

## Stability of Carboxyhaemoglobin in Blood Samples at Different Periods and Temperatures: A Forensic and Toxicological Tool for Diagnosis

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### Abstract

Carbon monoxide (CO) is one of the most toxic agents in clinical and forensic practices. Diagnosis of CO poisoning is a challenging task and needs a high level of suspicion. Carboxyhaemoglobin (COHb) level is considered the only established marker for diagnosis. The current work aims to determine the levels of COHb in blood samples collected from CO poisoned patients on admission and to re-estimate those levels after storage of samples for different periods and after incubation at various temperatures. The results showed that the mean concentrations of carboxyhaemoglobin at time of admission =  $23.05 \pm 13.44$ . Levels demonstrated insignificant change after either refrigerated storage of samples for different periods (one, two and three years) or after their incubation at different temperatures (37°C, 40°C and 50°C). It can be concluded that COHb concentration remains stable in refrigerated stored blood samples for up to 3 years as well as those present in high temperatures. It is recommended to immediately collect and store blood samples from patients suspicious of CO poisoning. When CO oximetry is not available, samples could be transported and sent to outside laboratory for analysis even after a long time has been passed. This could have great toxicological and medicolegal implications in cases of CO poisoning whether intentional or due to accidents and fires.

**Keywords:** Carboxyhaemoglobin; Stored blood; Temperature; Time; Forensic toxicology

### Introduction

Carbon monoxide is considered one of the most toxic agents in both clinical and forensic practices. It is produced as a result of incomplete combustion of hydrocarbons either from industrial source, incompatible engines or poorly functioning heating systems [1]. It may be responsible for more than half of all fatal poisonings all over the world [2,3]. In addition, carboxyhaemoglobin (COHb) level is considered a reliable indicator for contribution of this toxic gas to death in cases of intoxication and fires and would be helpful in determination of whether the patient was alive at the incident of fire or not [4-6].

In clinical practice, carbon monoxide (CO) poisoning may mimic many clinical diseases as symptoms and signs are often non-specific ranging from flu-like manifestations up to coma and death. Diagnosis is a challenging task and needs a high level of suspicion as misinterpretation may lead to a fatal outcome [1,7,8] which would be later on a problem facing both clinical toxicologists and forensic practitioners concerning the cause of death.

From a forensic point of view, CO has been considered a major contributing factor in fire deaths, aircraft accidents, and intentional exposure to autoexhaust. Measurement of COHb level is necessary as it is believed to be the only established marker for proper diagnosis of CO poisoning. Confirmation is done by reporting elevated COHb level more than 2% for non-smokers and more than 10% in smokers. It also assists to determine the cause of death whether it is due to fire or poorly functioning heating systems [9-12].

Hence, COHb detection is an important issue of medicolegal implications and has been a common diagnostic tool in emergency medicine and forensic toxicology. This is particularly valuable for evaluation of either the degree of CO toxicity in arsons, suicide, motor car and industrial accidents or its contributing role in deaths where COHb level is 10-50% [13-15].

There are controversial studies regarding the stability of COHb in blood samples at different conditions. Hence, the current work was

performed aiming to determine levels of COHb in blood samples collected immediately from CO poisoned patients on their admission and to assess stability of COHb and if it is reliable to re-estimate its concentration after storage of those samples for different periods and after their incubation at various temperatures particularly in cases with medicolegal implications.

### Patients and Methods

#### Patients

This work was conducted on blood samples of fourteen patients poisoned with carbon monoxide and presented to Mansoura Emergency Hospital, Toxicology Unit in the period between January 2005 and December 2006.

All patients underwent thorough medical examination and emergency treatment. Five ml blood sample was collected from each patient into heparinized test tubes for immediate baseline measurement of COHb levels. Samples were sealed tightly with rubber and parafilm, labeled and date of admission was recorded for every patient. Then, samples were refrigerated at 4°C until further analysis.

#### Ethical consideration:

A written informed consent was taken from all CO poisoned patients or their relatives to perform this study.

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## Methods

**Materials:** Two Haemoximetry Qualicheck ampoules were used (heat sterilized 2 ml each). One has 33.7% COHb and was used as low control while the other ampoule contains 37.3% COHb and was used as high control.

Teflon screw capped 12 ml test tubes.

Heparinized capillary tubes for injection of samples (interior: 1×1-1.2×75 mm), (exterior: 1.5-1.6 mm). Rinse solution 250 ml for OSM 2/3 (made in Denmark by Radiometer A/S).

