

Connecticut Department of Public Safety
Division of Scientific Services
Forensic Laboratory

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Document Title: Test for Urobilinogen
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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 (dH₂O)
 - d. Controls: known fecal stain and blank filter paper (include substrate control when 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.
 - 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 question samples.
- E. 2. d. bb. If limited question sample is available, run the controls prior to testing the question 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 question samples.
- e. Extract a portion of the stained material in a test tube with enough dH₂O 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).