Document Title: Screening Tests for Blood
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

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.

      aa. If controls yield the appropriate results then test the questioned sample. Record the results on the appropriate Quality Record Worksheet.

E. 2. a. 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_2O_2.

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_2O_2. 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_2O_2, indicating no blood was detected.

   c. *Inconclusive.* The appearance of a color change without the addition of 3% H_2O_2 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.

2. Hunt, A.C., C. Corley, B.E. Dodd and F.E. Camps. The identification of human blood stains: critical survey. J.Forensic Med. Vol. 7, 1960, pp. 112-130.

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.

F. 5. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 2-88 to 2-90.

6. SOP-GL-2 (Safety Manual).