



ATF-LS-FB05 Phenolphthalein Test for Blood	Published Online: March 2018
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1. Scope

This protocol is to be used when examining an exhibit of evidence for the presence of blood and is only a presumptive test. A presumptive test is one that is highly sensitive but not completely specific. The phenolphthalein test (also known as the Kastle-Meyer test) is a presumptive test for the presence of hemoglobin, a component of the red blood cells in blood. The phenolphthalein test is based on the peroxidase activity of a substance, in this case heme or heme derivatives, which catalyzes the reaction with peroxide which in turn oxidizes phenolphthalin to phenolphthalein. A positive reaction is observed when the colorless phenolphthalin solution turns pink as it is oxidized to phenolphthalein. The intensity of the pink color is directly proportional to the amount of hemoglobin and, therefore, blood that is present. Other substances such as plant peroxidases and chemical oxidants may also cause a positive reaction. Therefore, a positive phenolphthalein test only indicates the presence of blood but does not confirm the presence of blood.

2. References

- 2.1. EVIDENT Phenolphthalein Presumptive Blood Testing Kit.
- 2.2. R.E. Gaensslen, Identification of blood, in: Sourcebook in Forensic Serology, Immunology and Biochemistry, U.S. Department of Justice, Washington, D.C., 1983, pp. 73-133.
- 2.3. H.C. Lee, Identification and grouping of bloodstains, in: R. Saferstein, Forensic Science Handbook, Prentice Hall, Englewood Cliffs, 1982, pp. 267-337.

3. Equipment

- 3.1. Disposable gloves
- 3.2. Lab coat
- 3.3. Eye protection
- 3.4. Sterile swabs
- 3.5. Sterile water
- 3.6. Presumptive blood test kit (alcohol, phenolphthalein reagent, hydrogen peroxide – E.g. Evident Crime Scene Products: catalog #3658)
- 3.7. Known positive control (dried blood)
- 3.8. Known negative control
- 3.9. Scissors
- 3.10. Forceps
- 3.11. 70% ethanol or alcohol wipes
- 3.12. 10% bleach solution
- 3.13. Bench paper

4. Safety/Quality Assurance

- 4.1. Any utensils used to cut or manipulate swabs or other types of evidence must be cleaned between uses with 10% bleach solution followed by 70% ethanol or alcohol wipes.
- 4.2. Disposable gloves shall be worn when handling kit reagents and evidence.
- 4.3. Record the lot number of the phenolphthalein testing kit in the case notes. Do not use the reagents after the expiration date. Discard the phenolphthalein reagent if the colorless solution becomes pink.
- 4.4. At a minimum, each day the reagents are to be used, a negative control and positive control must be tested and documented in the analyst's notes. If either of the controls fail, a different lot of reagents shall be used.
- 4.5. This test is only a presumptive test and does not confirm the presence of blood. It is not human specific.
- 4.6. Lab coat and eye protection must be worn at all times while performing this procedure.
- 4.7. When practical, only one item of evidence shall be open at a time.
- 4.8. The laboratory bench surface shall be cleaned before and after use with 10% bleach solution or other sanitizing agent and may be followed by 70% ethanol. Fresh bench paper shall then be placed on the surface prior to examination.
- 4.9. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Procedure

- 5.1. Moisten a sterile swab with sterile water and lightly rub the suspected stain concentrating the sample on the swab tip. Alternatively, a cutting, filter paper rubbing or other appropriate method of sampling may be taken from the stain to be used for testing at the analyst's discretion.
- 5.2. Add one (1) drop of the alcohol reagent on the substrate surface.
- 5.3. Add one (1) drop of the phenolphthalein reagent. A pink color change at this point indicates the presence of other oxidative agents and the test is deemed inconclusive.
- 5.4. Add one (1) drop of the hydrogen peroxide reagent.
- 5.5. An immediate color change to vivid pink indicates a positive reaction. No color change indicates a negative reaction. Interpretation of results must be made immediately (within 5 seconds). A pink color change may occur after 30 seconds, which is a normal catalytic reaction that may occur without the presence of blood.
 - 5.5.1. Positive Result: an immediate color change to vivid pink (within 5 seconds)
 - 5.5.2. Negative Result: no color change within 5 seconds
 - 5.5.3. Inconclusive: color change to pink after the addition of the phenolphthalein reagent but before the addition of the hydrogen peroxide, color change to pink after 5 seconds, or any other anomalous result
- 5.6. Record results by noting the color change or lack of color change and the conclusion of the test.
- 5.7. A cutting or swabbing of an unstained area adjacent to the stained area may be tested as a substrate control at the analyst's discretion.