FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK

CARBON MONOXIDE
by
AVOXimeter 4000® CO-Oximeter

PRINCIPLE

Carbon monoxide (CO) is a colorless, odorless and tasteless gas produced by incomplete combustion. Many clinical and forensic toxicology laboratories use a CO-Oximeter for analysis of whole blood for the presence of total hemoglobin (tHb), % carboxyhemoglobin, % oxyhemoglobin, % methemoglobin and % reduced hemoglobin. Whole blood or postmortem blood, aliquoted through a small diameter pipette to avoid clots, is introduced into instrument specific cuvette which is placed into the test chamber of an AVOXimeter® 4000 CO-Oximeter. Light passes through the cuvette to a photodetector which measures five separate wavelengths. The instrument measures oxygenated hemoblogin (O_2Hb), carboxyhemoglobin (COHb), methemoglobin (mHb) and reduced hemoglobin (HhB) directly which are reported as %. Total hemoglobin (tHB) and $\%O_2Hb$ are calculated. Carboxyhemoglobin is stable in a refrigerated blood sample for many months. Other hemoglobins (O_2Hb, MetHb) are not stable.

SAFETY

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

INSTRUMENTATION

AVOXimeter® 4000 Whole Blood CO-Oximeter

DYMO SE300 Label Writer thermal printer

REAGENTS AND MATERIALS

1. RNA QC 253 bovine based CO-Oximeter Control Solutions Levels 1, 2 and 3 (RNA Medical). Individual controls or a combination control set are supplied as a package of 30 x 1.2mL ampoules. Store refrigerated. Three levels of COHb saturation* are used:

   Example:

Level 1	(2.8% - 10.8% COHb)
Level 2	(14.6% - 22.6% COHb)
Level 3	(51.5% - 59.5% COHb)

*Refer to manufacturer's insert for current ranges

Validate controls with new lot numbers against previously validated controls before use

2. Pasteur pipettes, 5 ¾' or 9"
3. 12 x 75 mm glass test tubes (or equivalent)
4. Disposable 1 mL plastic syringe without needle (or equivalent)
5. AVOXimeter® 4000 instrument specific single use cuvettes

PROCEDURE

Validation of Instrument (Each Day of Use)

The AVOXimeter® 4000 is checked on each day of use to verify that it is valid to use.

1. Press the red "Enter/On" key and wait for the lamp to warm up, about 20 min.
2. Check the LCD panel on the face of the instrument. The default screen is:

   ----Ready----

   Insert Cuvette

   Cal Code 28439*

   (*subject to change with a new lot number of cuvettes)

   Note: Refer to the AVOXimeter® 4000 Operator's Manual for instructions on changing the cuvette Cal Code and Lot No., or Calibration Level Lot Nos

3. Remove either the yellow or orange Optical QC filter from its holder. Insert into the test chamber.

   Note: The instrument performs the analysis first and then requires identification of the sample analyzed.

4. When analysis is complete, answer the following data entry queries:
   a. EnterUserID: no ID is currently necessary. Select OK
   b. Select OK again to verify that no user ID is necessary
   c. Select QC for Sample type
   d. Select Optical for QC type

e. Select optical filter color analyzed.

   Results displayed on the LCD screen will print.

5. Repeat from steps 3 and 4 for the other optical filter.
6. Review results for acceptability. The expected mean and range are noted on each filter and also on the report sheet. Acceptable results are those within the listed ranges., Document the results on the report sheet. If unacceptable, discontinue further analyses and consult a supervisor.

Analysis of Liquid Quality Control Samples

1. Remove liquid quality control materials (Level 1, Level 2 and Level 3) from the refrigerator and analyze as soon as possible.
2. Break ampoules and transfer contents into an appropriately labeled 12 x 75 mm test tube. AVOID BUBBLES

   Note: Use hand protection when breaking control ampoules

3. Remove an analysis cuvette from the ziplocked storage bag. Look through the clear sides of the cuvette to check for large scratches. Reject if found. Remove dust or lint from an acceptable cuvette by wiping gently with a tissue as needed. There are two vents (black circles) at the bottom of the cuvette. Orient the cuvette such that the vent which is duller and more raised is to the left.

   Note: The cuvettes need to be dry. There is a desiccant pack in the ziplock bag with indicator (three blue spots). If any of the indicator spots is changing to pink, replace the desiccant pack with a regenerated one. Regenerate the desiccant pack by heating in an oven until all spots are blue.

4. Unwrap a clean syringe and remove the cap from the tip.
5. Aspirate a small amount of liquid control into the syringe.

