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. sdH_2O: Sterile distilled water
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: positive (known bloodstain) and negative (blank filter paper)
   e. sdH_2O
   f. 0.5% ammonia
   g. Cotton swabs or spot plates

2. Procedure:
   a. Test a positive control and a negative control (blank filter paper) with sdH_2O according to the following procedure (steps 2.b. – 2.e.) prior to the questioned sample.

      aa. If controls yield the appropriate results, record on the appropriate Quality Record Worksheet and test the questioned samples.

E. 2. a. bb. If controls do not yield the appropriate results, review the procedure and retest the controls prior to beginning analysis on casework samples. If the controls still do not

yield the appropriate results, then determine the root cause and correct.

b. Moisten swab with sdH_2O to remove and test a portion of the questioned sample or stain. A portion of the questioned sample or stain may be removed and tested directly.

aa. 0.5% ammonia may be used for samples that are difficult to remove for testing.

bb. Both controls must be tested with 0.5% ammonia according to steps 2.a. – 2.e., prior to testing the questioned sample.

cc. If 0.5% ammonia is used, record on the appropriate Quality Record Worksheet and the General Reagent Sheet (FBQR-09).

c. Add one drop of phenolphthalin or o-Tolidine solution to the portion of questioned sample. *Caution:* Testing with o-Tolidine solution should be performed under the hood.

d. If no color change occurs, add one (1) drop of 3% H_2O_2.

e. 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.*
      aa. 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.

      bb. If no distinguishable color change is observed after the addition of 3% H_2O_2.

   d. Record the results on the appropriate Quality Record Worksheet. Note: The reason a result is determined to be inconclusive must also be recorded.

   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.

4. Record reagent(s) used on the General Reagent Sheet (FBQR-09).

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.

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

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