American Journal of Emergency Medicine (2008) **26**, 191–195

The American Journal of Emergency Medicine

www.elsevier.com/locate/ajem

Brief Report

Stability of carboxyhemoglobin in stored and mailed blood samples☆

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Received 9 April 2007; accepted 13 April 2007

Abstract

Background: Elevated blood carboxyhemoglobin (COHb) levels are used to confirm a clinical diagnosis of exposure to carbon monoxide (CO) and, in some instances, assess severity of poisoning. However, many hospital laboratories cannot measure COHb because they do not have CO-oximeters. In such instances, blood samples are often sent to outside laboratories or with a transported patient for measurement at the receiving hospital. This study was conducted to assess the stability of COHb in stored and mailed blood samples anticoagulated with heparin.

Methods: Adult human blood was drawn into standard sample tubes anticoagulated with sodium heparin. Carbon monoxide gas was infused to raise the COHb level to 25% to 35%. Samples were then refrigerated or stored at room temperature, and serial COHb determinations were performed for 28 days. Additional samples were measured after being mailed locally or across the United States and back.

Results: No significant changes in COHb levels were seen in samples stored either in refrigeration or at room temperature over a period of 28 days or in samples shipped without refrigeration locally or across the United States.

Conclusions: Carboxyhemoglobin levels in whole blood samples anticoagulated with heparin are stable with or without refrigeration for up to 4 weeks. If COHb measurement capability is not available, such samples may be shipped or transported with patients with confidence that the COHb level will be stable when measured at a later time.

1. Introduction

Carbon monoxide (CO) poisoning is common in the United States, accounting for an estimated 40 000 emergency department visits for diagnosed cases annually [1]. Because the signs and symptoms of CO poisoning are nonspecific, it is likely that many more cases are unsuspected or attributed to other etiologies and therefore go undiagnosed.

When CO poisoning is suspected clinically, measurement of blood carboxyhemoglobin (COHb) is typically performed. An elevated COHb level (>2% for nonsmokers and >9% for smokers) [2] documents exposure to exogenous CO and supports the clinical diagnosis. Carboxyhemoglobin is typically measured in hospital laboratories by multiwavelength CO-oximetry. Not all hospitals have laboratory CO-oximeters, often related to the expense of the equipment.

☆ The Edward H. Morgan Chair in Pulmonary and Critical Care Medicine, Virginia Mason Medical Center (Seattle, Wash) provided financial support for this study.

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doi:10.1016/j.ajem.2007.04.028

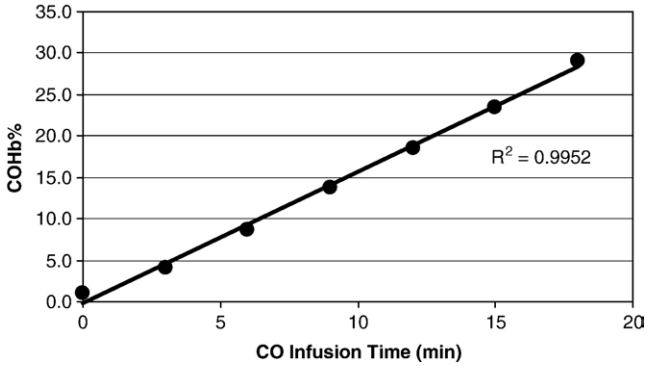

Fig. 1 Carboxyhemoglobin levels in pooled heparin-anticoagulated blood during infusion of 5000 ppm CO at 0.5 L/min.

When that is the case, blood samples can be sent to an outside laboratory for COHb measurement. Alternate methods that are sometimes used to demonstrate an individual's exposure to CO include measurement of exhaled CO [3] or noninvasive measurement of COHb through the use of a recently released fingertip pulse CO-oximeter [4].

When a patient suspected of having CO poisoning is being acutely transferred from an environment where COHb measurement is unavailable, it is sometimes suggested that an anticoagulated blood sample be drawn locally and sent with the patient for COHb determination upon arrival at the receiving hospital [5]. Both mailing blood samples to reference laboratories and sending samples with transported patients presume that such samples are stable. However, published data demonstrating stability of COHb in human blood stored in standard sample vials containing heparin, the anticoagulant preferentially recommended by CO-oximeter manufacturers, are not available in the literature. This study was performed to investigate the stability of COHb in stored and mailed human blood samples anticoagulated with heparin.

