

## FBS02 – Phenolphthalein Presumptive Chemical Test for the Presence of Blood

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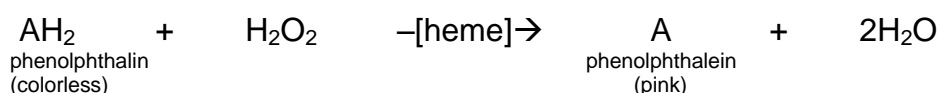
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### 1. Scope

- 1.1. This procedure is used to determine the possible presence of blood on evidentiary material.

### 2. Background

- 2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.
- 2.2. Blood can usually be located by the visual appearance of the stain (red-brown color). This is augmented by testing with presumptive tests for blood, such as the phenolphthalein (Kastle-Meyer) test. This test relies on the peroxidase-like activity of the heme groups associated with the hemoglobin contained in red blood cells. In the presence of hydrogen peroxide, this peroxidase-like activity will catalyze the oxidation of phenolphthalin, which is colorless in solution, into phenolphthalein resulting in a pink colored solution. The presence of a pink color is a positive test result indicating the presumptive presence of blood.



### **3. Safety**

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection) when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

### **4. Materials Required**

- 4.1. Phenolphthalin Working Solution (FBR16)
- 4.2. 3% Hydrogen Peroxide
- 4.3. Positive Control-Blood (FBR02)
  - 4.3.1. NOTE: Never use solutions directly from the stock bottles. Use Reagent SOPs for preparation and labeling instructions.

### **5. Standards and Controls**

- 5.1. The Positive and Negative Controls are tested prior to daily use. These results will be recorded in casework documentation.
  - 5.1.1. A known blood dilution test strip is tested as a Positive Control (FBR02). This control must exhibit a pink color within 10 to 15 seconds upon the addition of the 3% Hydrogen Peroxide up to the 1:512 dilution (or greater). If the 1:512 dilution tests negative, this indicates the reagents are losing sensitivity and the phenolphthalin and/or the hydrogen peroxide solution(s) need to be replaced.
  - 5.1.2. The unstained area on the known blood dilution test strip indicated as "Blank", is tested as the Negative control. This control should exhibit no pink color after 15 seconds upon the addition of the 3% Hydrogen Peroxide.

### **6. Calibration**

- 6.1. Not applicable

## 7. Procedures

- 7.1. Stain areas are located by a visual examination and/or with the aid of an alternate light source (ALS).
- 7.2. A small sample (e.g. swab, filter paper or cutting) of a suspected bloodstain is taken. The sampling method is dry or moist (diH<sub>2</sub>O) rubbing/swabbing of the stain area. Cuttings should only be taken if the stain is faint or weak (e.g., washed stain, small smear, etc) and stain size permits.
- 7.3. Add 1-2 drops of the phenolphthalin working solution to the sample.
- 7.4. Observe. No pink color should develop at this stage. If the sample exhibits a pink color, the result should be recorded as inconclusive due to the possible presence of other oxidative substances in the stain.
- 7.5. Add 1-2 drops of 3% Hydrogen Peroxide to the sample.
- 7.6. Observe the sample for 10-15 seconds for any color change. A pink color is a positive result and indicates the sample is presumptively positive for the presence of blood. No color development is a negative result and indicates the sample is presumptively negative for the presence of blood.

## 8. Sampling

- 8.1. Not applicable

## 9. Calculations

- 9.1. Not applicable

## 10. Uncertainty of Measurement

- 10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6)*.

## 11. Limitations

- 11.1. The phenolphthalein test is used for the presumptive identification of blood. A confirmatory test may be required to positively identify a stain as containing blood.
- 11.2. The phenolphthalein test depends upon the catalytic peroxidase-like activity of the heme group in hemoglobin. Because hemoglobin is used for oxygen and carbon dioxide transport in other organisms, this test will react with blood from animals as well as humans.
- 11.3. Insufficient sample quality and/or quantity could limit the development of a positive reaction.
- 11.4. A color change must be observed within 15 seconds due to the oxidative nature of the reaction. An unlimited detection time could lead to a false positive reaction.
- 11.5. Color development before the addition of hydrogen peroxide may be due to a chemical oxidant present in the sample.
- 11.6. Plant peroxidases react similarly to blood in catalyzing this reaction. Typically these stains are associated with plant tissue and can be visually distinguished from blood. Plant peroxidases tend to be unstable, losing their ability to oxidize the phenolphthalin reagent over time.

## 12. Documentation

- 12.1 FBU Serology Examination Worksheet
- 12.2 FBU Physical Evidence Recovery Worksheet
- 12.3 FBU Report of Examination

## 13. References

- 13.1. Camps, F. E., editor. Gradwohl's Legal Medicine. Baltimore: Williams and Wilkins (1968).

- 13.2. Culliford, Bryan J., *The Examination and Typing of Bloodstains in the Crime Laboratory*, National Institute of Law Enforcement and Criminal Justice PR71-7, Washington, DC, 1971, p. 41-52.
- 13.3. Gaensslen, R. E., *Sourcebook in Forensic Serology, Immunology, and Biochemistry*, U.S. Government Printing Office, Washington, DC, 1983, p. 103.
- 13.4. Lee, H. C. Identification and Grouping of Bloodstains. Saferstein, R., ed., In: *Forensic Science Handbook*, Prentice-Hall, 267-337 (1982).
- 13.5. FBR02 - Positive Control – Blood (Current Version)
- 13.6. FBR16 - Phenolphthalin Working Solution (Current Version)
- 13.7. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)
- 13.8. *FSL Departmental Operations Manuals* (Current Versions)
- 13.9. *FSL Laboratory Operations Manuals* (Current Versions)