Document Title: Test for Urobilinogen
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To determine the presence of urobilinogen in a Forensic sample, which indicates the presence of feces.

B. RESPONSIBILITY:

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

C. SAFETY:

Use appropriate measures for the proper handling of mercuric chloride according to SOP-GL-2 (Safety Manual).

D. DEFINITIONS:

1. ALS: Alternate Light Source
2. PBS: Phosphate Buffered Saline

E. PROCEDURE:

This test 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. Alcoholic mercuric chloride (saturated in ethanol)
   b. Alcoholic zinc chloride (saturated in ethanol)
   c. Distilled water (dH_2O)
   d. Controls: positive (known fecal stain) and negative (blank filter paper), include substrate control as needed
   e. Alternate Light Source (ALS)

2. Procedure:
   a. Prepare a saturated solution of mercuric chloride in a test tube with ethanol. Dissolve enough mercuric chloride in the ethanol until it no longer goes into solution.

   b. Repeat above step with zinc chloride.

   c. These saturated solutions must be prepared at the time the test is performed. Record on the General Reagent Sheet (FBQR-09).

E. 2. d. Test a positive and negative control with the following procedure (steps 2.e. – 2.m.).
   aa. The controls may be run concurrently with the questioned samples.

bb. If limited questioned sample is available, run the controls prior to testing the questioned sample. If controls yield the appropriate results then test the questioned sample.

cc. If controls do not yield the appropriate results, review the procedure and retest the controls prior to the questioned samples.

e. Extract a portion of the stained material in a test tube with enough dH_2O to cover the sample for a minimum of five minutes or longer as needed. Do not extract in PBS.

f. Remove substrate from test tube.

g. Add three (3) drops of extract to a 2nd test tube.

h. Add three (3) drops of alcoholic mercuric chloride to the test tube.

i. Add three (3) drops of alcoholic zinc chloride to the test tube.

j. Vortex the mixture.

k. Examine under ALS (blue or blue-green) and compare to controls.

l. Observe the color of the extract.

m. Discard any unused reagent.

3. Results:
   a. *Positive*. An apple green fluorescence is visible under ALS if urobilinogen is present.

   b. *Negative*. No color change is noted under ALS, which indicates that no urobilinogen is present or below detectable level.

   c. *Inconclusive.* No discernible color change and/or insufficient extraction of sample.

   d. It is important to compare results against the positive and negative controls.

   e. Record the results of the controls and samples on the appropriate Quality Record Worksheet.

   f. A 2nd examiner will observe and confirm results and initial the appropriate Quality Record Worksheet.

F. REFERENCES:

1. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 4-7.
2. SOP-GL-2 (Safety Manual).