

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
3. sdH₂O: Sterile distilled water

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. sdH₂O
 - d. Controls: positive (known fecal stain) and negative (blank swab), 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 sdH₂O 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-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).