2. Methods

After approval by the institutional review board of Virginia Mason Medical Center, the author NBH donated 120 mL venous blood drawn from an antecubital vein into 20 commercially available, green-top blood sample tubes (6 mL) anticoagulated with sodium heparin (Becton Dickinson, Franklin Lakes, NJ, #367871). The blood from all tubes was then combined in a beaker under a negative pressure hood and mixed with a magnetic stir bar. A 1-mL sample was obtained for baseline COHb measurement with a laboratory CO-oximeter (ABL 800 Series, Radiometer America, Westlake, OH). A mixture of 5000 ppm CO in nitrogen (Mesa International Technologies, Santa Ana, Calif) was then bubbled into the continuously stirred blood at a fixed rate of 0.5 L/min, and samples were obtained for COHb measurement after every 3 minutes of CO infusion.

Carbon monoxide infusion was stopped when the COHb concentration reached a level in the 25% to 35% range. Two milliliters of the anticoagulated, CO-saturated blood was then placed into each of 28 red-top blood sample tubes containing no additives (Becton Dickinson #366431) other than the heparin already present in the blood. Eleven tubes were placed into a rack for refrigerated storage (4°C). Eleven tubes were placed into a rack for storage at laboratory room temperature (22°C). One tube from each rack was withdrawn for baseline COHb measurement on day 0. Subsequent pairs of tubes were similarly analyzed on days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28.

Three pairs of tubes were sent on day 0 via the United Parcel Service to (1) the investigators at his Seattle employment address, (2) a colleague in Indiana, and (3) a colleague in Florida. The latter 2 were instructed to ship the packages back unopened. Carboxyhemoglobin levels were measured from each tube on the day of receipt by the author.

The technical support departments for 3 major manufacturers of laboratory CO oximeters were contacted by telephone regarding their recommendations for the preferred sample type for measurement in their instruments. To determine local laboratory preferences for blood COHb sample tube type and beliefs regarding sample stability, 2 commercial reference laboratories and 3 major medical center laboratories in the Seattle area were surveyed by telephone.

Descriptive statistics and regression analysis were used to analyze the data.

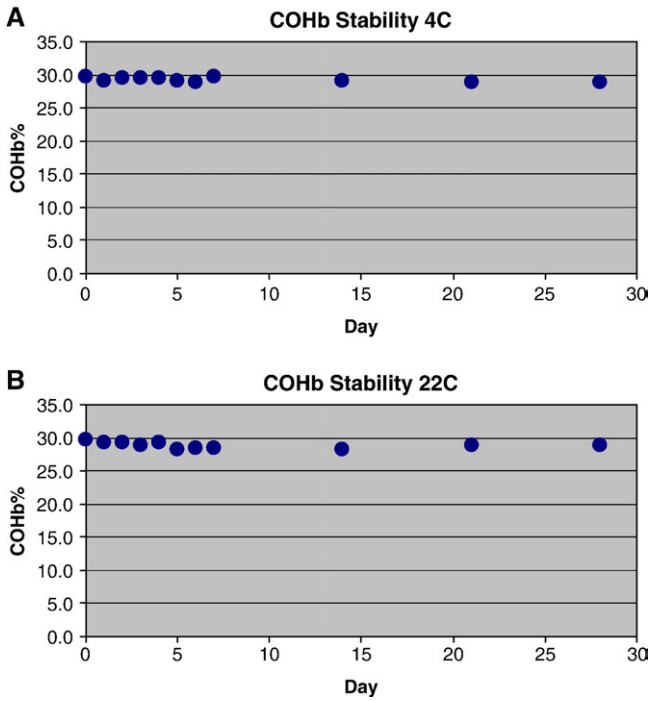

Fig. 2 A, Carboxyhemoglobin levels over 28 days in refrigerated blood samples (4°C). B, Carboxyhemoglobin levels over 28 days in blood samples stored at room temperature (22°C).

