

CONFIRMATION OF CARBOXYHEMOGLOBIN IN BLOOD BY CO-OXIMETRY

11.1 POLICY

This test method may be used to measure the percentage of carboxyhemoglobin (COHb) in whole blood samples. Only quantitative results within the reportable range of this procedure will be reported.

Any adjustments or deviations from the procedures below must be approved by a member of TLD Management, and appropriately documented in the batch file.

11.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the measurement of COHb present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, quality control, batch review and reporting of COHb.

11.3 PRINCIPLE

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 spectrophotometric analyzer. The analyzer is factory-calibrated and re-calibration is only conducted by the factory. Samples only come into contact with the cuvette and results are obtained within approximately 10 seconds.

11.4 SPECIMENS

11.4.1 Although the analysis can be performed using a minimum of 50 µl of sample, 0.5 mL is used as this is a more manageable volume for sample pretreatment.

11.4.2 The preferred specimen is whole blood. Other specimens (ex. spleen & tissue fluid) containing hemoglobin may be analyzed if whole blood is unavailable.

11.5 REAGENTS, MATERIALS AND EQUIPMENT

11.5.1 REAGENTS

11.5.1.1 Sodium Hydrosulfite (Na₂S₂O₄)

11.5.2 MATERIALS

- 11.5.2.1 CO-Oximeter diluent (Instrumentation Laboratories Inc., IL, part # 3311900)
- 11.5.2.2 Disposable 12 x 75mm tubes
- 11.5.2.3 Disposable pipette tips
- 11.5.2.4 Disposable safety closures for 16 x 75mm tubes
- 11.5.2.5 Plastic Luer taper syringes, 1 mL (Beckton Dickinson, BD)
- 11.5.2.6 Plastic sample cups (1 mL)
- 11.5.2.7 Quality control sample - Low (IL Multi-4 CO-Oximeter Control level 3, part # 3313250), store refrigerated
- 11.5.2.8 Quality control sample - High (IL Multi-4 CO-Oximeter Control level 1, part # 3315250), store refrigerated
- 11.5.2.9 Transfer pipettes (glass or polypropylene)

11.5.3 EQUIPMENT

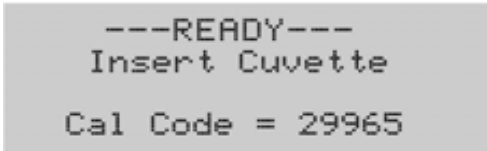
- 11.5.3.1 AVOXimeter® 4000 Whole Blood Oximeter (International Technidyne Corporation, ITC)
- 11.5.3.2 AVOXimeter® 4000 cuvettes (ITC)
- 11.5.3.3 Calibrated, adjustable, piston pipettes
- 11.5.3.4 Optical filters (yellow & orange, supplied with the AVOXimeter® 4000)
- 11.5.3.5 Vortex mixer

11.6 QUALITY CONTROL

The following quality control procedures shall be performed on each day case specimens are analyzed.

11.6.1 OPTICAL QUALITY CONTROLS

- 11.6.1.1 Verify that the instrument is ready to run a test and that the following is displayed on the screen.



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---READY---  
Insert Cuvette  
Cal Code = 29965
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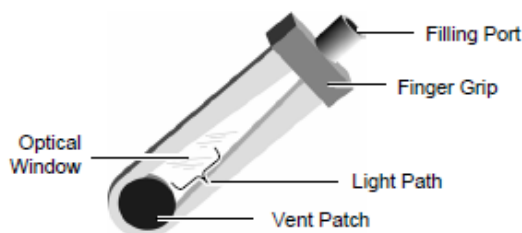
- 11.6.1.2 The cal code should correspond to the cuvettes in use. If it does not, follow the instructions for entering a cuvette calibration code as described in the AVOXimeter® 4000 Operator’s Manual.
- 11.6.1.3 Insert the yellow optical filter into the test chamber.
- 11.6.1.4 Respond to the on-screen prompts designating this as sample type “QC”, QC type “Optical” and Filter “Yellow”.
- 11.6.1.5 After the results have been printed out or recorded, remove the optical filter.
- 11.6.1.6 Repeat the process using the orange optical filter.
- 11.6.1.7 Verify that the results for each filter are within the ranges listed below.

