SOP-074 – Determination of Carbon Monoxide by Palladium Chloride

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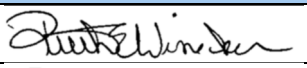
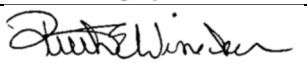
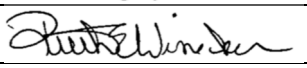
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**
Approving Authority Name	**Approving Authority Signature**	**Approval Date**
Ruth E. Winecker, Ph.D.		04/08/20158
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		12/06/2017

1. **Introduction and Principle of Assay**

1.1. Carbon monoxide binds to hemoglobin forming carboxyhemoglobin which prevents the binding of oxygen. In deaths associated with fire, inhalation of car exhaust or situations involving a faulty heater, carboxyhemoglobin concentrations may exceed 50% SAT. Deaths may occur at lower concentrations in the young, the elderly or in individuals with significant natural disease (e.g. COPD, heart disease).

1.2. This procedure describes the method for detecting the presence of carbon monoxide in blood specimens that had been analyzed by the UV-VIS Spectrophotometer and were deemed unsuitable for such analysis. The presence of a silvery film, due to the reduction of palladium ion to metallic palladium, in the inner well of the Conway dish indicates a positive test for carbon monoxide.

2. **Specimens**

2.1. This procedure is applicable to whole or hemolyzed venous, arterial or capillary blood. However, in the case of a fire, it may be necessary to squeeze blood out of the spleen in order to obtain a sample. Acceptable anticoagulants are EDTA and heparin. A minimum sample size is 500 µL.

3. **Reagents and Materials**

3.1. Palladium Chloride Solution

3.2. 10% Acetic Acid/Saturated with Lead Acetate

3.3. Deionized Water

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

4.1. Palladium Chloride Solution

4.1.1. In a 250mL flask, dissolve 0.22 grams of palladium chloride with 250mL 0.01 N Hydrochloric acid. Mix well. Heat if necessary to ensure good homogenous solution.

4.2. Level 1 ~ 60% saturated (Instrumentation Laboratories Kit)

4.3. Level 2 ~ 3% saturated (Instrumentation Laboratories Kit)

4.4. Level 3 ~ 23% saturated (Instrumentation Laboratories Kit)

4.5. 10% Acetic Acid Solution with Saturated Lead Acetate

4.5.1. In a graduated cylinder measure 90mL of water and add 10mL of acetic acid.

4.5.2. Pour this solution into a 250mL volumetric flask and saturate the solution with Lead Acetate.

5. **Equipment and Special Supplies**

5.1. Conway Diffusion Dishes with Covers

5.2. Disposable glass culture tubes (12 X 75mm)

5.3. Pasteur Pipettes

5.4. Wooden sticks

6. **Instrumentation and Parameters**

6.1. N/A

7. **Procedure**

7.1. Place Conway dishes on lab bench and label with tape or place on bench paper and label area below each with:

7.1.1. Level 1 ~ 60%

7.1.2. Level 2 ~ 3%

7.1.3. Level 3 ~ 23%

7.1.4. S# (Cases)

7.2. Obtain the CO standards from refrigerator 2601 – R1 (Additional boxes of standards may be found in 2603 – R2). Open and transfer the standards (using disposable glass Pasteur pipettes) into labeled disposable glass culture tubes (12 X 75mm).

7.3. If blood sample is coagulated or dried, weigh a 1 g aliquot of the blood in a 16x125 test tube and add 0.5mL of DI water and vortex for about 2 minutes.

7.4. Obtain the Palladium Chloride and Lead Acetate Solutions (Lab 2601).

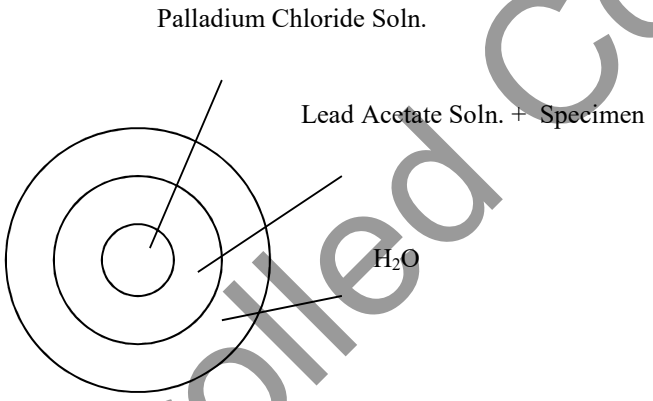
7.5. Pipette Palladium Chloride solution into the center well until full (about 0.75mL).
7.6. Add 0.5mL of the Lead Acetate solution into the 2nd ring using a micropipetter.

7.7. Add water drop wise around the outer ring using a Pasteur pipette (to make a seal).

7.8. Get out wooden sticks for mixing.

7.9. Pipette 0.5mL of each specimen into the middle 2nd ring with the lead acetate solution. Use the wooden sticks to gently mix the specimen and the lead acetate solution (see Figure 1).

7.10.



Conway Diffusion Dish

7.11. Figure 1

7.12. Cover each dish with a plastic cover, moving cover around to ensure a good seal. The seal is sufficient if there is a little tension on the cover when you try to pull it off. If there is no tension present, then move the cover around, add a little more water, and try again. Repeat until you have a good seal.

7.13. Let sit for 1.5-2 hours.

7.14. When it is time to make observations, remove plastic cover.

7.14.1. **Positive:** A very slick silvery metallic film over the inner well where the palladium was poured should be present.

7.14.2. **Negative:** There is no slick silvery metallic film over the inner well where the palladium was poured.

7.14.3. **Slightly Positive:** There is a slight silvery film over the inner well where the palladium was poured but it doesn't cover the well completely.

7.15. Ask a fellow analyst to sign as the observer.

8. **Calculations**

8.1. N/A

9. **Quality Control**

9.1. The quality control standards must be observed with the following results ordered most positive to least positive:

9.1.1.1. Level 1 – positive to very positive

9.1.1.2. Level 3 – slightly positive to positive

9.1.1.3. Level 2 – negative to slightly positive

9.1.1.3.1. An observed negative result from Level 1 or 3 or an inconsistent reaction level (e.g. Level II shows a stronger reaction than Level III) will result in a load failure and all specimens will be reanalyzed.

10. **Reporting**

10.1. Carbon monoxide – Less than 5% SAT

10.1.1. Negative to slightly positive color change observed ($\leq$ Level 2).

10.1.2. Consistent with result from instrumental analysis.

10.2. Carbon monoxide - (Approximately) ### % SAT

10.2.1. Slightly positive to positive color change observed

10.2.2. Report the result (if consistent) from instrumental analysis (or average if multiple results generated).

10.2.3. Include appropriate result comment.

10.2.3.1. E.g. Specimen matrix was unsuitable for exact measurement of carbon monoxide.

10.3. Carbon monoxide – Greater than 60% SAT

10.3.1. Positive to very positive color change observed.

10.3.2. Consistent with result from instrumental analysis.

10.4. Carbon monoxide – Specimen unsuitable

10.4.1. Color test results inconsistent with instrumental results.

10.4.2. Specimen matrix contains too little hemoglobin for analysis.

10.4.3. Include appropriate result comment.

10.4.3.1. E.g. Specimen was unsuitable for analysis due to low percentage of hemoglobin.

11. Preparation of Load

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 (A 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. Trinder and Harper. A colorimetric method for the determination of carboxyhaemoglobin over a wide range of concentrations. J. Clin. Path. 15:82-84. 1962.