**Equipment:** This study was performed using Gas analyzer radiometer (OSM 3) for the initial analysis of samples.

Gas analyzer radiometer is an automated visible spectrophotometric analyzer used for identification and quantification of different blood gases including COHb% in untreated blood samples. It is operated, calibrated and maintained according to the manufacturer's specification (Haemoximetry Qualicheck™ ©Radiometer A/S, Emdrupvej 72, DK-2400, Copenhagen NV, Denmark).

Radiometer's OSM3 haemoximeter is a computerized system measuring absorbances of different haemoglobin derivatives in a 35 µl whole blood sample at six fixed wavelengths (535, 560, 577, 622, 636, and 670). The COHb levels were then calculated automatically through a 6×6 matrix-equation [16].

**Analysis of COHb level:** An initial immediate baseline assay for each sample was done as an integrated step for initial toxicological evaluation of poisoned cases.

Sample number	COHb% at different storage durations			
	0	1 year	2 years	3 years
1	18.25	18.71	18.65	18.55
2	18.33	18.15	18.12	18.37
3	40.54	39.73	40.54	38.33
4	6.73	7.56	7.41	7.14
5	17.52	18.18	17.84	17.68
6	51.35	51.52	51.42	51.27
7	22.16	22.54	22.53	22.52
8	37.67	38.35	38.25	38.13
9	26.32	26.32	26.32	26.35
10	4.57	5.14	4.86	4.62
11	14.32	15.62	15.41	15.34
12	14.23	14.41	14.34	14.33
13	22.35	22.71	22.48	22.52
14	33.38	33.35	33.39	33.34

**Table 1:** The levels of carboxyhaemoglobin (COHb) in refrigerated stored blood samples measured at different periods (n=14 samples).

Sample	Mean ± SD	Paired t-test (pairs compared)	Sig. (2-tailed)
COHb on admission	23.05 ± 13.44		
COHb after 1 year	23.74 ± 12.98	COHb on admission and after 1 year	0.068
COHb after 2 years	23.68 ± 13.11	COHb on admission and after 2 years	0.078
COHb after 3 years	23.46 ± 12.92	COHb on admission and after 3 years	0.326
COHb at 37°C (12hs)	23.13 ± 13.15	COHb on admission and at 37°C	0.691
COHb at 40°C (5hs)	23.64 ± 12.92	COHb on admission and at 40°C	0.160
COHb at 50°C (1 h)	23.56 ± 12.97	COHb on admission and at 50°C	0.229

P>0.05 (insignificant)

**Table 2:** Comparison of carboxyhaemoglobin% (mean ± SD) in refrigerated stored blood samples at different periods and after incubation at various temperatures and durations (n=14).

Then, all samples were stored and refrigerated at 4°C till reanalysis of COHb level repeatedly after different periods for 3 times (after one year, 2 years and 3 years).

A further re-estimation of COHb% was done after incubation of blood samples at different temperatures as follows: 37°C for 12 hours, 40°C for 5 hours, and 50°C for 1 hour (Shell.Lab, Sheldon Manufacturing Inc., Model 1500 E Incubators).

The same was done for 4 control samples (two high and two low standards). Head space was fixed at 1 cm for control samples.

**Statistical analysis:** The statistical analysis of data was done by using SPSS program (Statistical Package for Social Sciences "SPSS" version 16, Inc, Chicago, IL). As the number of samples is less than 30, Kolmogrov-Smirnov test was used for analysis of normality of distribution of data and it was insignificant (P=0.2). Quantitative data were presented as mean ± SD. Paired t-test was used to examine significance between pairs of groups. Significance was set at p<0.05.

## Results

Table 1 illustrated the levels of carboxyhaemoglobin (COHb) in refrigerated stored blood samples measured at different periods.

The concentrations of carboxyhaemoglobin (COHb) (mean ± SD) in refrigerated stored blood samples measured at different periods and after incubation at various temperatures and durations are demonstrated in table 2. Comparison between pairs of samples is also shown and tailed t-test is insignificant at P>0.05. All the pairs displayed insignificant difference regarding the means of COHb concentration.

## Discussion

The aim of the current study is to determine levels of COHb in blood samples collected from CO poisoned patients presented to Mansoura Emergency Hospital, Toxicology Unit and to evaluate whether the storage of these samples for different periods and their incubation at various temperatures would affect the initial COHb concentration.

