CONFIRMATION OF CARBOXYHEMOGLOBIN IN BLOOD BY CO-OXIMETRY

11.1 METHOD

This test method may be used to measure the percentage of carboxyhemoglobin (COHb) in whole blood specimens. Exposure to carbon monoxide is determined through the analysis of the hemoglobin species, carboxyhemoglobin (COHb). Multi-wavelength analysis of whole blood samples is employed for the measurement of oxygenated hemoglobin (O2Hb), reduced hemoglobin (HHb), methemoglobin (MetHb) and COHb. Total hemoglobin (tHb), percent oxyhemoglobin (%O2Hb) and oxygen content (O2Ct) can then be calculated.

Testing is conducted with single use cuvettes inserted into the AVOXimeter® 4000 spectrophotometric analyzer.

11.2 SPECIMENS

The specimen volume is 0.3 mL whole blood for each analysis; specimens with COHb results ≥5% saturation from initial testing will have results confirmed in a second batch, from a fresh sampling of the specimen.

The preferred specimen containers are those containing EDTA or heparin (e.g., purple or green top tubes). The instrument manufacturer does not recommend gray top tubes for collection of sample specimens, as the accuracy of COHb results from testing performed on specimens collected in gray top tubes may be affected (see 11.9.7).

11.3 REAGENTS, MATERIALS AND EQUIPMENT

11.3.1 REAGENTS

- Blank blood (no sodium fluoride or potassium oxalate added)
- Deionized water (DI H_2O), laboratory general-use

11.3.2 MATERIALS

- Disposable 12 x 75 mm tubes or plastic sample cups
- Disposable pipette tips
- Plastic Luer taper syringes, 1 mL (Becton Dickinson, BD)
- Quality control samples – RNA Medical® Brand QC 253 Full Range CO-Oximeter control; Level 1 (84858), Level 2 (84954) and Level 3 (85050) or Multi-Level (848840), store refrigerated
- Transfer pipettes (glass or polypropylene)

11.3.3 EQUIPMENT

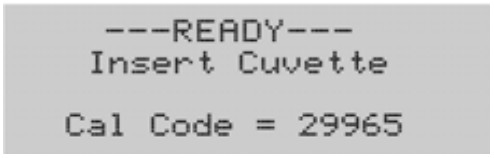
- AVOXimeter® 4000 Whole Blood Oximeter (International Technidyne Corporation, ITC)
- AVOXimeter® 4000 cuvettes (ITC)
- Calibrated, adjustable piston pipettes
- Optical filters (yellow & orange, supplied with the AVOXimeter® 4000)
- Vortex mixer

11.4 QUALITY CONTROL

The optical quality control procedures (yellow and orange optical filter) shall be performed on each day case specimens are analyzed, prior to the batch. The negative control, and Level 1, Level 2 and Level 3 positive quality control samples, must be run with each test batch (initial batch and confirmation).

11.4.1 OPTICAL QUALITY CONTROLS

11.4.1.1 After turning on the instrument, allow the instrument and QC samples to equilibrate for approximately five minutes. Verify that the instrument is ready to run a test and that the following is displayed on the screen.



11.4.1.2 Verify the cal code corresponds to the cuvettes in use. If it does not, follow the instructions for entering a cuvette calibration code as described in the *AVOXimeter® 4000 Operator's Manual*.

11.4.1.3 Insert the yellow optical filter into the test chamber.

11.4.1.4 Respond to the on-screen prompts designating this as sample type "QC", QC type "Optical" and Filter "Yellow." Press the "Enter/On" button to accept.

11.4.1.5 After the results have been printed, remove the optical filter. Press the "Enter/On" button to accept.

11.4.1.6 Insert the orange optical filter into the test chamber and repeat 11.4.1.4 and 11.4.1.5 for Filter "Orange."

11.4.1.7 Verify that the results for each filter are within the ranges listed below.

Optical Filter	Expected Range			
	tHb (g/dL)	$\%O_2Hb$	%COHb	%MetHb
Yellow	7.8 to 8.2	93.7 to 96.3	0.6 to 3.4	-0.4 to 2.4
Orange	16.7 to 17.3	37.8 to 40.2	20.0 to 23.0	0.2 to 1.8

11.4.1.8 If results are outside of the expected range for the optical filters, repeat the process. If results are still outside of the expected range, consult the troubleshooting section of the *AVOXimeter® 4000 Operator's Manual*.

