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.

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

11.4.3 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. The accuracy of COHb results from testing performed on specimens collected in gray top tubes may be affected (see 11.9.6).

11.5 REAGENTS, MATERIALS AND EQUIPMENT

11.5.1 REAGENTS

11.5.1.1 Deionized water (DI H_2O)

11.5.2 MATERIALS

11.5.2.1 Disposable 12 x 75 mm tubes

11.5.2.2 Disposable pipette tips

11.5.2.3 Disposable safety closures for 16 x 75mm tubes

11.5.2.4 Plastic Luer taper syringes, 1 mL (Becton Dickinson, BD)

11.5.2.5 Quality control sample - Low (IL Multi-4 CO-Oximeter Control level 3, part # 3313250), store refrigerated

11.5.2.6 Quality control sample - High (IL Multi-4 CO-Oximeter Control level 1, part # 3315250, store refrigerated

11.5.2.7 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)

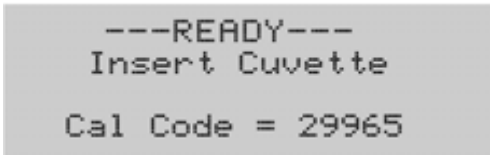
11.5.3.5 Vortex mixer

11.6 QUALITY CONTROL

The optical quality control procedures shall be performed on each day case specimens are analyzed. The Level 1 and Level 3 quality control samples must be run with each test batch (see also 11.7.12).

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.



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	Expected Range			
	tHb (g/dL)	$\%O_2Hb$	%COHb	%MetHb
Yellow	7.8 to 8.2	93.7 to 96.3	2.6 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

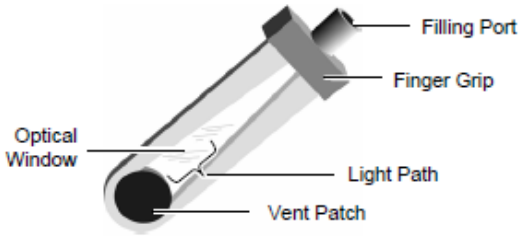
11.6.1.8 If results are outside of the expected range for the optical filters, repeat the process. If results are still outside of the expected range, consult the troubleshooting section of the AVOXimeter® 4000 Operator's Manual.

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 12 x 75mm tube.

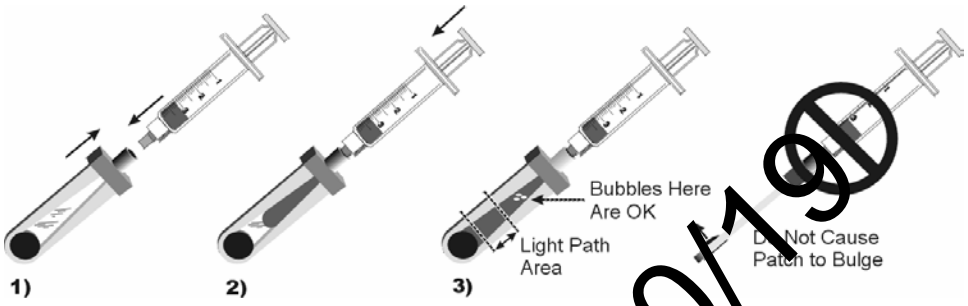
11.6.2.3 Draw the control into a syringe and connect to the filling port of an unused cuvette, holding the cuvette by the finger grip.



11.6.2.4 While holding the syringe and cuvette at a 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.]

11.6.2.5 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 any 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

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 with the case number of the sample.

11.7.3 Pipette 0.5 mL of the case sample into the tube.

11.7.4 Using a new syringe, draw up approximately 0.2 mL of the sample and fill a new cuvette as described in 11.6.2.

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

11.7.6 Respond to the on-screen prompts designating this as sample type "Patient", and then input the patient ID.

11.7.7 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 11.9.5).

11.7.8 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. Pipette 0.25 mL of the case sample and 0.25 mL DI H_2O into a labeled 12 x 75 mm tube. Cap the tube and briefly vortex-mix. Perform analysis as described in 11.7.4 – 11.7.7.

11.7.9 If the resulting COHb is <5% saturation (sat), no further analysis is necessary.

11.7.10 If the resulting COHb is ≥5% sat, re-sample the specimen from the 12 x 75 mm tube using a new syringe and a new cuvette and analyze.

NOTE: 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.

11.7.11 Verify that each COHb result agrees to 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 to establish agreement with one of the initial results (this may require a fresh sampling, as the initial volume may have been consumed in the first two tests).

11.7.12 Repeat the specimen testing process for the remaining cases.

NOTE: The total number of quality control samples (L1, L3) must make up at least 10% of the testing batch (number of individual case specimen tests performed, including analyses where a sample report is not produced – e.g. error messages). Additional L1 or L3 quality control samples may be used where testing batch size dictates use of more than the two quality control samples described in 11.6.2.

11.7.13 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).

11.7.14 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 All quality control samples shall be within the expected range for COHb (a failed control that has been remedied – see 11.6.2.14 – is considered acceptable).

11.8.3 Samples with COHb values of 5% sat or greater must have duplicate results that agree to within ±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 If the result is negative (or below 5% sat.), the result is reported as "<5% sat."

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

11.9.4 If duplicate COHb results that read between 5% and 75% sat are obtained (see 11.7.11), 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.

11.9.5 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. This tube type is not recommended by the manufacturer of the instrument used for this test.*

11.9.6 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.*

11.10 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.

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