SOP 100 - Salicylate Colorimetric Screen

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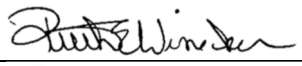
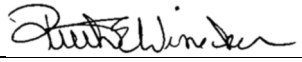
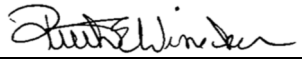
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**

Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/08/2015
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		08/29/2017

SOP 100 - Salicylate Colorimetric Screen

1. **Principle of Assay**

   1.1. This method is designed to detect the presence of salicylate in blood, urine, or tissue specimens by way of a colorimetric test. The specimens are prepared with Trinders reagent and a purple color indicates a positive result.

   1.2. The salicylates are a group of compounds used for the purposes of analgesia, fever reduction, and decreasing inflammation. In the blood acetyl salicylic acid (ASA) is rapidly hydrolyzed to salicylic acid (SA). Salicylate has a half-life that is dose-dependent and variable, ranging from 2-3 hours to 20-30 hours. In overdose, the half-life is greatly increased because the metabolic acidosis causes both a decrease in both blood and urine pH which reduces salicylate elimination.

2. **Specimens**

   2.1. Blood, vitreous, serum, Plasma, urine, bile, gastric contents, or tissue homogenate - volume varies depending upon the specimen availability, typically 1 mL of blood, urine, vitreous, bile, or gastric contents, or 1 g of tissue homogenate (1:4 dilution).

Specimen	Aliquot Volume
Blood	1.0 mL
Vitreous Humor	1.0 mL
Serum	1.0 mL
Plasma	1.0 mL
Urine/Bile/Gastric	1.0 mL
Tissue homogenate	1.0 g

3. **Reagents and Materials**

   3.1. Mercuric chloride

   3.2. Deionized water

   3.3. Ferric nitrate

   3.4. Salicylate standard (1000 mg/dL)

   3.5. Blood bank blood (drug-free).

   3.6. Hydrochloric Acid

4. **Standards, Controls, and Solutions**

4.1. Salicylate Standard (1000mg/dL)

4.1.1. Weigh 117 mg of sodium salicylate using an analytical balance.

4.1.2. Place in a 10 mL volumetric flask and dilute to the mark with deionized water. Mix Well

4.2. 1N Hydrochloric Acid

4.2.1. In a 250 mL volumetric flask, place about 100 mL of deionized water.

4.2.2. Slowly add 21 mL of concentrated hydrochloric acid and gently vortex.

4.2.3. Dilute to the mark with deionized water.

4.2.4. Invert several times to mix.

4.3. Trinders reagent

4.3.1. In a 2000 mL Erlenmeyer flask with a stir bar, place 40 g of mercuric chloride, and 850 mL of deionized water.

4.3.2. Heat and stir until warm to the touch.

4.3.3. Cool and add 120 mL of 1 N HCl and 40 g ferric nitrate.

4.3.4. Pour into a 1000 mL volumetric flask and dilute to the mark with deionized water. Mix well.

5. **Equipment and Special Supplies**

5.1. 5 mL Disposable Centrifuge Tubes

5.2. Vortex mixer

5.3. Centrifuge

5.4. Micro Pipette, air or positive displacement 10-100 µL, calibrated

5.5. Micro Pipette, air or displacement 100-1000 µL, calibrated

5.6. Macro Pipette, air displacement 1-5mL

6. **Procedure**

6.1. Label four 5mL Disposable Centrifuge Tubes “20”, “50”, “blank”, and “QC Std”. Do the same for all assigned specimens. Pipette 1mL of blank blood into the four control tubes.

6.2. Add 20uL of 1000mg/dL salicylate standard to tube labeled “20”.
Add 50uL of 1000mg/dL salicylate standard to tube labeled “50”.
Add 20uL of 1000mg/dL salicylate QC standard to tube labeled “QC Std”.

6.3. Pipette 1mL of specimen (unless otherwise directed upon assignment) into appropriately labeled 5mL Disposable Centrifuge Tubes.

6.4. Add 5mL Trinders Reagent to all standards and specimens, cap, vigorously shake for 30 seconds, and centrifuge at 2000 x g for 10 minutes.

6.5. Remove from centrifuge and observe.

6.6. A positive result is indicated by a purple color change. A negative result is indicated by no change in Trinders Reagent (yellow).

6.7. Have another analyst or toxicologist observe the results and initial the result sheet

7. **Calculations**

7.1. N/A

8. **Quality Control**

8.1. For an analysis to be acceptable the following criteria must be met:

8.1.1. A positive result (purple color change) must be observed in the 20 and 50mg/dL standards as well as the 20mg/dL QC.

8.1.2. A negative result (no color change to the yellow Trinders reagent) must be observed in the negative control.

8.1.3. If the criteria above are not met, the specimens will be re-analyzed.

9. **Load Assignment Packet Preparation**

9.1. After completion of the analysis, the analyst will organize the data in the following order:

9.1.1. The Load Checklist - should be initialed and dated to acknowledge completion of load.

9.1.2. Load assignment sheets, followed by any additional notes to file pertaining to load.

9.1.3. Load specimen sheet.

9.1.4. Chain of Custody.

9.1.5. Aliquot Chain of Custody.

9.1.6. Standard and Control Worksheet.

9.1.7. Results worksheet – a copy for each case in the load and one for the Tox Folder.

10. **References**

10.1. Levine, Barry. *Principles of Forensic Toxicology*. Washington, D.C.: American Association for Clinical Chemistry, 1999. Print.

10.2. Clarke's Analysis of Drugs and Poisons. 3rd Edition. Moffat, Osselton and Widdop eds. Pharmaceutical Press, London. 2004. P. 296.