Optical Filter	tHb (g/dL)	Expected Range		
		%O ₂ Hb	%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

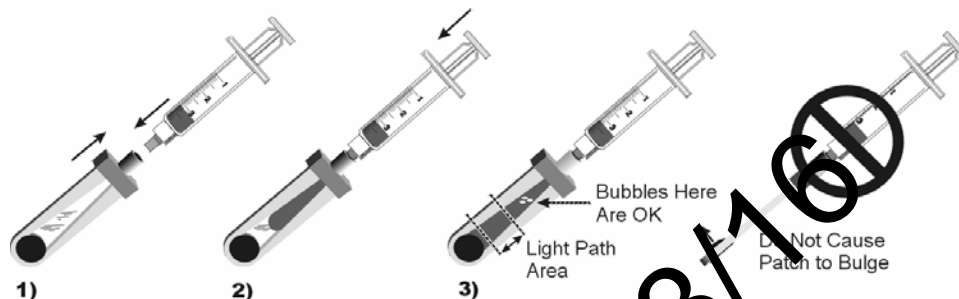
- 11.6.1.8 If results are outside of the expected range for the optical filters, repeat the process. If results are still outside of the expected range, consult the troubleshooting section of the AVOXimeter® 4000 Operator’s Manual.

11.6.2 LEVEL 1 AND LEVEL 3 QUALITY CONTROL SAMPLES

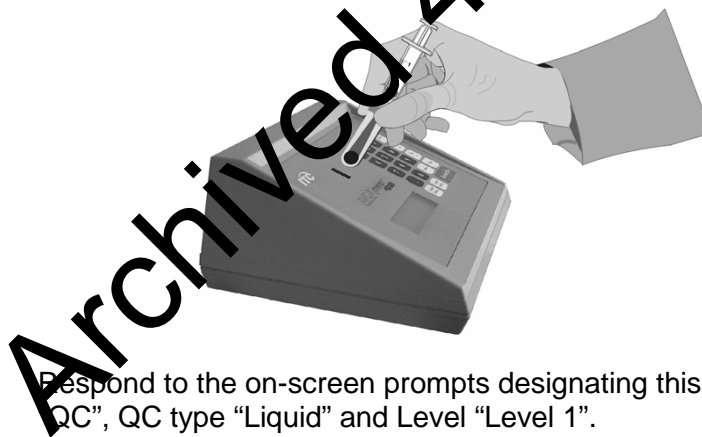
- 11.6.2.1 Verify that the instrument is ready to run a test.
- 11.6.2.2 Transfer the contents of a level 1, Multi-4 CO-Oximeter control to a plastic sample cup.
- 11.6.2.3 Draw the control into a syringe and connect to the filling port of an unused cuvette, holding the cuvette by the finger grip.



- 11.6.2.4 While holding the syringe and cuvette at a 45° 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.]
- 11.6.2.5 Stop filling the cuvette when the sample reaches the vent patch. Do not cause the vent patch to bulge.
- 11.6.2.6 Verify that the light path is free of bubbles and remove any liquid from the exterior of the cuvette.



- 11.6.2.7 Leave the syringe and cuvette attached and insert the cuvette into the test chamber.



- 11.6.2.8 Respond to the on-screen prompts designating this as sample type "QC", QC type "Liquid" and Level "Level 1".
- 11.6.2.9 Select the lot number for the control and then verify the cuvette lot number or enter a new lot number. [The procedure for entering lot numbers for the quality control samples is described in the AVOXimeter® 4000 Operator's Manual.]
- 11.6.2.10 After the results have been printed out or recorded, remove the cuvette. [NOTE: Grasp the finger grip when removing the cuvette.]
- 11.6.2.11 Verify that the Level 1 quality control results are within the expected ranges (see manufacturer's package insert).
- 11.6.2.12 Proceed with testing of case specimen(s), as described in 11.7.

- 11.6.2.13 Following analysis of all case specimens(s), repeat the process described above for the Level 3 quality control. NOTE: On the instrument, the Level 3 control is entered as control level 2.
- 11.6.2.14 If either 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.

