PHENOLPHTHALIN (KASTLE – MEYER) COLOR TEST

Background

This protocol outlines a presumptive test for blood. Phenolphthalin in the presence of hemoglobin and hydrogen peroxide produces a pink color reaction.

Summary of Procedure

A sampling of the forensic evidence is treated with alcohol and phenolphthalin. In the absence of a color change, 3% H_2O_2 is added. A pink color change at this step is indicative of the presumptive presence of blood

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.
Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling.**

Warnings and Precautions

A false positive can be caused by the following:

- Chemical oxidants and catalysts such as copper, nickel salts, rust, hypochlorite (bleach), iodine, lead oxides, etc.
- Plant sources such as vegetable peroxidases (i.e.-apple, apricot, bean, blackberry, artichoke, horseradish, potato, turnip, cabbage, onion and dandelion root).

A false negative can be caused by the presence of a reduction compound capable of interfering with the oxidation process (i.e.-ascorbic acid/lemon juice).

Reagents and Materials

See **Appendix B** for reagent preparation

10% Bleach
MBG Water
100% Ethanol
3% H_2O_2
Phenolphthalin working solution
Disposable bench paper

Lab Coat
Kimwipes
Gloves
Protective Eyewear
Swabs
Sample handling tools (scissors, scalpel blades, forceps, etc.)
Coors color test plate, clear microfuge tubes or whatman #1 filter paper
15ml Dropper bottles

Reagents and Materials – Storage and Handlings

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Evidence Examination Worksheet** or other appropriate worksheet is required for documentation. All information must be completed.

The phenolphthalin working solution shall be tested successfully against a positive blood control and a negative control to ensure detection efficacy prior to use.

All results must be verified by a second qualified analyst. If a second qualified analyst is unavailable photo documentation of the result is acceptable.

Positive Control

Phenolphthalin Blood Positive Control (included in presumptive blood kit)

Negative Control

Blank substrate (e.g. filter paper, swab, etc.)

Procedure

1. Transfer stained material to the substrate (e.g. filter paper, swab) through cutting or rubbing.
2. Add one drop of alcohol to the substrate.
3. Add one drop of phenolphthalin working solution to the substrate.
4. Observe the substrate for a color change for approximately 30 seconds. If a pink/red color change occurs, this indicates of the presence of a contaminant (False Positive test), stop the test and proceed to the Hematrace test. If no color change occurs proceed.
5. Add one to two drops of 3% H_2O_2 solution to the substrate.
6. Observe the substrate for a color change within 10-15 seconds. The substrate will develop a color within several minutes even if no blood is present.
7. Note the results on the **Evidence Examination Worksheet** or other appropriate worksheet.

Results and Conclusions

Positive Result: A pink color change within 10-15 seconds indicates the presence of blood which is a positive result. This would be reported as blood is indicated on the appropriate report.

Negative Result: No color change within 15 seconds indicates the absence of blood which is a negative result. This would be reported as no blood is indicated on the appropriate report.

Inconclusive Result: A color change (other than pink) may indicate the presence or absence of blood which is not a conclusive or decisive result. This would be reported as the test for blood was inconclusive on the appropriate report.

Reference

Identification and Grouping of Bloodstains – Henry C. Lee, Ph.D., Connecticut State Police

Criminalistics; An Introduction to Forensic Science Ed. 8 Richard Saferstein
(p. 336)