TEST FOR FECAL MATERIAL

18.1 PURPOSE

18.1.1: To determine the presence of urobilinogen in Forensic samples.

A. Theory

The urobilinogen test for the presence of fecal material relies on the formation of a green fluorescent zinc-urobilin complex. Urobilinogen is oxidized to urobilin, which is soluble in alcohol. In the presence of neutral alcoholic zinc salts, the green complex is formed and is visualized under an alternate light source or ultraviolet light.

B. Limitations

Screening for fecal material based on color may be misleading. Although it is usually brown in color, in infants, it is yellow, partly due to the presence of unchanged bilirubin and partly to the milk diet. Newborn infants produce meconium for the first few days after birth. This is dark green/brown to black and sticky.

Urobilinogen may be absent in stools from infants under 6 months old.

18.1.2: To quality control new mercuric chloride and zinc chloride chemicals.

18.2 RESPONSIBILITY

18.2.1: Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in the discipline of testing for urobilinogen according to FB SOP-26 (Training Manual and Checklist).

18.2.2: Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New chemicals are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory (Appendix 3) in the FB folder on the shared drive.

18.3 SAFETY

Use appropriate measures for the proper handling of biohazardous materials, mercuric chloride and zinc chloride according to GL-2 (Safety Manual) and the Safety Data Sheets.

18.4 DEFINITIONS

A. ALS: Alternate Light Source

B. PBS: Phosphate Buffered Saline

18.5 TEST PROCEDURE

A. This test will be performed at the discretion of the examiner, with input from the Unit Lead(s), based on the submitting agency requests, case information and the condition of the evidence.

B. A sample is considered limited when it appears to be of low quantity and compromised when it appears to be in poor condition. The conditions the evidence may have been exposed to prior to submission shall be considered when assessing the sample tested and/or collected.

18.5.1: Materials

A. Alcoholic mercuric chloride (saturated in ethanol)
B. Alcoholic zinc chloride (saturated in ethanol)
C. dH_2O
D. Controls: positive (known fecal stain) and negative (blank swab), include a substrate control from the evidence as needed
E. ALS: Blue/Green (B/G) 460-510nm

18.5.2: 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).

D. Test a positive and negative control with the following procedure (steps 17.52.E - 17.5.2.M).
   1. The controls may be run concurrently with the questioned samples.

   2. If the questioned sample is limited/compromised, run the controls prior to testing the questioned sample. If the controls yield the appropriate results then immediately test the questioned sample.

   3. If the controls do not yield the appropriate results, review the procedure and retest the controls prior to the questioned sample. If the controls still do not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

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. Agitate to ensure sample is submerged.

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. The positive and negative controls are used to aid in the interpretation of the results.

N. Discard any unused reagent in a designated waste disposal container.

O. Record the results of the controls and sample(s) on the appropriate Quality Record Worksheet (Appendix 1).

P. A 2nd examiner will observe and confirm results and initial the appropriate Quality Record Worksheet (Appendix 1).

18.5.3: Results and Conclusions

A. Positive

1. An apple green fluorescence is visible under ALS if urobilinogen is present.

2. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Screening - Urobilinogen	Positive	Fecal material indicated

Appendix:
Urobilinogen is a component of fecal material.

b. *[ ] gave a positive result(s) with a color screening test for the presence of urobilinogen, a component of fecal material.*

B. Negative

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

2. Suggested Report Wording:

a.

Testing Performed	**Result**	**Conclusion**
Screening - Urobilinogen	Negative	Fecal material not detected

Appendix:

Urobilinogen is a component of fecal material.

b. *A color screening test for the presence of urobilinogen, a component of fecal material, was performed on [ ]. Fecal material was not detected with this test.*

18.6 QC PROCEDURE

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

A. Test the new lots before use according to the test procedure and the Urobilinogen Reagent Log Sheet. Record the required information.

B. If the appropriate results are not obtained, review the procedure and repeat the test. If the reagent still does not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

C. If the lots are acceptable for use, record the date received, date opened and examiner's initials on the bottles.

The lots are acceptable for use when a positive result is obtained with the fecal control and a negative result is obtained with a blank/negative control.

D. Store at room temperature.

18.7 REFERENCES

A. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 4-7.
B. GL-2 (Safety Manual)
C. GL-6 (Purchasing)
D. Safety Data Sheets