11.7 SPECIMEN TESTING

Postmortem, whole blood specimens can have elevated methemoglobin values. Treatment of whole blood with sodium hydrosulfite prior to measurement reduces both methemoglobin and oxyhemoglobin to hemoglobin leaving the COHb measurement unaffected.

If the circumstances of the case reveal exposure to oxidizing agents such as nitrates or nitrites or topical disinfectants such as Lysol®, or drug analysis reveals elevated levels of the topical anesthetics dibucaine, lidocaine, benzocaine or tetracaine (particularly in children) the CO shall be repeated without pre-treatment of the blood.

If the blood has a chocolate-brown color or if methemoglobinemia is suspected, it shall be analyzed both with and without pre-treatment.

- 11.7.1 Verify that the instrument is ready to run a test.
- 11.7.2 Working with only one case sample at a time, label a new 12 x 75mm tube and a new plastic sample cup, with the case number of the sample.
- 11.7.3 Transfer approximately 50 mg of sodium hydrosulfite into the 12 x 75mm tube. This is equivalent to approximately a spatula-tip full of this chemical.
- 11.7.4 Pipette 0.5 mL of the case sample and 0.5 mL of CO-Oximeter diluent into the tube. Cap the tube and vortex mix the contents briefly.
- 11.7.5 Transfer the contents of the tube into the sample cup.
- 11.7.6 Using a new syringe, draw up approximately 0.5 mL of the reduced sample and fill a new cuvette as described in 11.6.2.
- 11.7.7 Leave the syringe and cuvette attached and insert the cuvette into the test chamber.
- 11.7.8 Respond to the on-screen prompts designating this as sample type "Patient", and then input the patient ID.
- 11.7.9 After the results have been printed out or recorded, remove the cuvette.

NOTE: If specific error messages appear (e.g. THb < 4.0%) when analyzing a specimen, the instrument will not print a sample report. Note the error on the worklist and in LIMS (see 11.9.7).

- 11.7.10 If the resulting COHb is <5% saturation (sat), no further analysis is necessary.
- 11.7.11 If the resulting COHb is $\geq 5\%$ sat, re-sample the specimen from the sample cup using a new syringe and a new cuvette and analyze.

NOTE: If the initial result is $\geq 5\%$ sat, but the second result is <5% sat, the results are not averaged, and are reported as <5% sat.
- 11.7.12 Verify that each COHb result agrees to within $\pm 5\%$ (not % sat) of the mean value. If values do not agree, re-sampling or analysis of an alternate sample is appropriate.
- 11.7.13 Repeat the specimen testing process for the remaining cases.
- 11.7.14 Analyze the level 3 quality control as described in 11.6.2 and verify that results are within the expected ranges (see manufacturer's package insert).
- 11.7.15 Shutdown 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.

11.8 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 Both quality control samples shall be within the expected range for COHb.
- 11.8.3 Samples with COHb values of 5% sat or greater must have duplicate results that agree to within $\pm 5\%$ (not % sat) of their mean value.

11.9 REPORTING

- 11.9.1 Results are reported in units of percent saturation (% sat).
- 11.9.2 Results are truncated to their whole integer value.
- 11.9.3 Truncated values are averaged together and the average is truncated to the whole integer value.
- 11.9.4 If the result is negative (or below 5% sat.), the result is reported as "<5% sat."
- 11.9.5 If the average value is between 5 and 75% sat, it is reported to no more than two significant figures.
- 11.9.6 If the average result is greater than 75% sat, it is reported as ">75% sat."

- 11.9.7 If no results (and no sample report) are obtained due to specimen quality (e.g. high %THb), performance of the test will be documented in LIMS, as described below:
- a. In the confirmation data screen, add carbon monoxide as analyte
 - b. Under units, select nothing (there is a blank placeholder above % sat)
 - c. In the report annotations box, add [RPT-T] *Unable to obtain a result due to poor sample quality*

11.10 INSTRUMENT SPECIFICATIONS

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

11.10.2 ACCURACY (COHB): $\pm 2\%$ sat

11.10.3 PRECISION (COHB): $\leq 1\%$ sat

11.11 REFERENCES

- 11.11.1 AVoximeter® 4000 Whole Blood Oximeter, Operator's Manual, AP4001, October, 2007.

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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

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