SCREENING OF BIOLOGICAL SPECIMENS FOR SALICYLATES USING TRINDER'S REAGENT COLOR TEST

40.1 POLICY

This test method may be used to presumptively identify salicylates 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.

40.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the presumptive identification of salicylates 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.

40.3 PRINCIPLE

Rapid presumptive tests are simple colorimetric tests that may be performed directly on a biological sample with little or no previous sample preparation. The specimen is treated with Trinder's reagent, an acidic solution of ferric nitrate and mercuric chloride. The free carboxylic acid of salicylate reacts with Fe^{3+} to form a violet complex.

40.4 SPECIMENS

40.4.1 The specimen volume is 0.5 mL.

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

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

40.5 REAGENTS, MATERIALS AND EQUIPMENT

40.5.1 REAGENTS

40.5.1.1 Certified blank blood (and/or other matrices)

40.5.1.2 Deionized water (DI H_2O)

40.5.1.3 Ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$)

40.5.1.4 Hydrochloric acid (HCl, concentrated 12N)

40.5.1.5 1M Hydrochloric acid

Add 400 mL DI H_2O to a glass flask. Carefully add 42 mL concentrated HCl (12N). Dilute with DI H_2O to a final volume of 500 mL. Store the acid at room temperature in a glass container for up to one year. Volumes may be adjusted provided that the proportions remain constant.

40.5.1.6 Mercuric chloride ($HgCl_2$)

40.5.1.7 Methanol (MeOH)

40.5.1.8 Trinder's reagent

Dissolve 40 g $HgCl_2$ in 850 mL DI H_2O. Add 120 mL of 1M HCl. Add 40 g $Fe(NO_3)_3 . 9H_2O$ and apply magnetic stirring to dissolve. Dilute to a total volume of 1 L with DI H_2O. Store the solution at room temperature in a plastic bottle for up to one year. Volumes may be adjusted provided that the proportions remain constant.

40.5.2 MATERIALS

40.5.2.1 Disposable 16 x 125 mm tubes with closures

40.5.2.2 Disposable pipette tips

40.5.2.3 Glass and plastic storage bottles

40.5.2.4 Laboratory glassware (graduated cylinders, flasks, beakers)

40.5.2.5 Magnetic stir bar

40.5.2.6 Magnetic stir plate

40.5.2.7 Volumetric glassware (flasks, pipettes)

40.5.3 EQUIPMENT

40.5.3.1 Calibrated, adjustable piston pipettes

40.5.3.2 Centrifuge

40.5.3.3 Vortex mixer

40.6 STANDARDS, BLANK AND CONTROLS

40.6.1 STANDARDS

40.6.1.1 Reference material (referred to interchangeably in this method as stock standard) is used for the preparation of the control working standard, which is then used to produce positive controls.

40.6.1.2 Control working standard (5 mg/mL)

a. Using an analytical balance, weigh out 50 mg salicylic acid and add to a 10 mL class A volumetric flask.
b. Add methanol to the flask to the designated volume.
c. The final concentration of the working standard is 5 mg/mL. The control working standard is stored in an amber bottle in the freezer and expires one year from the date of preparation. Volumes may be adjusted, provided that proportions remain constant.

40.6.2 BLANK

40.6.2.1 Certified blank testing matrix is used as a matrix blank, for comparison to the negative control. This assay does not employ the use of internal standard.

40.6.3 CONTROLS

40.6.3.1 Negative Control

a. One negative control for each testing matrix is analyzed with every batch. The negative control is prepared using certified blank matrix.

40.6.3.2 Positive Control (100 mg/L)

a. One positive control for each testing matrix is analyzed with every batch. The positive control is prepared using certified blank matrix to which the designated volume of control working standard has been added.
b. The preparation of the positive control is detailed in 40.7 SAMPLE PREPARATION.

NOTE: Batch requirements described in *Quality Assurance Principles (PQ12703)* 4.2.3.1 do not apply to visual color tests.