In the present work, the concentrations of carboxyhaemoglobin in the collected samples were high at time of admission (mean ± SD=23.05 ± 13.44) supporting the provisional diagnosis of CO poisoning based upon clinical data.

In the clinical field, the finding of an elevated COHb level is considered a powerful proponent for confirmation of clinical diagnosis of carbon monoxide intoxication [10,17]. The half-life of COHb is 4-5 hours [7] so; immediate collection and analysis of patient's samples were done to reflect the real COHb saturation in their blood at time of admission.

In the current study, determination of stability of carboxyhaemoglobin was performed through successive serial measurement of the refrigerated stored samples after different periods (one, two and three years). Comparison of levels of COHb showed insignificant differences (p>0.05).

Likewise, Hampson [9] stated that either refrigerated heparinized blood samples or those stored at room temperature (22°C) for up to one month have shown stable levels of carboxyhaemoglobin. Moreover, Aberg et al. [18] found that COHb concentration has not changed in donated blood of smokers which was stored for 1 week in a refrigerator.

Kunsman et al. [14] showed stability of COHb analysis, but their work was performed in postmortem blood samples. They also defined

CO stability as a second analysis result that is within the range of the first analysis giving a similar interpretation of COHb level. This could be attributed to strong union of CO to haemoglobin even in low concentrations [19,20].

Storage of blood samples for a long duration is not an obstacle to re-estimate COHb level. This could be explained by reliability of measurement of COHb levels in putrefied blood samples as stated by Lee et al. [21]. The authors used six-wavelengths CO-oximetry to examine ten putrefying blood or blood-containing cavity fluid samples, originating from CO poisoning cases. They found that this type of CO-oximeters is appropriate for the determination of COHb levels in putrefied blood. Hence, even if decay occurred in the specimen, it is still possible to estimate COHb levels. However, the studied blood samples didn't show such putrefaction.

In the present study, incubation of blood samples at different temperatures for variable durations did not affect the levels of COHb. No significant difference was found between incubated samples and refrigerated ones. There was an insignificant change of COHb levels with incubation at 37°C for 12 hours, 40°C for 5 hours and 50°C for an hour ( $p>0.05$ ). Additionally, the volume of head space above the blood samples did not affect the concentration of COHb and interpretation of results.

In contrast, previous studies claimed that head space volume and storage temperature could affect the stability of CO "the bigger the surface area and increased temperature, the greater the loss of CO" [22,23]. In agreement with our work, Kunsman et al. [14] stated that the initial COHb% was not significantly affected by volume of head space in refrigerated postmortem blood samples following specimen collection at autopsy. More or less similar, Seto et al. [24] claimed that COHb is relatively resistant to heat denaturation. They conducted an *in vitro* experiment which demonstrated that overnight incubation of blood samples as well as their mild heating at 54°C for 3 hours showed insignificant change of COHb levels.

Hampson et al. [4] mentioned that hospitals and health care units in small towns and villages have few resources with no available laboratory CO-oximetry. In developing countries like Egypt, COHb% determination is not always available in all health care settings. It may be only performed in very few big institutions which have CO-oximetry. In addition, blood samples could be exposed during transportation to high temperatures which may reach more than 40°C especially in Upper Egypt.

So, heparinized blood samples could be collected, sealed tightly and transported to be analyzed for COHb% with the assumption that its concentration will be stable when measured later on. This is true for either samples transported for long distances at various higher temperatures or those refrigerated samples stored for up to three years at 4°C particularly in cases with questionable forensic issues. In Egypt, many legal problems may take years in the court to be solved or old cases might be re-investigated for a final decision with the possible need for reanalysis of stored samples after a long time has been passed.

## Conclusions and Recommendations

Based upon the present results, it can be concluded that COHb concentration remains stable in both refrigerated stored blood samples for up to 3 years and those present in high temperatures for variable durations. Stability of COHb in blood warranted the immediate collection and storage of blood samples from patients suspicious of CO poisoning to be sent to outside laboratory when CO oximetry is not

available. They can be analyzed after a long time has been passed even after death.

It is also recommended that the medicolegal expert should not reject the non-refrigerated heparinized blood samples transported to the forensic laboratory even those subjected to temperature up to 50°C to measure COHb saturation. This might have great medicolegal implications in cases of CO poisoning whether intentional or due to accidents and fires so as to determine COHb level and to decide if the poison would play a role in causation of death.

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