SCREENING OF BIOLOGICAL SPECIMENS FOR ACETAMINOPHEN BY O-CRESOL RAPID COLOR TEST

31.1 POLICY

This test method may be used to presumptively identify acetaminophen in biological samples. Reporting of presumptive results following the application of this method will be contingent upon a thorough review of the batch and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by a member of TLD Management, and appropriately documented on the work list.

31.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the presumptive identification of acetaminophen present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, criteria for acceptance and presumptive reporting of the specified compounds.

31.3 PRINCIPLE

Rapid presumptive tests are simple colorimetric tests that may be performed directly on a biological sample with little or no previous sample preparation. In this colorimetric assay, acetaminophen is hydrolyzed to p-aminophenol, which forms a blue color in the presence of o-cresol.

31.4 SPECIMENS

31.4.1 The specimen volume is 0.5 mL.

31.4.2 Specimens include whole blood, serum, plasma, urine, and tissue homogenate.

31.4.3 Dilutions of specimens may be analyzed at the Forensic Scientist's discretion; however, this should be done in addition to testing the standard specimen volume, unless sample quantity dictates otherwise.

31.4.4 Analysis of larger specimen volumes must be approved and documented.

31.5 REAGENTS, MATERIALS AND EQUIPMENT

31.5.1 REAGENTS

31.5.1.1 Ammonium hydroxide (NH_4OH), concentrated 10N

31.5.1.2 Certified blank blood

31.5.1.3 Deionized water (DI H_2O)

31.5.1.4 Hydrochloric acid (HCl), concentrated 12N

31.5.1.5 Methanol

31.5.1.6 O-cresol

31.5.1.7 1% O-cresol

Add 10 mL o-cresol to 800 mL DI H_2O in a 1 L glass flask. Dilute to 1 L with DI H_2O and mix. Allow to stand for 24 hours prior to initial use. Store solution in a glass bottle at room temperature for up to two years. Adjustments to final volume are permitted as long as the proportions are maintained.

31.5.1.8 Trichloroacetic acid (TCA)

31.5.1.9 6.25% Trichloroacetic acid

In a fume hood, dissolve 6.25 g TCA in 80 mL DI H_2O, in a glass flask. Dilute to 100 mL with DI H_2O and mix well. Store solution in a glass bottle at room temperature for up to two years. Adjustments to final volume are permitted as long as the proportions are maintained.

31.5.2 MATERIALS

31.5.2.1 Disposable 12 x 75mm tubes with closures

31.5.2.2 Disposable 16 x 100mm tubes with closures

31.5.2.3 Disposable pipette tips

31.5.2.4 Disposable serological pipettes (borosilicate glass)

31.5.2.5 Laboratory glassware (graduated cylinders, flasks)

31.5.2.6 Volumetric glassware (flasks)

31.5.3 EQUIPMENT

31.5.3.1 Calibrated, adjustable piston pipettes

31.5.3.2 Centrifuge

31.5.3.3 Oven, dry bath or wet bath

31.5.3.4 Vortex mixer

31.6 STANDARDS, BLANK AND CONTROLS

31.6.1 STANDARDS

31.6.1.1 Reference materials (referred to interchangeably in this method as stock standards) are used for the preparation of working standards which in turn are used to produce positive controls.

31.6.1.2 Working standard (1.0 mg/mL)

a. Using a calibrated balance, weigh 25 mg of acetaminophen and add to a 25-mL class A volumetric flask.
b. Add methanol to the flask to the designated volume.
c. The final concentration of the working standard is 1.0 mg/mL. The working standard is stored in the freezer in an amber bottle and expires one year from the date of preparation. Volumes may be adjusted provided that proportions remain constant.

31.6.2 BLANK

31.6.2.1 A water blank is prepared with each batch, for comparison to the negative control (testing matrix). This colorimetric assay does not employ the use of internal standard.

31.6.3 CONTROLS

31.6.3.1 Negative Control

a. At least one negative whole blood control is tested with every batch. The negative control is prepared using certified blank blood.
b. When testing different sample types, wherever possible, include a negative control prepared from that matrix. (For example, when analyzing whole blood and urine samples the batch shall include at least one negative whole blood control and at least one negative urine control.)

