Carbon Monoxide Analysis in Biological Specimens

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1 INTRODUCTION

Carbon monoxide (CO) is a colorless, odorless gas produced by the incomplete combustion of organic fuels. It is also a common trace pollutant in the atmosphere. CO is present in the body at low concentrations, where it is bound to hemoglobin (Hb), to form carboxyhemoglobin (COHb).

When CO is inhaled, it competes with oxygen (O2) for the hemoglobin in red blood cells. The affinity of CO for hemoglobin is approximately 250 times that of O2. When COHb is formed, O2 cannot be transported to the tissues that need it, putting those tissues in a state of anemic hypoxia. Overexposure to CO produces headache, tremor, nausea, weakness, confusion, stupor and coma. Carbon monoxide poisoning may occur in fire victims or as a result of inhalation of automobile exhaust, heating system/stove waste products or other combustion gases.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation
Matrices	Blood (0.2 mL CO-Oximeter, 0.33mL HS-GC-TCD)
Analytes	Carbon Monoxide (CO)
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Samples are screened and quantitated by spectrophotometry using a CO-Oximeter.

Headspace gas chromatography with thermal conductivity detection (GC/TCD) may also be used to analyze samples. Sulfuric acid is added to liberate the CO from the hemoglobin. An automated headspace sampler then samples and injects a portion of the headspace onto the HS-GC/TCD system.

4 SPECIMEN CRITERIA

Blood, spleen or other blood-rich organs can be analyzed by these procedures.

5 EQUIPMENT

5.1 Equipment

A. Vortex mixer
B. Pipettors: 5 µL - 1000 µL

5.2 Consumables

A. Disposable syringes: 1mL
B. Disposable Test Cuvettes for AVOXimeter® 4000
C. Kimwipes® and/or BloodBloc® pads
D. 20 mL headspace vials (HSV)
E. Magnetic crimp caps for HSV

5.3 Instruments

5.3.1 Agilent 6890N Gas Chromatograph

5.3.1.1 Detectors

Thermal Conductivity Detector

5.3.1.2 Column

J&W HP-Molesieve 30 m x 0.32 mm x 12 µm, or equivalent

5.3.1.3 Headspace Autosampler

Gerstel MPS2 or equivalent

5.3.2 CO-Oximeter

AVOXimeter® 4000 Whole Blood CO-Oximeter with QC filters

5.4 Software

Windows XP SP2; Chemstation E.02.00.493; Xcalibur 2.0.7 SP1

5.5 Chemicals/Reagents

5.5.1 Purchased

Item	Supplier*	Description	Part Number*
Sulfuric acid, conc.	Fisher	≥Reagent grade	A300-500
Formic acid	Fisher	≥89%	A118P-100
Water	In-House	Deionized, 18mΩ	n/a

*use of an equivalent product is allowable

5.5.2 Prepared

Depending upon the batch size, the absolute amounts may be adjusted so long as the ratios of components are maintained.

1 M sulfuric acid solution

Step	Action	Amount	Component/Information
1	Acquire	1	Graduated cylinder, 100 mL
2	Add	80 mL	Water
3	Add	5 mL	Sulfuric acid
4	QS	90 mL	Deionized water
5	Mix		
6	Transfer		Glass
7	Storage		Ambient
8	Stability		≥ 1 year
9	Prepares	90 mL	

5.6 Standards/Controls

5.6.1 Purchased

%COHb Controls

Analyte	Supplier*	Description	Part Number*
COHb	RNA Medical	Levels 1, 2, and 3	QC 253

*use of an equivalent product is allowable

5.6.2 Prepared

0.05 M formic acid solution (GC-TCD System Performance Check)

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 10 mL
2	Add	22 µL	Formic acid
3	QS	10 mL	Deionized water
4	Mix		
5	Transfer		Glass
6	Storage		Ambient
7	Stability		1 month
8	Prepares	10 mL	(200 samples)

6 PROCEDURE

6.1 Screening and Quantitation of %COHb by CO-Oximeter

CO-Oximeter System Performance Check

Activity	Note		Reference/Lot
☐	1. Turn the AVOXimeter on and wait for the "---READY--- Insert Cuvette" message to appear.		
☐	2. Insert the yellow optical quality control filter.		
☐	3. "Select Sample Type" screen, type "2/Enter" for QC.		
☐	4. "Select QC Type" screen, type "2/Enter" for Optical.		
☐	5. "Select Filter" screen, type "1/Enter" for Yellow. Hit "Enter" or OK if prompted.		
☐	6. The results will appear within ten seconds. Press "Print" to print a copy. (Sometimes "Print" must be pressed twice.)		
☐	7. Verify that the results are within the specifications on the sticker on the filter. Record whether the results Pass or Fail in the instrument logbook. (Target value ranges are printed on each filter.)		
☐	8. Repeat steps 1-7 for the orange filter. (Type "2/Enter" in step 5 for the orange filter.)		
Analyzing Samples and Controls by CO-Oximeter			
☐	1. Acquire RNA Medical Co-Oximeter Controls (3 Levels) and case specimens.		
☐	2. Verify that the "---READY--- Insert Cuvette" message appears.		
☐	3. After mixing the blood sample via inversion, draw approximately 0.2 mL of a sample or control into a disposable syringe.		
☐	4. Insert the syringe into the cuvette.		
☐	5. Hold the syringe and cuvette at 45° and gently press the plunger. Stop pressure when the sample reaches the vent patch (do not allow vent patch to bulge).		
☐	6. Verify that the light path area is free of bubbles.		
☐	7. Clean any drops of blood off the exterior of the cuvette with a Kimwipe or BloodBloc pad. If blood has broken through the vent patch, discard the cuvette and prepare a new one.		

