Evaluation of the Avoximeter 4000® to Measure the Stability of Carboxyhemoglobin in Different Blood Collection Tubes Over Time

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Abstract

Introduction: Carboxyhemoglobin (COHb) is typically monitored by toxicology laboratories in persons who die in house fires or are found dead in automobiles. Carbon monoxide (CO) displaces oxygen bound to hemoglobin with a binding affinity approximately 300 times that of oxygen. As a result, even low levels of CO for extended periods of exposure will result in toxic to lethal levels of COHb. At Cuyahoga County Medical Examiner's Office, COHb is determined using an Avoximeter 4000® (International Technidyne Corporation) with confirmations performed spectrophotometrically. The Avoximeter is a CO-oximeter which uses disposable/fillable cuvettes. There is no tubing or pump to malfunction and the analysis is complete in < 1 min. The current study evaluated the Avoximeter and the stability of COHb levels in different blood collection tubes routinely used to collect specimens during autopsy. Included were gray top (NaF and potassium oxalate) red top (no additives), lavender top (EDTA), green top (heparin), and blue top tubes (K_2EDTA).

Materials and Methods: A unit of red cells and a unit of plasma were obtained from University Hospitals in Cleveland. Both units had expired and were to be discarded. The red cells and plasma were combined to make whole blood. Sodium dithionite (500 mg) was added to convert hemoglobin to deoxyhemoglobin. CO gas (99.5%) was then bubbled through the whole blood to convert hemoglobin to carboxyhemoglobin; Dissolved CO was removed from solution by bubbling with air for an extended period. Carboxyhemoglobin was monitored using an Avoximeter. The level of COHb for time zero was adjusted to 69.8 % by addition of oxyhemoglobin. The Avoximeter has an upper limit of measure of 75% COHb. Confirmation of COHb concentration was made spectrophotometrically by the method of Rodkey. Blood was dispensed into five replicates of the different blood tubes and the level of COHb measured for 14 consecutive days. Evaluation of statistical differences was by single factor analysis of variance using an Excel® spreadsheet.

Results: Levels of COHb in red, green, purple and blue blood collection tubes remained essentially unchanged from Time 0 during the 14 day period (range 67.2 to 71.4 %). The %CV ranged from 1.12 to 1.97. COHb levels in gray top collection tubes were significantly lower ($p < 0.05$) with a mean of 63.6 % and %CV = 2.80. Results of the control procedure measured 69.2 % COHb which confirmed the accuracy of the Avoximeter.

Discussion/Conclusion: The results of this study confirm earlier findings of Kunsman, et.al., 2000 demonstrating the stability of COHb in blood collection tubes stored under refrigerated conditions. Why gray top collection tubes produced a somewhat lower value is unknown at this time. No interactions of NaF or potassium oxalate with COHb have been reported. This study shows that measurement of COHb from different types of blood tubes is an acceptable practice. The Avoximeter was found to be accurate, precise, quick and extremely easy to use. The stability of COHb will continue to be tracked in the different blood collection tubes for even longer periods of time.

Keywords: Avoximeter, Carboxyhemoglobin, Stability

Objectives

- To determine which blood collection tubes gives the most stable readings for percent COHb over time.
- To increase familiarity with the Avoximeter 4000® and show emphasis of its reliable analysis of CO.

Introduction

- Carbon monoxide (CO) gas results from incomplete combustion of carbon, with complete combustion producing CO_2. CO is not physiologically detectable because it is an odorless, colorless and tasteless gas[1]. Daily exposure to low levels of CO come from cigarette smoke, exhaust from engines, or ventilation system malfunctions. Lethal exposure to CO usually occur during house fires, from faulty gas fired hot water heaters and furnaces, or through intentional exposure to automobile exhaust (suicides).

Carbon Monoxide Toxicity:

- Effects from CO toxicity can range from headache and flushed skin (at low percent saturation levels), to coma, respiratory paralysis, and death at high saturation levels [1].
- Inhalation of CO results in formation of carboxyhemoglobin in red blood cells, with one mole of CO binding one mole of the ferrous ion in heme. As a result, hemoglobin is no longer available to deliver oxygen to tissues. The binding affinity of CO to heme Fe^{2+} is approximately 300 fold that of O_2, so being in an atmosphere of low CO concentration for an extended period of time can also be deadly[1] .
- A lethal level of carboxyhemoglobin (COHb) is generally thought to be around 50% saturation. If the person is a child or in declining heath, then lower levels of COHb may produce death.

Measuring Carboxyhemoglobin:

- Determination of carboxyhemoglobin is a test that is not performed on all cases, but when the test is needed, it is an assay that must be performed STAT to allow pathologists to quickly establish the cause of death. Many methods have been developed for quantifying COHb[2-5]. This presentation describes quantification of COHb using the Avoximeter 4000 which is easy to use and provides rapid results.

