

DNA Analyst Training Laboratory Training Manual

Protocol 2.13
Fecal Matter Indication: Urobilinogen



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Urobilinogen is formed in the intestine by reduction of bilirubin. Urobilinogen is oxidized to urobilin which is soluble in alcohol. This test relies on the formation of a green fluorescent zinc-urobilin complex formed in the presence of neutral alcohol zinc salts.

SAFETY CONSIDERATIONS

Mercuric Chloride - Warning! Severe Irritant!
Danger! Highly Toxic!

Zinc Chloride - Caution! Irritant!
Caution! Moderately Toxic!

Methanol - Caution! Slightly Toxic!

PREPARATIONS

Solution #1: 40% Alcoholic mercuric chloride solution

Mercuric chloride 4 g.
Methanol 10 ml.

Mix and store in stoppered bottle.

Solution #2: 40% Alcoholic zinc chloride solution

Zinc chloride 4 g.
Methanol 10 ml.

Mix and store in stoppered bottle

Solution #3: Amyl alcohol

INSTRUMENTATION

No Instrumentation Required.

MINIMUM STANDARDS & CONTROLS

A known fecal stain and a substrate sample (when available) should be tested each time the testing is performed. Use distilled water as a negative control.

PROCEDURE OR ANALYSIS

1. Extract the suspected stain and an unstained control in 3 drops of water in separate test tubes.
2. Place an equal amount of water in a third test tube for the negative control.
3. Add 3 drops of 40% alcoholic mercuric chloride to each tube.
4. Add 3 drops of amyl alcohol to each tube.
5. Shake to mix.
6. Centrifuge and collect the clear supernatant.
7. Examine under UV long-wave light for any fluorescence. No fluorescence should be visible at this point in the analysis.
8. Add 3 drops of 40% alcoholic zinc chloride to each tube and shake.
9. Incubate at room temperature for 30-60 minutes.
10. Examine under long-wave UV light. The appearance of a green fluorescence is considered a positive test for urobilinogen, indicative of the presence of fecal matter. This test is not confirmatory for fecal matter.

Note:

Safety eyeglasses which absorb ultraviolet radiation must be worn when examining for fluorescence with UV light.

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