SOP 106 - Arsenic Detection by Reinsch Test

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1. Principle

1.1. This method is designed to detect the presence of arsenic in blood, urine, or tissue specimens. The specimens are heated in the presence of strong acid, and the arsenic collects on a copper wire immersed in the sample. A dull deposit on the wire indicates a positive result.

2. Specimens

2.1. Blood, urine, vitreous, bile, gastric contents, or tissue (not tissue homogenate) volume varies depending upon the specimen availability, typically 4 mL of blood, urine, vitreous, or bile, or 1 g of gastric contents or tissue.

3. Reagents and Materials

- 3.1. Concentrated nitric acid
- 3.2. Concentrated hydrochloric acid
- 3.3. Water (HPLC grade)
- 3.4. Coiled copper wire strips
- 3.5. Blood bank blood (drug-free)

4. Standards, Controls, and Solutions

4.1. Arsenic standard (100 µg/mL)

4.2. 2 N Hydrochloric Acid

- 4.2.1. In a 25 mL volumetric flask, place approximately 10 mL of deionized water. Slowly add 4.2 mL of concentrated hydrochloric acid and gently vortex. Dilute to the mark with water. Mix well.
- 4.3. Arsenic Standard (1000 µg/mL)
 - 4.3.1. In a 10 mL volumetric flask, place 133mg of arsenic ³⁺. Add approximately 5mL Di H₂O. Slowly add 1.6 mL of concentrated hydrochloric acid. Dilute to the mark with deionized water. Mix well.

4.4. Arsenic Standard (100 μg/mL)

4.4.1. In a 10 mL volumetric flask, place 1mL of the 1000 μ g/mL arsenic standard. Dilute to the mark with 2 N hydrochloric acid. Mix well.

5. Equipment and Special Supplies

- 5.1. Hot plate
- 5.2. Water bath

6. **Procedure**

6.2.1.1.

- 6.1. Label a 16x125mm screw-top test tube for QC, Blank, and each specimen.
- 6.2. Pipet 1g of blank tissue homogenate or 4mL of blank blood or urine (matrix match specimens) into the tubes labeled "QC " and "Blank".
 - 6.2.1. Multiple QCs and Blanks may be needed if multiple matricies are represented in the case specimens.

Specimen Matrix	Associated QC Matrix	~?
Blood		
Vitreous Humor	4mL	
Urine	Blood	
Bile		
Gastric Contents	1 gram	
Tissue homogenate	Tissue Homogenate	

- 6.3. Case Specimens: Weigh 1g tissue (shaved) or gastric, or 4mL of blood, urine, vitreous, or bile into a 16X125mm screw-top test tube labeled with the case number.
 - 6.3.1. Note: Tissue must be cut into small pieces to maximize surface area. Analyzing large "chunks" of tissue may produce a false negative result.
- 6.4. To the sample labeled "QC", add 40μ L of the 100 µg/mL arsenic standard. The final concentration will be 4 µg/g (tissue/gastric) or 1ug/mL (blood, urine, vitreous, or bile).
- 6.5. Add 3mL DiH2O to each tube containing tissue or gastric, **do not add water to blood**, **urine**, **vitreous**, **or bile final volume should be** ~4mL.
- 6.6. Add 0.8 mL of concentrated hydrochloric acid to all samples and vortex for 5 seconds.
- 6.7. Cut one 3-inch length of copper wire for each test tube.
- 6.8. Clean the copper wires by immersing them in concentrated nitric acid for approximately 5 seconds. Rinse well with deionized water.
- 6.9. Place a clean copper wire into each test tube and screw on cap.

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- 6.10. Add approximately 300 mL of hot tap water into a 500mL beaker.
- 6.11. Place test tubes into beaker and place the beaker on a hot plate set to 300-400 °C.
- 6.12. Bring water bath to a boil and allow specimens to incubate for 1 hour.
- 6.13. At the end of incubation, turn off the hot plate and allow the specimens to cool for 15 to 30 minutes.
- 6.14. Label a bench sheet or white paper towel with specimen info leaving enough space for the corresponding copper wire.
- 6.15. Remove the copper wire from each tube, rinse with DiH2O, and place on the bench sheet under the corresponding label (Figure 1).
 - + Ctrl Black
 - 6.15.1. Figure 1: Arrows point to submerged end of copper wire.

6.16. Examine the wires carefully. A dull deposit on the submerged portion of the copper wire indicates a positive result. Record the results on the assignment sheet.

7. Quality Control

- 7.1. For an analysis to be accepted, the following criteria must be met:
 - 7.1.1. A positive result (a dull discoloration of the submerged copper wire) must be observed in the positive (4 ug/mL) control.

- 7.1.2. A negative result (no discoloration of the submerged copper wire) must be observed in the negative control.
- 7.1.3. If the criteria above are not met, the specimens will be re-analyzed.

8. Validation

8.1. See: <u>SOP 106V - Reinsch Validation Summary 102616</u> loacated in <u>S:\toxicology\QAQC\SOP</u>.

9. Reporting

- 9.1. Specimens positive for arsenic:
 - 9.1.1. In Toxlog, report as "Arsenic Present".
 - 9.1.2. Include the result suffix "Reinsch".
- 9.2. Specimens negative for arsenic:
 - 9.2.1. In Toxlog, report as "Arsenic None Detected".
 - 9.2.2. Include the result suffix "Reinsch"

10. Load Assignment Packet Preparation

- 10.1. After completing result sheet and reviewing for corrections, the analyst will assimilate the data in the following order:
 - 10.1.1. The Load Checklist should be initialed and dated to acknowledge completion of load.
 - 10.1.2. Load assignment sheets, followed by any additional notes to file pertaining to load.
 - 10.1.3. Chain of custody.
 - 10.1.4. Aliquot chain of custody
 - 10.1.5. Standard and control worksheet.
 - 10.1.6. Verified result sheet (An additional copy of the result sheet should be made for each case assigned).

11. References

- 11.1. Levine, Barry. Principles of Forensic Toxicology. Washington, D.C.: American Association for Clinical Chemistry, 1999. Print.
- 11.2. Baselt, Randall C. Disposition of Toxic Drugs and Chemicals in Man. 9th Edition. Foster City, CA: Chemical Toxicology Institute, 2011. 112-116. Print.
- 11.3. Clarke's Analysis of Drugs and Poisons. 3rd Edition. Volume 1. Moffat, Osselton and Widdop eds. Pharmaceutical Press, London. 2004. P. 261.

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