TEST FOR FECAL MATERIAL

18.1 PURPOSE

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

A. Theory

Urobilinogen is a component of fecal material. It is a colorless product formed in the intestine when bilirubin is metabolized by intestinal bacteria. Urobilinogen is further oxidized by intestinal bacteria to form urobilin which gives fecal material its characteristic color.

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 and/or electronically in the Forensic Biology Unit. New chemicals are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory located in Appendix 3.

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
C. QRW(s): Quality Record Worksheet(s) (Appendix 1)

18.5 TEST PROCEDURE

A. This test will be performed at the discretion of the examiner, with input from the Unit Lead(s) when applicable, 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. Mercuric chloride
B. Zinc chloride
C. Ethanol
D. dH_2O
E. Test tubes
F. Controls: positive (known fecal stain) and negative (blank swab), include a substrate control from the evidence as needed
G. ALS: Blue/Green (B/G) 460-510nm

18.5.2: Procedure

A. Saturated solutions of mercuric chloride and zinc chloride will be prepared at the time of use.

1. In a test tube, dissolve enough mercuric chloride in ethanol until it no longer goes into solution. Repeat for zinc chloride.

2. Record on the General Reagent Sheet (FBQR-09).

B. Test a positive and negative control with the following procedure below.

1. The controls may be run concurrently with the questioned samples as an intermediate check. Reagent QC is always conducted prior to use on case 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.

C. Extract a portion of the stained material in a test tube (appropriately labeled) 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.

D. Remove three (3) drops of extract and add to a 2nd test tube (appropriately labeled).

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

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

G. Vortex the mixture.

H. Examine under ALS (blue-green) and observe the color of the extract.

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

J. Record the results of the controls and sample(s) on the appropriate QRW.

K. A 2nd qualified examiner will observe and confirm results and initial the appropriate QRW.

L. Discard sample/reagent mixtures and any unused reagent in a designated waste disposal container.

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

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

A 2nd qualified examiner will observe and confirm results and initial the Urobilinogen Reagent Log Sheet.

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 chemicals at room temperature according to the manufacturer's instructions.

E. Discard/replace according to the manufacturer's expiration date or according to 21.4.3.E in FB SOP-21 (General Chemical and Reagent QC).

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

18.7 REFERENCES

A. Li, Richard, Forensic Biology, Second Edition, CRC Press, FL, 2015, Chapter 17, "Identification of Urine, Sweat, Fecal Matter and Vomitus", pp. 318-323 (17.3 Identification of Fecal Matter).
B. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 4-7.

C. GL-2 (Safety Manual)
D. GL-6 (Purchasing)
E. Safety Data Sheets