MEASUREMENT 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 either the State Toxicologist, a Manager, or a Supervisor, 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, technical 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 (O_2Hb), reduced hemoglobin (HHb), methemoglobin (MetHb) and COHb. Total hemoglobin (tHb), percent oxyhemoglobin ($\%O_2Hb$) and oxygen content (O_2Ct) 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 in less than 10 seconds.

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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 ($Na_2S_2O_4$)

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

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, air-displacement pipettes

11.5.3.4 Optical filters (yellow & orange, supplied with the AVOXimeter® 4000)

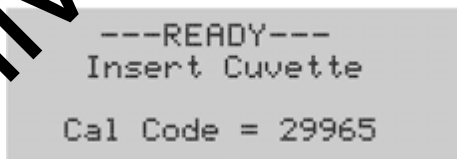
11.5.3.5 Vortex mixer

11.6 QUALITY CONTROL

The following quality control testing shall be performed on each day of testing.

11.6.1 Optical Quality Control

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.

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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

11.6.1.8 Submit the optical quality control results for review.

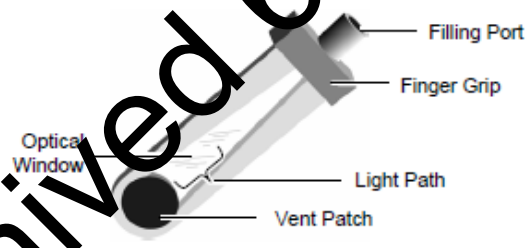
11.6.1.9 If results are outside of the expected range for the optical filters, repeat the process. If results are still outside of the expected range, consult the troubleshooting section of the AVOXimeter® 4000 Operator's Manual.

11.6.2 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.

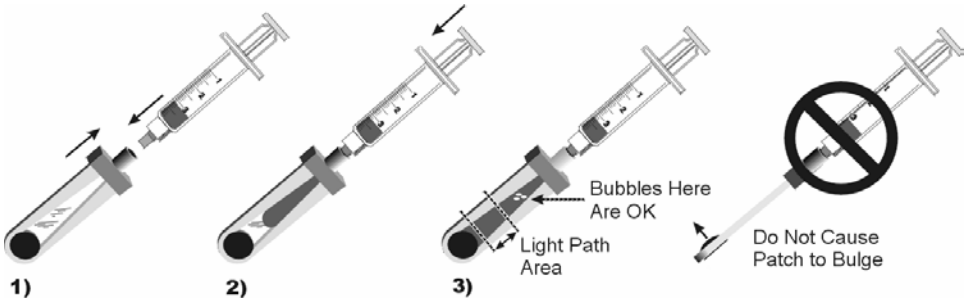
11.6.2.3 Draw the control into a syringe and connect to the filling port of an unused cuvette, holding the cuvette by the finger grip.



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.



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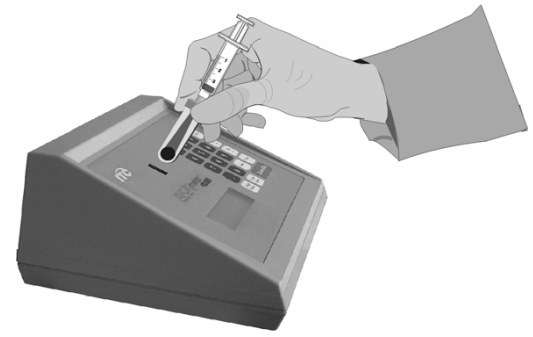
11.6.2.7 Leave the syringe and cuvette attached and insert the cuvette into the test chamber.



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 control result is within the expected range.

11.6.2.12 Repeat the process using the level 3 control.

11.6.2.13 Submit the quality control results for review.

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.

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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.

11.7.10 If the resulting COHb is greater than 5%, re-sample the specimen from the sample cup using a new syringe and a new cuvette.

11.7.11 Verify that each COHb result agrees to within ±5% of the mean value. If values do not agree, re-sampling or analysis of an alternate sample is appropriate.

11.7.12 Repeat the specimen testing process for the remaining cases and submit all results and quality control results for technical review.

11.7.13 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 TECHNICAL 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% or greater must have duplicate results that agree to within ±5% of their mean value.

11.9 REPORTING

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

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 average result is negative or below 5%, the result is reported as "<5%".

11.9.5 If the average value is between 5 and 75% it is reported to no more than two significant figures.

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11.9.6 If the average result is greater than 75% it is reported as ">75%".

11.10 INSTRUMENT SPECIFICATIONS

11.10.1 Measurement Range (COHb): 0 – 75%

11.10.2 Accuracy (COHb): ±2%

11.10.3 Precision (COHb): ≤1%

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
03/09/12	Method approved by Washington State Toxicologist. See DRA dated 02/29/12. Method released for use in evidentiary testing on 03/09/12.	All
06/19/12	Corrected the syringe description from Luer-lok™ to Luer taper in 11.5.2.5	2

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