11.4.2 QUALITY CONTROL SAMPLES

The RNA Medical® Brand QC 253 Full Range CO-Oximeter Control, provided in three levels, is used for positive quality control within each batch. An aliquot of blank blood, specified for use with CO testing (no sodium fluoride or potassium oxalate added) is used as the negative control.

The total number of quality control samples (negative, Levels 1, 2 and 3) must make up at least 10% of the testing batch (number of individual case specimen tests performed).

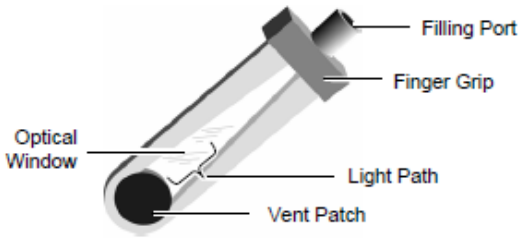
11.4.3 SAMPLE ANALYSIS (QUALITY CONTROL SAMPLES AND SPECIMENS)

The procedure below describes preparation of a quality control sample or specimen for analysis.

11.4.3.1 Verify that the instrument is ready to run a test and there are enough supplies (syringes, cuvettes) for the batch size (see 11.4.1.1).

11.4.3.2 Transfer the contents of the control ampoule, or use a calibrated pipette to measure 0.3 mL case specimen, into a 12 x 75 mm tube or sample cup.

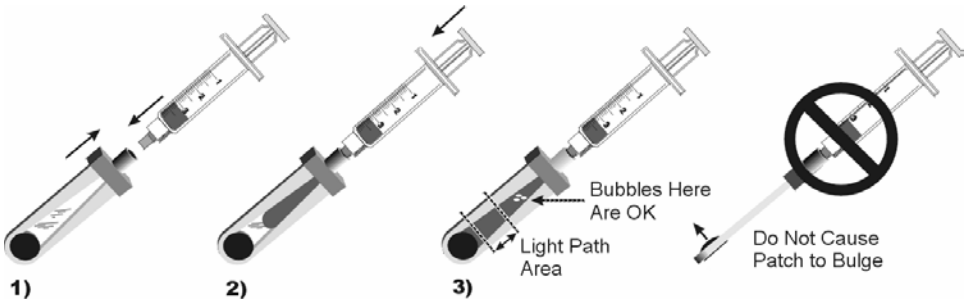
11.4.3.3 Using a new syringe, draw up approximately 0.2 mL of the sample and connect to the filling port of an unused cuvette, holding the cuvette by the finger grip.



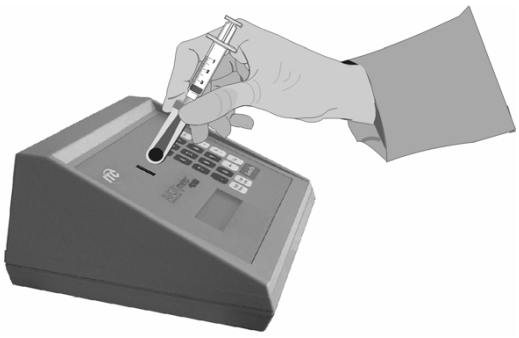
11.4.3.4 While holding the syringe and cuvette at a 45º angle, gently fill the cuvette by pressing the syringe plunger. [NOTE: Do not force the sample into the cuvette. If it does not fill easily, discard the cuvette and use another one.]

11.4.3.5 Stop filling the cuvette when the sample reaches the vent patch. Do not cause the vent patch to bulge.

11.4.3.6 Verify that the light path is free of bubbles and remove any liquid from the exterior of the cuvette. If necessary, wipe the exterior of the cuvette with a Kimwipe to remove any dust or fingerprints.



11.4.3.7 Leave the syringe and cuvette attached and insert the cuvette into the test chamber.



11.4.3.8 Respond to the on-screen prompts, designating the sample as follows:

11.4.3.8.1 Quality controls

- For negative controls, designate as sample type “Patient,” and input the patient ID as “0000.” Press the “Enter/On” button to accept.
- For positive quality controls, designate as “QC,” QC type “Liquid” and Level (Level 1, 2 or 3).

a) Select the lot number for the control and then verify the cuvette lot number or enter a new lot number. Press the "Enter/On" button to accept. [The procedure for entering lot numbers for the quality control samples is described in the *AVOXimeter® 4000 Operator's Manual*.]

