 |  |  |
| Unknown 2 |  |  |

Part 2 Procedure: Describe the role of the chemicals fused in the Kastle-Meyer test.

**Data Table 2: The Role of Chemicals in Blood sample Analysis**

|  |  |
| --- | --- |
| **Chemical** | **Function** |
| Distilled Water |  |
| Ethyl Alcohol |  |
| Phenolphthalein |  |
| Hydrogen Peroxide |  |

Questions:

1. Explain why you need a positive control when testing samples.
2. Explain why you need a negative control when testing samples.
3. Should the pink color be seen when adding phenolphthalein to a cotton swab? Explain.
4. Should the pink color be seen when adding hydrogen peroxide to a cotton swab? Explain.
5. If animal blood is different from human blood how is it possible to get a positive reaction with the Kastle-Meyer test using dog blood?
6. List two types of substances that might produce a false positive test when performing the Kastle-Meyer test for the presence of blood.
7. Why is it important to use a cotton swab when conducting this test?
8. Why aren’t the chemicals applied directly to the blood stain?
9. Suppose that a red stain was found in a bath tub along with bath water. Would it be possible to detect the blood even if it is diluted by the water?