

**TITLE: Screening Test for Blood**

**A. Purpose:**

To perform screening tests for the presence of blood in Forensic samples.

**B. Responsibility:**

Forensic Science Examiners from the Connecticut State Forensic Science Laboratory who have been trained in the discipline of blood screening according to SOP-FB-31 (Training Manual).

**C. Safety**

Use appropriate measures for the proper handling of the o-Tolidine solution according to SOP-GL-2 (Safety Manual).

**D. Definitions**

1. PBS: Phosphate Buffered Saline
2. KM: Kastle-Meyer Test

**E. Procedure:**

These tests will be performed at the discretion of the examiner based on the submitting agency requests, case information and the condition of the evidence.

1. Materials:

- a. Phenolphthalin solution (KM test)
- b. o-Tolidine solution
- c. 3.0% Hydrogen peroxide
- d. Controls: known blood stain and blank filter paper
- e. PBS
- f. 0.5% ammonia
- g. Cotton swabs or spot plates

2. Procedure:

- a. Test a positive control and a PBS reagent/filter paper blank with the following procedure (steps 2.b. – 2.e.) prior to the question sample.

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- aa. If controls yield the appropriate results then test the questioned sample. Record the results on the appropriate Quality Record Worksheet.
  - bb. If controls do not yield the appropriate results, review the procedure and retest the controls prior to the question samples.
  - b. Remove and test a portion of the questioned sample or stain.
  - c. The questioned sample may be extracted in 0.5% ammonia and test the extract either directly or on a cotton swab.
  - d. Add one drop of phenolphthalin or o-Tolidine solution to the portion of questioned sample. Testing with o-Tolidine solution should be performed under the hood.
  - e. If no color change occurs, add one (1) drop of 3% H<sub>2</sub>O<sub>2</sub>.
  - f. Observe any color change within 10 seconds.
3. Results:
- a. *Positive.* If blood or "other peroxidase-type material" is present, a color change will occur after the addition of 3% H<sub>2</sub>O<sub>2</sub>. Phenolphthalin (KM test) will turn pink; o-Tolidine will turn blue.
  - b. *Negative.* A negative reaction will show no color change within 10 seconds of the addition of 3% H<sub>2</sub>O<sub>2</sub>, indicating no blood was detected.
  - c. *Inconclusive.* The appearance of a color change without the addition of 3% H<sub>2</sub>O<sub>2</sub> may indicate the presence of a chemical oxidant in the stain.
  - d. Record the results on the appropriate Quality Record Worksheet.
  - e. If the quantity of stain is insufficient to perform a confirmatory test, a positive chemical screening test is sufficient to forward the stain directly for DNA analysis.

### F. References:

1. Holland, V.R.B., C.Saunders, F.L.Rose and A.L.Wulpoll. A safer substitute for benzidine in the detection of blood. Tetrahedron, Vol.30, 1974, pp. 3299-3302.

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3. Koan, J. and T. O'Kelly. An ortho-tolidine method for the detection of occult blood in feces. J. Clinical Pathol. Vol.8, 1955, pp. 249-251.
4. Ruttan, R.F. and R.H.M. Hardisty. A new reagent for detecting occult blood. Can. Med. Associat. J. Vol.41(n.s.2), 1912, pp. 995-998
5. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 2-88 to 2-90.
6. SOP-GL-2 (Safety Manual).