11.4.3.8.2 Case specimens

- Designate as sample type "Patient," and input the patient ID as the case ST number. Press the "Enter/On" button to accept.

11.4.3.9 After the results have been printed, remove the cuvette and press the "Enter/On" button. [NOTE: Grasp the finger grip when removing the cuvette.]

11.4.3.10 For quality control samples, positive results shall fall within the expected range (see the manufacturer's *Expected Values Chart* in the package insert) and negative results are < 5% sat.

11.4.3.11 If any control result is outside of its expected range, repeat the process for that control. If a result is still outside of the expected range, obtain a fresh quality control sample for testing or consult the troubleshooting section of the *AVOXimeter® 4000 Operator's Manual*.

11.5 INITIAL BATCH ANALYSIS

11.5.1 When handling case specimens, work with only one specimen at a time, labeling a new 12 x 75 mm tube or sample cup with the respective case number.

11.5.2 Following completion of the optical quality control tests (11.4.1), analyze the negative control, followed by the Level 1 positive control.

11.5.3 Analyze case specimen samples, analyzing the Level 2 quality control midway through the number of case specimens. If only one specimen is being analyzed, the Level 2 quality control may be analyzed prior to the specimen (following Level 1) or after the specimen (preceding Level 3).

11.5.4 Analyze the Level 3 quality control at the end of the batch.

11.5.5 If the initial analysis of a specimen results in an error message (e.g., THb > 25%), dilute a fresh sample of the specimen and re-analyze.

a. Using a calibrated pipette, pipette 0.2 mL of the case specimen and 0.2 mL DI H_2O into a labeled 12 x 75 mm tube. Cap the tube, briefly vortex-mix and analyze.

NOTE: Should error messages appear (e.g., THb < 4.0%) when analyzing a specimen, the instrument will not print a sample report. Note this error on the worklist and in LIMS (see 11.9.6).

11.5.6 If the initial case specimen COHb result is <5% saturation (sat), no further

analysis is necessary.

11.5.7 If the initial COHb result is ≥5% sat, a confirmation analysis of the case specimen is performed.

11.6 CONFIRMATION BATCH ANALYSIS

11.6.1 Perform a second batch analysis for those case specimens with initial results ≥5% sat. The batch consists of a negative control, Level 1 positive control, case specimens, Level 2 positive control, case specimens and Level 3 positive control, as described in 11.5.

NOTE: Confirmation analysis is performed on a fresh (independent) sampling from each case specimen tube.

11.6.2 Verify that case specimen COHb results agree within ±5% (not % sat) of the *mean value*. If values do not agree, re-sampling or analysis of an alternate sample is appropriate.

NOTE: If a specimen is tested in duplicate, with one result >75% sat and another with a value (≤75% sat), perform an additional analysis, on a fresh sampling from the specimen tube, to establish agreement with one of the initial results. Specimens that require a third analysis may be bracketed by the Level 3 control that concludes the second batch and another positive control (Level 1 or Level 2); a full batch, as described in 11.6.1 is not required.

11.6.3 Shut down the instrument after testing has been completed. This can be done by pressing the “Main Menu” button, selecting option “4” and then pressing the “Enter/On” button.

11.7 REPORTS/DOCUMENTATION

11.7.1 Batch documentation includes instrument report printouts for all members of the test batch. For case specimens for which no report was printed (due to errors), this is documented on the worklist.

11.7.2 The analyst performing the test reviews the instrument reports, adding initials/date to each printout and signs and dates the worklist.

11.7.3 *AVOXimeter® 4000* instrument reports print on register tape paper, in black ink that can fade over time. To ensure the information on the reports remains readable, create photocopies of the instrument reports for all members of the test batch. The original register tape reports are also retained.

11.8 BATCH REVIEW

11.8.1 The optical quality control results shall be within their expected range for all values for both optical filters.

11.8.2 All positive quality control samples shall be within the expected range for COHb (a failed control that has been remedied – see 11.4.3.11 is considered acceptable). Negative controls shall have results < 5% sat.

