

# CARBOXYHEMOGLOBIN

Spectrophotometric determination  
of Carboxyhemoglobin on whole blood  
Rodkey method, modified by Beutler and West

5 x 5 ml

REF CM06-25

## Intended use

Kit for quantitative *in vitro* determination of Carboxyhemoglobin on whole blood.

## Principle

The sample is incubated with a reducing agent which converts all hemoglobin into carboxyhemoglobin and reduced hemoglobin. This procedure eliminates any possible interference from oxyhemoglobin and methahemoglobin. COHb % is spectrophotometrically determined by Rodkey simplified formula, which is obtained by the result of a system based on two equations, representing the molar Hb and COHb absorptivities applied to Lambert-Beer law. For reliable determinations, accurately calibrate the spectrophotometer and verify the presence of a small passing-through band.

## Sample

Whole blood anticoagulated with heparin or EDTA.

STABILITY: at least 5 days at room temperature or at 2-8°C.

## Reagents

Kit components:

### REAGENT 1

Buffer pH 6.85

\*REAGENT 2/A (predosed)

Reducing reagent

### REAGENT 2/B

Buffer pH 6.85

REF CM06-25

CM06-25R1: 2 x 35 ml

CM06-25R2: 5 vials

CM06-25R3: 5 x 5ml

(\* ) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 20-25°C, sealed reagents are stable up to the expiration date on the label. Store away from light.

## Preparation of Reagent 2

Right before starting the procedure, pour the contents of one vial of Reagent 2/B into one vial of Reagent 2/A with a Pasteur pipette. Shake gently by inversion and avoid incorporating air.

WARNING: Reagent 2 fear humidity.

STABILITY: use Reagent 2 within 60 minutes after it is prepared.

## Manual Assay Procedure

Method: spectrophotometric  
Wavelength: 420 nm, 432 nm  
Optical path: 1 cm  
Temperature: room temperature (20-25°C)  
Reaction time: 10 minutes  
Reading: against Reagent 2

## Preparation of the hemolysate

Pipette into a small glass tube:

Reagent 1	3.0 ml
Sample	25 µl

Mix by inversion two or three times. Let it stand for 5 minutes to allow complete cellular lysis.

## Determination of COHb %

Pipette into a small tube:

Reagent 2	1.15 ml
Hemolyzed sample	100 µl

Mix gently by inversion and incubate for at least 10 minutes at room temperature. Do not wait more than 60 minutes.  
Pour the contents of the glass tube into a glass cuvette and read the absorbance at 420 nm and 432 nm against Reagent 2.

## Calculation

Calculate the  $A_r$  ratio between the absorbances at 420 nm and 432 nm:

$$A_r = A_{420} / A_{432}$$

Put the obtained value ( $A_r$ ) in the following equation:

$$\% \text{ COHb} = [1 - (A_r \times F_1)] \times 100\% / [A_r \times (F_2 - F_1) - F_3 + 1]$$

where:

$F_1$  = molar absorptivities  $Hb_{432} / Hb_{420}$

$F_2$  = molar absorptivities  $COHb_{432} / Hb_{420}$

$F_3$  = molar absorptivities  $COHb_{420} / Hb_{420}$

If the spectrophotometer is calibrated, the following values of molar absorptivities published by Rodkey are correct, for human hemoglobin. They correspond to ratio the  $F_1, F_2$  and  $F_3$  as follows:

Wavelength	AHb	ACOHB	$F_1 = 1,3330$
420 nm	0,988	1,970	$F_2 = 0,4787$
432 nm	1,317	0,473	$F_3 = 1,9939$

## Control of the Calibration

As control standards are not available on the market, to check the method efficiency, compare smokers and nonsmokers blood and note the difference. However, sometimes it is advisable to verify the spectrophotometer calibration, with the following procedure:

Pipette into two class A glass balloons, respectively labeled COHb and Hb:

	COHb	Hb
Nonsmoker hemolyzed blood	2 ml	2 ml
Saturation with CO	YES	---
Saturation with air	---	YES
Reagent 2 up to volume of	25 ml	25 ml

Read immediately at 420 and 432 nm against Reagent 2 and calculate  $F_1, F_2$  e  $F_3$  as described above.

Prepare the hemolysate as describe in the technical sheet, in sufficient quantity (for example: 50 µl + 6 ml of Reagent 1).

## Reference Values

Indication of carbon monoxide intoxication:

% COHb	
< 4 %	nonsmoker
3 - 10 %	smoker
< 10 %	light intoxication
30 - 40 %	serious intoxication
> 50 %	coma, death

## Reference

1. E. Beutler et C. West, "Clin. Chem.", 30 (6), 871-874 (1984).
2. G. Heinemann, K. Löschenko et H. Schievelbein, "J. Clin. Chem. Clin. Biochem.", 17 (10), 647-651 (1979).

## KEY SYMBOLS

	In Vitro diagnostic medical device
	batch number
	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD

CE

Ed. 01 - Jul 2022

## MANUFACTURER



FAR

Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY

phone +39 045 6700870

website <http://www.farddiag.com>

e-mail: [order@farddiag.com](mailto:order@farddiag.com) e-mail: [farddiag@farddiag.com](mailto:farddiag@farddiag.com)