40.7 SAMPLE PREPARATION

40.7.1 Label a clean 16 x 125 mm tube for the matrix blank, positive control, negative control and case samples.

40.7.2 Using a calibrated pipette, add 0.5 mL certified blank testing matrix into the negative control tube and blank matrix tube.

40.7.3 Using a calibrated pipette, add 490 µL certified blank testing matrix into the positive control tube.

40.7.4 Using a calibrated pipette, add 10 µL control working standard to the positive control tube.

40.7.5 Using a calibrated pipette, sample 0.5 mL of each case sample into its respective tube.

40.7.6 Add 4 mL Trinder's reagent to each tube, cap and vortex mix.

40.7.7 Centrifuge the tubes for 5 minutes at 2000 rpm.

40.7.8 Evaluate and document any color change (or lack of color change) on the worklist.

40.7.9 A second analyst evaluates the tubes for color change (or lack of color change) and indicates agreement with the testing analyst's original observations by signing/dating the worklist as the reviewer.

40.7.10 Discard of all waste in appropriate chemical waste container for proper disposal.

40.8 CRITERIA FOR BATCH ACCEPTANCE

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

40.8.1 Blank

40.8.1.1 No color change should be observed in the matrix blank.

40.8.2 Controls

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

40.8.2.2 There should be a distinct color change (violet) observed in the positive control.

40.9 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.

40.9.1 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 violet color in the supernatant constitutes a presumptive positive result for salicylates.

40.10 REPORTING

40.10.1 Qualitative Reporting

40.10.1.1 Results of this presumptive test may be reported as either negative or presumptive positive, as appropriate, using this colorimetric assay. If a specimen is presumptive positive from this test, it will be determined whether confirmation/quantitation is warranted, as indicated by case circumstances. As necessary, a separate sampling of the specimen will be sent to an external reference laboratory for confirmation/quantitation.

40.10.2 Reporting Examples

a. If result from this color test 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 this color test 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 this color test 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.

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

40.11 TRACEABILITY

40.11.1 Traceability of reference materials is provided through the certificate of analysis provided by the approved reference material supplier.

40.12 REFERENCES

40.12.1 Porter, W.H., Moyer, T.P.: Clinical Toxicology (Salicylate). *In*: Tietz Fundamentals of Clinical Chemistry, 4th ed., C.A. Burtis, E.R. Ashwood, Eds., W.B. Saunders, Philadelphia, 1996, pp. 440-442.

40.12.2 A Rapid and Simple Color Test for Detection of Salicylate in Whole Hemolyzed Blood. Asselin, W.M.; Caughlin, J.D. *J. Anal. Toxicol.* **1990**, 14, 254-255.

40.12.3 Widdop, B.: Hospital Toxicology and Drug Abuse Screening (Salicylic Acid). *In*: Clarke's Isolation and Identification of Drugs, 2nd ed., A.C, Moffat, J.V. Jackson, M.S. Moss, B. Widdop, Eds., The Pharmaceutical Press, London, 1986, p. 26.

40.12.4 Baselt, R.C.; Disposition of Toxic Drugs and Chemicals in Man, 6th ed., Biomedical Publications, Foster City, CA, 17-20.

LIST OF CHANGES

Revision Date	Description	Page Number
10/4/16	Method approved by Washington State Toxicologist. See DRA dated 9/9/16. Method released for use in evidentiary testing 10/4/16.	All
10/1/17	Removed wording regarding dilutions in 40.4 and added "control" to working standard description in 40.6.1 and 40.7.4. Edited 40.6.3 and 40.7.2 and 40.7.3 for use of any matrix. Added NOTE in 40.6.3.2 indicating that visual color tests are not required to meet batch control criteria (10% of number of specimens). Specified the use of calibrated pipettes for measurement of blank blood, specimens, and standards in section 40.7 SAMPLE PREPARATION. Added evaluation of results by a second analyst documenting review/agreement on the worklist. Added 40.11 for traceability. Other minor edits throughout.	1-5