3. Results

Carboxyhemoglobin levels in pooled blood during the CO infusion are shown in Fig. 1. The rate of rise of COHb was linear ($R^2 = 0.9952$) with infusion at a constant rate. The target COHb range of 25% to 35% was achieved with 18 minutes of infusion.

Baseline COHb levels from 2 samples were 29.7 and 29.6 (mean ± SD, 29.6 ± 0.07). Regression analysis showed no significant change in either refrigerated or unrefrigerated samples over the course of 28 days ($P = .0546$ and $P = .2613$, respectively). Refrigerated samples ranged from 28.8% to 29.6% and averaged 29.2% ± 0.3% (mean ± SD) (Fig. 2A). Samples stored on the laboratory bench at room temperature ranged from 28.2% to 29.3% and averaged 28.8% ± 0.4% (mean ± SD) (Fig. 2B).

Unrefrigerated blood samples that were shipped locally or transcontinentally and measured 2 to 7 days later demonstrated COHb ranging from 28.1% to 29.2% and averaging 28.5% ± 0.3% (Table 1).

Results from telephone surveys of CO-oximeter manufacturers and local laboratories are listed in Tables 2 and 3.

Table 1 Carboxyhemoglobin levels in heparin-anticoagulated blood sample tubes after shipping

Shipped to	Days in transit	Sample 1 (%)	Sample 2 (%)	Average temperatures in destination city during time of transit (°C)
Seattle, Wash	2	28.1	29.2	11/5
Jacksonville, Fla	5	28.7	28.8	24/13
Indianapolis, Ind	7	28.7	28.6	17/1

Table 2 Anticoagulants recommended by major CO-oximeter manufacturers for use in their instruments

CO-oximeter manufacturer	Heparin	EDTA	Citrate
Instrumentation Laboratories	Yes for IL 682 Yes for GEM OPL	Yes for IL 682 No for GEM OPL	No for IL 682 No for GEM OPL
Radiometer America	Yes for ABL 800 Series	No for ABL 800 Series	
Siemens Medical Systems Diagnostics	Yes for M400 Series	No for M400 Series	No for M400 Series
	Yes for M800 Series	No for M800 Series	No for M800 Series

Source of information was the operation manual for the respective instruments and personal telephone communication with technical support personnel for each company.

4. Discussion

The Centers for Disease Control and Prevention recently published case definitions for various types of chemical poisoning, including that of CO [6]. Although these were designed to facilitate uniform reporting of illness resulting from chemical exposure among public health agencies, the importance of demonstrating an elevated blood COHb level to advance a case from "probable" (based on clinical grounds) to "confirmed" is emphasized. In that definition, a COHb concentration of greater than 5% in nonsmokers and greater than 10% in smokers, as determined by hospital or commercial laboratory tests, is considered confirmatory.

However, a recent survey of acute care hospitals in a 4-state region found that only 44% had CO-oximetry capability [7]. Hospitals in smaller communities were less likely to have a laboratory CO-oximeter than those in more populated areas. When they did not, most hospital laboratories indicated that they send samples to outside laboratories for COHb measurement. In some instances, this involves the transport of blood across town or to a nearby town by courier, taking from minutes to a few hours. Some, however, send samples for COHb measurement to contracted reference laboratories more than 1000 miles away, taking days. Although the utility of the latter practice in the management of a patient acutely poisoned with CO is questionable, it nonetheless occurs.

The most common instance that a blood sample would be drawn and sent with a transported patient for COHb measurement at the receiving hospital would be referral for hyperbaric oxygen treatment of suspected severe CO poisoning. Carbon monoxide binds to hemoglobin reversibly and in competition with oxygen. Such a patient would be

Table 3 Opinions of regional clinical laboratories regarding appropriateness of different anticoagulants for COHb measurement and stability of COHb in whole blood samples