   Note; the cuvette only requires 50 micro liters of sample

6. Carefully introduce liquid quality control into the cuvette until it is full. Reject any cuvette and sample with bubbles in the bottom ½ of the cuvette.

   Note: Do NOT apply significant pressure when filling the cuvette. Too much pressure will cause blood to flow through the vents in the bottom of the cuvette which could soil and disrupt the optics of the instrument.

7. **Leave the syringe attached to the cuvette** and place the cuvette in the test chamber.
8. When the analysis finishes, answer the queries on the user ID screens as follows:
   a. Select Liquid for QC type

b. Select the appropriate control level.

c Verify that the lot number on the screen matches the lot number on the selected QC level. Select OK to confirm, or Enter New Value to change.

d. Verify that the cuvette lot number on the screen is correct. Select OK to confirm, or Enter New Value to change

e. Verify that displayed lot numbers are correct and confirm by selecting OK.

d Results displayed on the screen will print. Document the CO results on the worksheet and review for acceptability.

9. Press Enter (or any key) to return to the default screen
10. Repeat steps 3 through 7 for the next two control levels.
11. Acceptable controls will fall within the stated ranges of the lot numbers currently in use. If any control level fails, repeat it. If it fails twice, stop and consult a supervisor.

> ***Note:*** *Though not optimal, out of date controls may be used if they meet acceptance criteria.*

12. If all control levels meet criteria, proceed to analyze specimens

Analysis of Unknown Blood Samples

1. **Using a 5" or 9 ¾" Pasteur pipette,** transfer a small aliquot of blood (approx. 0.5 to 1 mL) to a 12 x 75 mm glass test tube

> ***Note:*** *a thin nosed Pasteur pipette is used to eliminate clots from the blood.*

2. To analyze the unknown, perform steps 4 through 7 in the Analysis of Liquid Quality Control Samples section.
3. When the analysis is complete, select Patient ID and enter the case number.

> ***Note:*** *Only digits are available on the touch pad. Twelve digits can be used. Enter both the year and case number for the sample. If further information is needed (e.g. femoral, repeat, dilution) WRITE this information on the printout!*

Format: YrToxnum Example: 120155

The number entered will display. Verify that this is the correct number and confirm by selecting OK Results displayed will print.

Make any necessary notations on the printout and record the result on the manual worksheet.

4. If two bloods from a case are analyzed, annotate the blood type on the printout and the worksheet. Similarly, if a repeat analysis is necessary, write repeat on the printout and worksheet. Record any other useful information on the printout. For additional space press the paper feed button at the base of the printer
5. Press Enter to return to the default screen.

Error Messages

1. If a sample does not meet internally preset criteria on the instrument, the LCD on the instrument will display one or more error messages* and **no results will print.** Check the error messages by pressing Enter until no new messages appear.

   *tHB <4 or > 25; %O_2Hb <-10.0%; %CO <-7.0; %MetHb >107.o%;%HHb>107%; % Scat <-15%

2. When the ONLY error message is "tHb (total hemoglobin) >25%", the sample can be diluted and re-analyzed. Note the original result on the report sheet and dilute the original aliquot 1:2 with deionized water in a new 12 x 75 mm glass test tube. Re-analyze. If tHb is still high analyze a higher dilution. If acceptable results are returned, proceed normally to complete the analysis. Annotate the printout and the worksheet with the dilution(s).
3. When error messages preclude printing do the following:

   a. Write E (for Error) on the report sheet for that sample (tHb excepted as above).

   b. Determine the result of the Conway analysis. If negative, stop. If positive, notify a supervisor to schedule an alternative spectrophotometric method.

4. When analyses are completed, remember to seal the cuvette bag tightly.
5. Turn off the instrument. Press Menu key, select Turn Off and press Enter.

ACCEPTANCE CRITERIA

1. All optic controls must fall within the ranges noted on the filter.
2. All liquid controls must fall within the expected ranges for the lot numbers currently in use.

REPORTING

1. Results less than 5% COHb are reported as "carbon monoxide less than 5% saturation".
2. Results equal to 5% COHb or greater are reported as "carbon monoxide *X* % saturation".
3. If two blood specimen types are analyzed on one case, report them separately. Do not average the two readings.

INTERFERING SUBSTANCES

Bilirubin and hemolysis have a less than 1% affect on %COHb.

REFERENCE

AVOXimeter® 4000 Whole Blood CO-Oximeter Operator's Manual, International Technidyne Corp. Edison, NJ, 2007.