Hemoglobin

Drabkin. Colorimetric

Quantitative determination of hemoglobin

CE IVD

PACKAGING

Ref.: 101-0368	Cont.: 50 x 4 x 5 mL

Store at 2 - 8º C

CLINICAL SIGNIFICANCE

The hemoglobin is a protein that contains iron and that the red color to the blood. The hemoglobin is in red globules and it is the one in charge of oxygen transport by the blood from the lungs to weaves.
When the level of hemoglobin appears underneath the normal levels is describing an anemia that can be of different origins: primary anemia, cancer, pregnancy, renal diseases, and hemorrhages.
If the hemoglobin levels appear high it can be due to: cardiopathies, dehydratation and stays in places of much altitude[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Hemoglobin is oxidized by potassium ferricyanide into methaemoglobin, which is converted into cyanomethaemoglobin, by potassium cyanide.
The intensity of the color formed is proportional to the hemoglobin concentration in the sample[1,2].

REAGENTS

HEMOGLOBIN 50x	Potassium ferricyanide Potassium cyanide Dihydrogen potassium phosphate	0.60 mmol/L 77 mmol/L 2 mmol/L

Optional

HEMOGLOBIN CAL	Hemoglobin Standard 15 g/dL Animal origin

PRECAUTIONS

Potassium cyanide: Harmful (Xn): R20/21/22: Harmful by inhalation, in contact with skin and if swallowed. R32: Contact with acids liberates very toxic gas.
Environmentally dangerous (N): R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S7: Keep container tingly close. S28: After contact with skin, wash immediately with plenty of water.S29: Do not empty into drains.
S45: In case of accident or if you feel unwell, seek medical advice immediately. S60: This material and its container must be disposed of as hazardous waste. S61: Avoid release to the environment. Refer to special instructions/safety data sheets.
Cyanide (poison): The amount of cyanide in the Reagent Concentrate (50x) is appreciably less than the minimum lethal dose for an adult. Gaseous hydrogen cyanide will be released on contact with acids.

PREPARATION

Working reagent (WR):
- For 5 mL 4.9 mL of distilled water + 2 drops of Reagent
- For 250 mL 245 mL of distilled water + 1 vial (5 mL) of Reagent

Mix well.
Stability: 2 months at 2 - 8º C, protected from the sunlight.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm ≥ 0.01.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 540 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Venous or capillary blood[1].
Use anticoagulants like EDTA, heparin or oxalate.
Stability of the sample: 1 week at 2 - 8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 540 nm
   Cuvette: . . . . . 1 cm. light path
   Temperature . . . . . 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette:

A) MACRO METHOD:

	Blank	Standard	Sample
WR (mL)	5.0	5.0	5.0
Calibrator (µL)	--	20	--
Sample (µL)	--	--	20

B) MICRO METHOD:

	Blank	Standard	Sample
WR (mL)	2.5	2.5	2.5
Calibrator (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 3 min. at room temperature (15 - 25º C).
5. Read the absorbance (A) of the samples and calibrator, against the Blank.

CALCULATIONS

With factor[2]:

(A) Sample x 36.77 = g/dL hemoglobin in the sample

With calibrator:

$$\frac{(A)\,Sample}{(A)\,Standard} \times 15\ (Standard\ conc.) = g/dL\ hemoglobin\ in\ the\ sample$$

QUALITY CONTROL

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Men 14 - 18 g/dL ≅ 8.7 – 11.2 mmol/L
Women 12 - 16 g/dL ≅ 7.5 – 9.9 mmol/L

These values are for orientation purpose; each laboratory should establish its own reference range.

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PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0.1 g/dL to *linearity limit* of 20 g/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (g/dL)	8.00	15.2	7.81	15.1
SD	0.29	0.33	0.19	0.26
CV (%)	3.59	2.19	2.51	1.74

Sensitivity: 1 g/dL = 0.027 A.

Accuracy: Results obtained using CHRONOLAB reagents did not show systematic differences when compared with other commercial reagents.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

A list of drugs and other interfering substances with hemoglobin determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

1. Franco R S. Hemoglobin. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1294-1296 and 418.
2. Van Kampen EJ et al. Standarization of hemoglobinometry Clin. Chim 1961;6: 438-544.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed. AACC 2001.
5. Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 1995.

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