080 - Methanol/Formaldehyde Detection by Chromotropic Acid Screen

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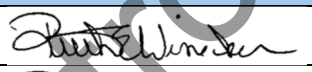
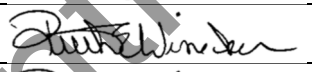
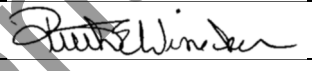
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:** 7.13 – Changed amount of Chromotropic Acid added to reflect practice.	**Revision Date/Initials:** MSF – 09/22/15

Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/08/2015
Ruth E. Winecker, Ph.D.		06/27/2016
Ruth E. Winecker, Ph.D.		08/29/2017

080 - Methanol/Formaldehyde Detection by Chromotropic Acid Screen

1. **Principle of Assay**

    1.1. This method is designed to detect the presence of methanol and formaldehyde in blood, urine, or tissue specimens. The specimens are prepared in duplicate and potassium permanganate is used to oxidize the methanol in one set into formaldehyde. Chromotropic acid and sulfuric acid are added to all specimens to form a reaction product that renders the formaldehyde purple as a positive test.

2. **Specimens**

    2.1. Blood, urine, vitreous, bile, gastric contents, or tissue homogenate - volume varies depending upon the specimen availability, typically **2 mL of specimen** or (2 g of gastric contents or 1:4 dilution tissue homogenate).

3. **Reagents and Materials**

    3.1. Methanol standard (100 mg/dL), prepared fresh daily

    3.2. Deionized water (dH_2O)

    3.3. Trichloroacetic Acid (20% w/v)

    3.4. Potassium permanganate solution

    3.5. Sodium bisulfite

    3.6. Chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid)

    3.7. Concentrated sulfuric acid

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

    4.1. Methanol Standard (100 mg/dL)

        4.1.1. In a 200 mL volumetric flask, place approximately 100 mL of deionized water. Add 0.2 g of methanol (HPLC grade or better). Dilute to the mark with deionized water and mix well. Prepare fresh daily.

    4.2. Trichloroacetic Acid (20% w/v)

        4.2.1. In a 100 mL volumetric flask, place approximately 50 mL of deionized water. Add 20 g of trichloroacetic acid. Dilute to the mark with deionized water and mix well.

4.3. Potassium Permanganate Solution

4.3.1. In a 100 mL volumetric flask, add approximately 50 mL of deionized water. Slowly add 15 mL of 85% phosphoric acid. Dilute to the mark with deionized water and mix well. Add 3 g of potassium permanganate to this solution and mix well.

5. **Equipment and Special Supplies**

5.1. 16 X 125 Culture Tubes

5.2. 5mL Conical Tubes

5.3. Pasteur pipettes

5.4. Small spatula

5.5. Vortex mixer

5.6. Centrifuge

6. **Instrumentation and Parameters**

6.1. N/A

7. **Procedure**

7.1. Label three 16 x 125 Culture Tubes “20”, “50”, and “blank”. Label additional 16 X 125 Culture Tubes with the S#’s of the assigned specimens. Prepare the standards as follows:

7.2. To tube labeled “20”: add 0.4mL 100mg/dL methanol standard + 1.6mL dH_2O

7.3. To tube labeled “50”: add 1.0mL 100mg/dL methanol standard + 1.0mL dH_2O

7.4. To tube labeled “Blank”: add 2.0mL dH_2O

7.5. Pipet 2mL of specimen (unless otherwise directed upon assignment) into appropriately labeled16 x 125 Culture Tubes.

7.6. Add **4 mL of trichloroacetic acid (TCA)**, and vortex for 5 seconds. Centrifuge at 3000 RPM (2000 x g) for 5-10 minutes.

7.7. While specimens are centrifuging, label two 5mL conical tubes for each control and specimen. Designate one set of tubes to be oxidized to

formaldehyde and the other set to be non-oxidized. Separate the tubes accordingly.

7.8. Remove the 16 x 125 Culture Tubes from the centrifuge.

7.9. Pipette 1mL of each control/specimen into the designated oxidized set.

7.10. Pipette 1mL of each control/specimen into the designated non-oxidized set.

7.11. Add 5 drops of potassium permanganate to each of the designated oxidized set. Vortex for 5 seconds and let sit for approximately 2 minutes. This set should turn purple. Note: If the oxidized set is not purple in color, a new batch of potassium permanganate solution should be prepared and analysis repeated.

7.12. Add approximately 10 mg of sodium bisulfite to both sets and vortex. If color remains in the oxidized tubes, add more sodium bisulfite until the liquid turns clear.

7.13. Add approximately 20 mg of Chromotropic acid to both sets and vortex for 2 seconds. The liquid in both sets should turn a slight yellow.

7.14. Slowly underlay the solution in each tube with approximately 2-3 mL of concentrated sulfuric acid using a Pasteur pipet.

7.15. Observe each sample. A purple ring at the interface of the two liquids is a positive test for formaldehyde. Record the results on the assignment sheet.

7.16. Have another analyst or toxicologist observe the results and initial the assignment sheet.

8. **Calculations**

8.1. N/A

9. **Quality Control**

9.1. Results for Formaldehyde

9.1.1. A purple ring in the tubes of the designated non-oxidized set indicates the specimen is positive for formaldehyde.

9.1.2. No reaction (lack of purple ring) in the designated oxidized and non-oxidized sets indicates the specimen is negative for methanol.

Oxidized	Non-oxidized	Result
-	-	Methanol - Negative Formaldehyde - Negative
+	-	Methanol - Positive Formaldehyde - Negative
+	+	Formaldehyde - Positive
-	+	Repeat

9.2. Acceptance/Rejection Criteria of Assay

9.2.1. A positive reaction should be observed with the oxidized set of positive controls. Load failure will occur when no reaction is observed (lack of purple ring) for the oxidized "20" and "50" positive controls, or if a positive reaction is observed in either the oxidized/non-oxidized "Blank" or non-oxidized "20" and "50" controls.

10. **Reporting**

10.1. Specimens positive for Formaldehyde

10.1.1. Report as "Formaldehyde Present"

10.1.2. Report Methanol from Volatiles assay as "Present"

10.2. Specimens negative for Formaldehyde

10.2.1. Report as "Formaldehyde None detected"

10.2.2. Report calculated methanol result from Volatiles assay (repeat at dilution if necessary).

11. **Load Assignment Packet Preparation**

11.1. After completing all data generation and reviewing for corrections, the analyst will assimilate the data in the following order:

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

11.1.2. Load specimen sheet.

11.1.3. Chain of Custody.

11.1.4. Standard and control worksheet.

11.1.5. Verified result sheet (An additional copy of the result sheet should be made for each case assigned).

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

12. **References**

12.1. West, P. W., and B.Sen.1956. Spectrophotometric determination of traces of formaldehyde. Journal of Analytical Chemistry153: 12–18.