FORENSIC TOXICOLOGY LABORATORY
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CARBON MONOXIDE
by
Microdiffusion (qualitative)

PRINCIPLE

The method of microdiffusion analysis, the apparatus and many of the procedures were devised by Conway over the period of 1933-1944. The general principal of microdiffusion analysis concerns the absorption by simple gaseous diffusion of a volatile substance, from the outer chamber of the Conway microdiffusion cell where it exerts a certain tension, into an absorbing solution in the inner chamber where its tension is zero at the surface of the absorbing liquid. Feldstein and Klendshoj have applied the Conway microdiffusion cell to determination of volatile compounds encountered in toxicologic analysis. Qualitative and semi-quantitative determination can be carried out in 30 minutes to 2 hours.

Carbon monoxide is released from carboxyhemoglobin by adding acid to the blood sample in the outer chamber of the Conway microdiffusion cell. The released carbon monoxide diffuses into the inner chamber of the Conway microdiffusion cell and reduces the palladium chloride to metallic palladium.

$$PdCl_2 + CO + H_2O = Pd + 2HCl + CO_2$$

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

APPARATUS

Modified Conway microdiffusion cell

Serologic pipettes capable of accurately pipetting 1.0, 2.0 and 3.0 mL

REAGENTS

All chemicals should be analytical reagent grade or better.

1. Hydrochloric acid, 0.1N
   Dilute 8.3 mL of concentrated hydrochloric acid to one liter with water.
2. Palladium chloride, 0.005N

Dissolve 0.44 g of palladium chloride in 500 mL of 0.1N hydrochloric acid in a one-liter flask. Mix the solution, allow it to stand overnight, then dilute it to one liter with 0.1N hydrochloric acid.

3. Lead acetate-acetic acid solution
   Dilute 10.0 mL of glacial acetic acid to 100 mL with water. Saturate this solution with lead acetate.

PROCEDURE

1. Label covers for Conway cells with case numbers.
2. Include IL MULTI-4 CO-Oximeter Controls (Level I, II, III).
3. Pipet water all around the outside well of the Conway cell. The purpose is to form a water seal for the cover and create a closed system for the ensuing reaction.
4. Pipet 3.0 mL of palladium chloride into the center well of each Conway cell.
5. Pipet 2.0 mL of lead acetate into one side of each middle well.
6. Pipet 1.0 mL of blood sample into the other half of each middle well.
7. Cover each Conway cell with the corresponding top as soon as pipetting is completed.
8. Mix the contents of the middle well with gentle rotation of the Conway cell.
9. Allow the cell to stand for approximately one (1) hour at room temperature. Evaluate the change of color in the center well.

ACCEPTANCE CRITERIA

1. Only samples which have been tested along with successful controls may be reported.
2. Negative and positive controls must give expected results.

INTERPRETATION

A qualitative or semiquantitative estimate of carboxyhemoglobin can be made by observing the extent of the reduction that has occurred. The silver film of metallic palladium that forms is a function of the amount of carbon monoxide released from the specimen. Limit of detection (LOD) is 3 - 5% saturation.

REPORTING

Readings of less than 5% COHb are reported as “less than 5% saturation”.

Readings of 5% COHb or higher are reported as “detected”.

All blood specimens with COHb results reported as detected are scheduled for quantitative analysis by CO-Oximeter.

INTERFERING SUBSTANCES

Sulfides can interfere if present in large quantities, as found in putrefied blood. This interference, however, can be eliminated by substitution of lead acetate-acetic acid solution for 3.6N sulfuric acid as the liberating solution.

REFERENCES

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