

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK**

**ACETAMINOPHEN
(COLOR TEST)**

PRINCIPLE

A simple, rapid color test for acetaminophen can be performed on urine. Acetaminophen and its metabolites are hydrolyzed in acid solution to p-aminophenol, which is then coupled with o-cresol to give a distinctive indophenol blue.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines detailed in the safety manual.

REAGENTS

All chemicals should be ACS grade or better.

1. Ammonium hydroxide, 4N
Add 284 mL of concentrated ammonium hydroxide to a one liter volumetric flask and Q.S. with distilled water.
2. Saturated o-cresol reagent
Mix 10 mL of o-cresol with approximately 500 mL deionized water in a one liter volumetric flask. Q.S. with deionized water. Allow to stand for 24 hours before use.
3. Concentrated hydrochloric acid (HCl)
4. Distilled or deionized water

PROCEDURE

1. Number test tubes.
2. Aliquot approximately 1 mL of unknown urine into a 12 x 75 mm test tube.
3. Remove the required number of positive and negative controls from the freezer, thaw and include in the batch(s). (A positive control is included with each hydrolysis batch.)
4. Add 2 drops (0.2 mL) concentrated HCl.
5. Mix by Vortex.
6. Hydrolyze in simmering water bath for a minimum of 10-15 minutes.

7. Add 0.5 mL 4N ammonium hydroxide.
8. Add 1 mL o-cresol.
9. Mix by Vortex.
10. Blue color will develop if acetaminophen is present.

LIMIT OF DETECTION

Limit of detection (LOD) for acetaminophen is 15 mg/L in urine.

ACCEPTANCE CRITERIA

1. Only specimens that have been analyzed with successful controls can be reported.
2. Negative control must not react with o-cresol.
3. Positive control must produce a blue color in the presence of o-cresol.

REPORTING

Samples which do not cause a color reaction with o-cresol are reported as "acetaminophen not detected."

Samples which cause a color reaction with o-cresol are reported as "acetaminophen detected" and are scheduled for confirmation by HPLC.

INTERPRETATION

The formation of the indophenol blue is extremely sensitive. A strong positive can be obtained with urine from volunteers who have taken 1g of acetaminophen 24 hours previously.

INTERFERING SUBSTANCES

Acetaminophen and p-aminophenol are metabolites of phenacetin. Hence, this reaction can give positive results after ingestion of phenacetin.

REFERENCE

Curry, A., *Poison Detection in Human Organs*. Charles C. Thomas Publisher; Springfield, IL, 1969

Sunshine, I., *Methodology for Analytical Toxicology*. CRC Press, Inc., Boca Raton, FL, 1975