Laboratory type	Heparin tube (green top)	EDTA tube (purple top)	COHb stability (refrigerated)	COHb stability (room temperature)
Commercial reference 1	Preferred	Accepted	7 d	8 h
Commercial reference 2	Not accepted	Preferred	5 d	
Medical center 1	Required	Not accepted	4 h	
Medical center 2	Required	Not accepted	<1 h	
Medical center 3	Required	Not accepted	2 h	30 min

transported on high-flow supplemental oxygen, which accelerates the clearance of COHb from the circulation. If the transport takes hours, it is possible that circulating COHb could normalize by arrival. An elevated COHb level is primarily used to support the diagnosis of CO poisoning and not necessarily direct management. The Undersea and Hyperbaric Medical Society recommends hyperbaric oxygen therapy for CO-poisoned individuals with the greatest mortality and morbidity risks [8]. These include patients with transient or prolonged unconsciousness, neurologic signs, cardiovascular dysfunction, or severe metabolic acidosis, irrespective of the degree of elevation of their COHb levels. It is noted, however, that most hyperbaric physicians do use hyperbaric oxygen to treat patients with less severe symptoms when presenting COHb levels are elevated to the range of 25% to 30% [9]. To assess this accurately, it is necessary to draw blood samples as soon as possible after CO poisoning is suspected.

Either of these practices (sending blood samples to outside laboratories or with transported patients) presumes that COHb is stable in whole blood samples obtained from a living person. Previous work has demonstrated COHb stability in highly processed hemolysate made from blood collected in tubes containing EDTA [10], in samples collected postmortem in tubes containing sodium fluoride or EDTA [11], and in samples of human donor blood anticoagulated with EDTA [5]. However, at least 3 major manufacturers of laboratory CO oximeters recommend using samples anticoagulated with heparin (green-top tubes) for 1 or more of their instruments (Table 2). Acids such as EDTA have the potential to lower the sample pH, alter the binding of CO to hemoglobin, and thereby change the COHb concentration. Furthermore, EDTA and citrate have the potential to damage sensor electrodes present in some instruments. Despite the fact that heparin is the preferred anticoagulant for COHb samples, no studies have previously examined stability using it.

Laboratory requirements for COHb samples and beliefs regarding their stability vary widely. In our survey of laboratories in the Seattle area, information obtained differed greatly (Table 3). Heparin-anticoagulated specimens were preferred, accepted, or not accepted, depending on the laboratory. Similar responses were obtained for EDTA-anticoagulated samples.

In the present study, COHb levels were very stable in heparin-anticoagulated whole blood samples either refrigerated or stored at room temperature for up to 4 weeks. Levels were also stable in samples shipped at ambient temperature for distances of up to 5000 miles over transit times of up to 1 week. The average level for all samples measured was 29.0% ± 0.4%. In comparison, when pooled samples were sent by the American College of Pathologists to 4031 laboratories using a total of 20 different CO oximeters in 2006, the average was 24.8% ± 1.3% [12]. Carboxyhemoglobin values seen in this study appear to be within the error of laboratory CO-oximetry testing.

The stability of COHb in heparin-anticoagulated blood sample tubes demonstrated in this study supports the concept that it is reasonable to send samples to other laboratories for measurement when CO-oximetry is unavailable. Furthermore, such samples do not appear to require refrigeration. When evaluating a patient for acute CO poisoning, it is probably only necessary to know that the sample will be stable for up to a day. However, there may be other nonemergent situations where longer delay because of distant shipment of samples is not unreasonable. Because stability was demonstrated for up to 1 month, it would seem that all such circumstances would be encompassed by the present findings.

It has been previously demonstrated that hospitals without laboratory CO-oximetry are located in smaller towns and are presumably smaller institutions with fewer resources [7]. A new pulse CO-oximeter capable of measuring heart rate, oxygen saturation, and also COHb was recently marketed [4]. In an emergency setting, this would certainly provide a reasonable alternative to blood sample shipment, as it would provide immediate results. It may provide a convenient solution for many hospitals in the future. If the device is not available, however, heparin-anticoagulated samples in sealed tubes can be transported with confidence that the COHb level will be stable when measured at a later time.

Acknowledgment

The author thanks Karen A. Boyd, NCA (CLS), and Donna Glaser, CCT (NRCT), for their expert technical laboratory support and Drs Manfred Mueller and Scott Silvers for their assistance with the shipped blood samples.

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