11.8.3 Samples with COHb values of 5% sat or greater must have duplicate results that agree to within ±5% of their ***mean value*** (not within ±5% sat) to be reported.

11.8.4 If an instrument report is not included for a case specimen, any information regarding errors is documented on the worklist.

11.8.5 Batch review is documented by the reviewer adding initials/date to each report printout and signing/dating the worklist.

11.8.6 After review, the worklist and reports for the optical quality controls, negative control and positive quality controls (both original tapes and photocopies) are retained in the case file folder for the first specimen tested in the batch.

11.9 REPORTING

11.9.1 Results are reported in units of percent saturation (% sat).

11.9.2 If the result is negative (or below 5% sat), the result is reported as "<5% sat."

11.9.3 If the initial result is ≥5% sat, but the second result is <5% sat, the results are not averaged, and are reported as <5% sat.

11.9.4 If duplicate results of >75% sat are obtained, the result is reported as ">75% sat."

11.9.5 If duplicate COHb results that read between 5% and 75% sat are obtained (see 11.6.2), a mean is calculated. Each individual result is first truncated to its whole integer value prior to calculating the mean. The calculated mean is then truncated to the whole integer value and reported.

11.9.6 If no results (and no sample report) are obtained due to error messages (e.g., high %THb), performance of the test will be documented in LIMS, as described below:

a. In the confirmation data screen, add carboxyhemoglobin as analyte

b. Under units, select nothing (there is a blank placeholder above % sat)

c. In the report annotations box, add [RPT-T] *Results unable to be obtained for this sample.*

11.9.7 For results reported from testing performed on specimens collected in gray top tubes, the following comment will be included on the test report:

a. In the report annotations box, add [RPT-T] *Gray top blood collection tubes may affect the accuracy of carboxyhemoglobin results. This tube type is not recommended by the manufacturer of the instrument used for this test.*

11.9.8 Other information regarding the testing (e.g., error messages, tube type) may be added to the comments section of the final report, if needed.

11.10 INSTRUMENT SPECIFICATIONS

11.10.1 MEASUREMENT RANGE (COHb): 0 – 75% sat

11.10.2 ACCURACY (COHb): ±2% sat

11.10.3 PRECISION (COHb): ≤1% sat

11.11 REFERENCES

11.11.1 AVOximeter® 4000 Whole Blood Oximeter Operator's Manual, AP4001, October, 2007 (original) or updated revision.

11.11.2 RNA Medical Brand QC 253 Full Range CO-Oximeter Control Expected Values Chart (package insert).

LIST OF CHANGES

Revision Date	Description	Page Number
3/9/12	Method approved by Washington State Toxicologist. See DRA dated 02/29/12. Method released for use in evidentiary testing on 03/09/12.	All
6/19/12	Corrected the syringe description from Luer-lok™ to Luer taper in 11.5.2.5	2
6/1/15	Changed 11.6.2.13 and 11.7.14 to describe that positive COHb controls will bracket case specimens. Edited 11.7.9 and 11.9.7 to describe procedure when no results are obtained/printed. Edited 11.7.10 and 11.7.11 to detail results and reporting for negative samples (results <5% sat). Other minor edits throughout (see DRA dated 5/26/15).	All
4/18/16	Added description of preferred specimen collection containers in 11.4.3. Removed references to sample treatment with sodium hydrosulfite and chemical diluent and replaced use of sample cups with 12 x 75 mm tubes in 11.7. Added procedure for specimen dilution with DIH_2O in 11.7.8 and note in 11.7.11. Added note to describe that 10% of the testing batch must consist of quality control samples in 11.7.12. Edited comment that appears on reports when no result is obtained (11.9.5.c) and added use of comment when results from testing performed on specimens collected in gray top tubes are reported (11.9.6). Other minor edits throughout. See DRA dated 3/23/16.	1-3, 5-8
5/30/19	Removed policy, purpose and principle sections, summarizing under new section METHOD. Specimen volume changed in 11.2, with wording added to specify initial and confirmation testing. Use of negative control (blank blood without preservative and anticoagulant) and three levels of positive QCs, sourced from RNA Medical, specified in procedure. Specified in 11.6 that specimens are initially tested, with positives confirmed in a second complete batch from a fresh sampling from the specimen tube. Added section 11.7 for reports/documentation and added information to 11.8 for review of the batch. Other minor edits throughout.	All