☐	8. Insert the cuvette (with syringe still attached) into the AVOXimeter.		
☐	9. At the "Select Sample Type" screen, type "1/Enter" for Patient.		
☐	10. The results will appear within ten seconds. Press "Print" to print a copy. (Sometimes "Print" must be pressed twice.) Label the printouts with the correct sample name or control level. See Section 8 for Decision Criteria.		
☐	11. After each sample is analyzed, the cuvette should be disposed of in biohazard waste.		
☐	12. Analyze all case samples and control(s) in duplicate, using a fresh cuvette each time.		
☐	13. Record results via scan/photo since the paper printouts are less readable over time (can discard printouts after capture).		

6.2 %COHb by GC/TCD

Carbon Monoxide System Performance Check

	Activity	Note	Reference/Lot
☐	1. To a 20 mL HSV, add 1 mL of concentrated sulfuric acid.		
☐	2. Add 50 µL 0.05 M formic acid solution. Immediately crimp-seal the HSV and vortex for 10 seconds.		
☐	3. Incubate HSV at 100°C for 60 minutes in a laboratory heating block or a GC oven.		
☐	4. Analyze the headspace as per the instrumental conditions provided in Section 7 of this procedure.		
☐	5. Verify that the Decision Criteria for the Testmix defined in Section 8.1.2.1 of this procedure are met before continuing.		
	Analysis of Controls and Case Samples		
☐	1. Acquire RNA Medical Co-Oximeter Controls (3 Levels) and case specimens.		
☐	2. For each control and case sample, label a clean 20 mL HSV with the sample name (quantitation in duplicate)		
☐	3. Using an adjustable pipettor, aliquot 0.33 mL portions of the appropriate blood sample or control into each HSV.		
☐	4. Add 0.33 mL liberating agent (1M sulfuric acid) to HSV, immediately sealing each vial with a crimp cap.		
☐	5. Uniformly vortex each HSV for 30 seconds using a moderate setting. Avoid excessive splashing of sample onto crimp cap.		
☐	6. Analyze each HSV using the GC/TCD using the instrumental parameters in Section 7 of this procedure.		

7 ANALYTICAL PARAMETERS

7.1 Agilent Gas Chromatograph

7.1.1 Oven – Standard Conditions

Step	Temperature (°C)	Hold (min)	Ramp (°C/min)
1	40	5	0 (isothermal)
Equilibrium Time (min)	0.2		

Total Run Time (min): 5.00

7.1.2 Inlet/Carrier/Column

Inlet		**Carrier**		**Column**	
Temperature (°C)	250	**Gas**	ultrapure helium	**Type**	HP-Molesieve
Injection Mode	Split	**Mode**	constant flow	**Length (m)**	30
Split Ratio	3.1	**Flow (mL/min)**	5.0	**Internal Diameter (mm)**	0.32
				Film Thickness (µm)	12

7.2 Thermal Conductivity Detector

Temperature (°C)	205
Reference Flow (mL/min)	20
Make Up Gas	helium
Make Up Flow (mL/min)	2.5

7.3 Autosampler

Mode	Headspace	**Syringe**	
Incubation		Temperature (°C)	70
Temperature (°C)	50	Sample Fill Volume (mL)	0.9
Time (min)	20	Sample Fill Rate (mL/sec)	0.9
Agitator Speed (rpm)	250	Sample Fill Strokes	5
Agitator Timing (s) On/Off	30/2	Sample Injection Speed (mL/sec)	0.9
Cycle Time (min)	5.3	Flush Time (min)	2

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 CO-Oximeter

8.1.1.1 Performance Check

Results from both optical filters should be within the manufacturer's specification ranges. If they are not, contact IOSS or the instrument manufacturer for assistance.

8.1.1.2 Control Values

The CO-Oximeter controls are supplied at approximately the following levels (the actual values and ranges vary; consult the product insert for each lot):

Level	total Hb, g/dL Average Value	total Hb, g/dL Range	COHb, % Average Value	COHb, % Range
1	8.3	7.6-9.0	5.7	1.7-9.7
2	13.7	12.6-14.7	16.1	11.6-20.6
3	17.2	15.9-18.5	44.2	38.9-49.5

The control results (average value of duplicates) for all levels must pass for assay validity.

8.1.2 HS-GC-TCD

8.1.2.1 Performance Check Decision Criteria

The CO peak should be well separated from the nitrogen and oxygen peaks (>0.5 min baseline separation) and have a peak area greater than 200 units.