31.6.3.2 Positive Control

a. One positive whole blood control is tested with every batch. The positive control is prepared using certified blank blood to which the designated volume of working standard has been added.
b. The preparation of the positive whole blood control is detailed in 31.7 SAMPLE PREPARATION.
c. When testing different sample types, wherever possible, include at least one positive control prepared from that matrix.

31.7 SAMPLE PREPARATION

31.7.1 Label a clean 16 x 100mm tube for each member of the test batch. (e.g., water blank, positive control, negative control, case samples).

31.7.2 Add 0.5 mL DI H_2O to the water blank tube.

31.7.3 Add 0.5 mL of certified blank whole blood into the positive and negative control tubes.

31.7.4 Add 25 µL of the working standard to the positive control tube. Final concentration of the positive control is 50 mg/L.

31.7.5 Sample 0.5 mL of each case sample into its respective tube.

31.7.6 Add 2 mL of 6.25% TCA to each tube. Cap and vortex mix.

31.7.7 Centrifuge the tubes for 15 minutes at 2500 rpm.

31.7.8 Transfer 200 µL of the supernatant to clean, labeled 12 x 75mm tubes.

31.7.9 Add 200 µL concentrated HCl to each tube. Cap and vortex mix.

31.7.10 Incubate the tubes at 100°C for 30 minutes.

31.7.11 Remove the tubes from heat and allow them to cool to room temperature.

31.7.12 In a fume hood, add 0.5 mL of 1% o-cresol reagent.

31.7.13 Add 0.5 mL concentrated NH_4OH.

31.7.14 Cap and Vortex.

31.7.15 Allow tubes to stand 10 minutes and evaluate color change.

NOTE: Evaluation of supernatant color change (or lack of color change) should be observed after 10 minutes. All tubes standing longer than 10 minutes may develop blue color (including water blank or negative control).

31.7.16 Dispose of all waste in appropriate chemical waste container for proper disposal.

31.8 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

31.8.1 Blank

31.8.1.1 No color change should be observed in the water blank.

31.8.2 Controls

31.8.2.1 No color change should be observed in the negative control (supernatant should match that observed in the water blank).

31.8.2.2 There should be a distinct color change (blue) observed in the positive control.

31.9 CRITERIA FOR CASE SAMPLE ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are met.

31.9.1 A comparison of the color change of the specimen(s), relative to the negative and positive control is used to determine whether confirmation and quantitation is necessary. Development of a blue color within 10 minutes constitutes a presumptive positive result for acetaminophen.

31.10 REPORTING

31.10.1 Qualitative Reporting

31.10.1.1 Results of this rapid presumptive test (RPT) may be reported as either negative or presumptive positive, as appropriate, using this colorimetric assay. If reported as presumptive positive, a separate sampling of the specimen will be tested by a more specific assay for confirmation and/or quantitation.

31.10.1.2 Reporting Examples

a. If result from RPT is presumptive positive, and the subsequent confirmation assay result is positive:

- Both tests and respective results will be entered in LIMS and appear on the final report.

b. If result from RPT is presumptive positive, and the subsequent confirmation assay result is negative:

- Only the confirmation test with “none detected” as the result will be entered in LIMS and appear on the final report.

c. If the result from RPT is negative, and the subsequent confirmation assay result is positive:

- Only the confirmation test and results will be entered in LIMS and appear on the final report, provided that an additional confirmation is performed.

31.10.1.3 A presumptive positive result from this colorimetric assay must be confirmed using a more specific test method on a separate sampling prior to reporting.

31.11 TRACEABILITY

31.11.1 Traceability of the reference materials to SI units is provided through the certificate of analysis provided by the approved reference material supplier.

31.12 REFERENCES

31.12.1 B.E. O’Reilly, in-house method development.

31.12.2 Virginia Department of Forensic Sciences, Rapid Presumptive Test Method.

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LIST OF CHANGES

Revision Date	Description	Page Number
8/18/14	Method approved by Washington State Toxicologist. See DRA dated 07/17/14. Method released for use in evidentiary testing on 8/18/14.	All
9/30/16	Modified deviation approval in 31.1 to “TLD Management.” Changed “air-displacement” to “piston” in pipette description in 31.5.3.1. Added “Printed Copies are Uncontrolled” to the footer.	All