Avoximeter 4000:

- Whole blood oximeter
- No sample prep needed
- Sample injected into disposable cuvettes
- Result in less than ten seconds

Materials and Methods

Materials:

- A unit of red cells and a unit of plasma were obtained from University Hospitals in Cleveland, OH.
- Five identical sets of BD Vacutainer blood collection tubes were made (Gray, Lavender, Green, Royal Blue and Red).

	Blood Collection Tubes Used	
Tube Color	Preservative	Capacity
Gray	Sodium Fluoride/Potassium Oxalate	10.0ml
Lavender	K_2EDTA	4.0ml
Green	Sodium Heparin	4.0ml
Royal Blue	K_2EDTA	6.0ml
Red	Silicone Coated	10.0ml

- Avoximeter Cuvettes
- Canister of 99.5% purity carbon monoxide from Praxair, Inc.

Methods:

- Red cells and plasma were combined to make whole blood. After this step, sodium diothionite (500mg) was added to convert hemoglobin to the Fe^{2+} state (deoxyhemoglobin).
- CO gas (99.5% purity) was bubbled through the whole blood for an extended time to saturate the solution with CO.
- Since the Avoximeter has an upper reporting limit of 75%[6],the high level of COHb was lowered to 69.8% by bubbling with air.
- In order to confirm the initial COHb level, a spectrophotometric analysis was performed[2] with COHb levels measured as 69.2%. At time zero, the average COHb level measured with the Avoximeter was 69.5%.
- For analysis of the blood over the time periods, 50µl of the blood samples were used per analysis. A total of five replicate sets of different blood collection tubes were measured. One set was used per week and rotated throughout the time period.
- To obtain the daily and weekly CO values, the blood tube was inverted five times and if a second reading was needed, the tubes were inverted two additional times.

Avoximeter® 4000

Results

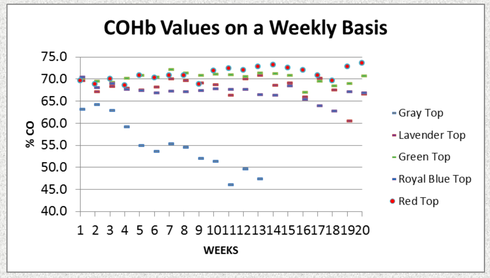
Table 1: Statistical Variability During Daily Assay, Weeks 1 and 2

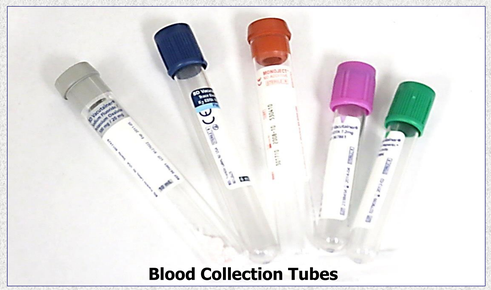
- This table shows the average CO values, Standard Deviation and %CV during the first 14 days of the study.

	Gray Top	Lavender Top	Green Top	Royal Blue Top	Red Top
Mean	63.6	69.2	69.3	69.3	69.6
St. Dev.	1.78	1.14	1.07	1.37	0.78
%CV	2.80	1.65	1.54	1.97	1.12

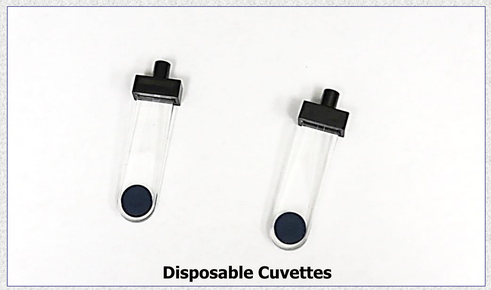
Figure A

- This figure shows the CO values measured weekly for 20 weeks.





Blood Collection Tubes



Disposable Cuvettes

Conclusions

- The data shows that gray top tubes in both the daily and weekly analysis had a mean CO percentage significantly lower ($p < 0.001$, Single Factor ANOVA) than measured at time zero.
- From the daily analysis (Two Weeks Data), the blood in Red Top tubes showed the smallest variability (%CV = 1.12) with a mean CO percentage of 69.6.
- During the duration of storage (20 weeks), COHb in green top tubes was the most stable with a mean CO percentage of 70.3 with a %CV of 1.71.
- Blood in gray top tubes could not be measured past week 14, possibly as a result of decomposition. The error message reported by the Avoximeter was % Scat < -15.0%.
- Data show that four out of five different blood collection tubes were satisfactory for COHb analysis. Green top blood tubes had the smallest statistical variability during weekly analysis.
- These findings are not consistent with Kunsman, et.al., 2000 who reported that COHb in gray top tubes was stable under refrigerated conditions for two years [7].
- Gray top tubes were found to be unsatisfactory for CO measurement.
- The Avoximeter is an instrument which is simple to use, requires a small sample size, and is easy to calibrate and maintain.

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