8.1.2.2 Blood Controls

A detectable CO peak will be obtained from each control. Using the calculations described in Section 9, the Level 1 and Level 3 controls should calculate to within 5% (absolute) of the target value for Level 1, within 10% (relative) of the target value for Level 2, and within 20% (relative) of the target value for Level 3.

8.1.2.3 Case Specimens

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs.

Additionally, the retention time of the peak should be within ±2% of the retention time obtained from injection of the testmix or positive control.

Using the calculations described in Section 9, the case specimen results should agree within 20% (relative) of the average value obtained from the CO-Oximeter.

9 CALCULATIONS

%COHb is estimated from the HS-GC/TCD CO peak and the tHb amount measured by the CO-Oximeter as described in the example below:

COHb = saturation of carbon monoxide
TCD = GC/TCD area counts
tHB = total hemoglobin (CO-Oximeter)

$COHb_{specimen} = (TCD_{specimen} / TCD_{level2}) X (tHb_{level2} / tHb_{specimen}) X (COHb_{level2target})$

Assume that the Level 2 Control Target Value (from the insert) is accurate. (For the example below, this value is 18.6%.) Assume that the Hb average amount for each sample calculated by the AVOXimeter is correct.

$COHb_{specimen} = ((2036623/16.6) X (18.6)) / (65409/13.6)$
$COHb_{specimen} = 47.45$

10 REPORTING

10.1 Case Specimen Interpretation

Interpretation of results will consider variables such as:

- History of potential exposure to a carbon monoxide source
- Symptoms consistent with CO poisoning such as headache, dizziness, nausea/vomiting, confusion, fatigue, chest pain, shortness of breath, and loss of consciousness.
- Elevated COHb level (>3-4% in nonsmokers, or >10% in smokers)

10.2 Case Specimen Reporting (CO-Oximetry)

- Duplicate measurements must be within 10% (absolute) of the average value in order to report.
- Results less than 5% COHb are reported as “carbon monoxide less than 5% saturation”.
- Results equal to 5% COHb or greater are reported as “carbon monoxide X % saturation”.
- If two different blood specimens are analyzed from one case, report them separately. Do not average results from two different specimens.
- Reference Section 10.3 for guidance on measurement uncertainty.

10.3 Measurement Uncertainty

CO-Oximeter data: Two control levels (purchased from RNA Medical) were analyzed in triplicate over five days during the validation period. The “true” value of the controls was taken from the package insert. Our testing showed an average 2.7% positive bias for the two levels and gave an overall standard deviation value of 1.3%. Since there is no certified reference material that we can base our calibration on, we must account for both the bias and the precision of these

measurements to estimate our uncertainty. Therefore, these values will be summed to achieve a value of 4%. This value will be used as the historical uncertainty for the method, rather than the 1.3% value. Any positive case specimens will therefore be reported with a +/-12% uncertainty (relative) at a 99.7% confidence level. (For example, COHb was identified at a saturation of 50% ± 6%, 99.7% CL, k=3.396).

11 CORRECTIVE MEASURES

11.1 Co-Oximeter

11.1.1 Case Specimen Suitability

Specimens that are putrefied, highly viscous, lipemic or otherwise degraded may not be suitable for analysis via co-oximetry. Indications that a sample is not suitable for analysis may include an inability to load the cuvette properly or an error message upon an attempt at analysis.

11.1.2 CO-Oximeter Error Messages

If a specimen does not pass internally preset criteria, the CO-Oximeter LCD will display one or more error messages, and no results will print. Review the error messages by pressing "Enter" until no new messages appear. Error messages can include:

- tHB <4 or > 25
- %O2Hb <-10.0%
- %CO <-7.0
- %MetHb >107.0%
- %HHb>107%
- % Scat <-15%

When the only error message is "tHb (total hemoglobin) >25%", the sample may be diluted 1:2 with deionized water and re-analyzed. A higher dilution may be used if tHb >25%.

11.2 HS-GC-TCD

The GC column used in this procedure is a molecular sieve column, which may retain water. The column may be reconditioned by heating the GC oven to 225°C for >4 hours or overnight. Insufficient column conditioning results in poor chromatographic separation between the CO and air peaks.

12 Performance Characteristics

12.1 Method Performance

Analyte	Level	Bias (%)	Repeatability (%)	Precision (%)
%COHb	Low	4.17	5.42	8.62
	Medium	2.90	1.26	1.33
	High	2.75	0.96	1.16
AVOXimeter; based on 15 measurements at each level on 5 days				

12.2 Carryover

No carryover was detected during validation.

13 Limitations

- Specimens to be analyzed should be rich in red blood cells.
- Serum-separated or "spun-down" blood samples are not appropriate for CO analysis.
- Samples that do not give acceptable results by the AVOXimeter may be considered unsuitable for %COHb measurement.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the FBI Laboratory Safety Manual for guidance.

15 Revision History

Revision	Issued	Changes
04	02/11/2022	Document reformat. Updates to scope statement and clarifications on reporting and measurement uncertainty.