HEMOGLOBIN
Insert

Ref.: 43

Intended use . System for hemoglobin determination in total blood by end point colorimetric reaction.

Test principle . Fe (II) of heme group of hemoglobin, oxyhemoglobin, and carboxyhemoglobin is oxidized to ferric by ferricyanide yielding hemiglobin (Hi), what reacts to ionized cyanide yielding hemiglobin cyanide (HiCN) that is measured at 540 nm.

Summary . The Color Reagent pH is low to accelerate the reaction velocity. It was also added a non-ionic detergent to avoid presenting turbidity.

The comparison of imprecision found in the repetitivity and reproducibility tests demonstrate that the system of measurement is very robust in the regions of significant concentrations for the clinical use, indicating a safe performance daily.

The system is easily applied to most automated and semi-automated equipments capable to measure with accuracy the absorbance at 540 nm.

Methodology . Hemiglobin cyanide (HiCN).

Reagents

1. R 1 **- Color Reagent - Stock - Store at 15 - 25 ºC.**
Reagent label bears expiration date. Potassium ferricyanide (60.7 mmol/L), Potassium cyanide (76.8 mmol/L) and surfactant. Do not refrigerate. It is poisonous. Handle with care.

Precautions and warnings

For in vitro diagnostic use.

Disposal of all waste material should be in accordance with local guidelines.

The usual security cares should be applied on the reagent handling.

The Color Reagent contains potassium cyanide that is poisonous. Avoid ingestion. In case of eyes contact, immediately flush eyes with plenty of water and get medical assistance.
Do not dispose of the Color Reagent (Stock, Working, or the material used for measuring) in the sink that contains residues of sulfuric acid.

Do not refrigerate the reagent. Do not store in plastic bottle.

Storage and stability . Unopened reagents, when stored at indicated temperature, are stable up to expiration date shown on the label.

Deterioration . Microbial or chemical contamination may decrease reagents stability.
Color Reagent in Use is not suitable in case of contamination signs or if it develops turbidity.

Specimen collection and preparation

Use total blood (citrate, EDTA or oxalate) what can be stored for about 7 days at 4 ºC.
False increased hemoglobin values may occur with a prolonged tourniquet use while collecting the sample.

No known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

Interference

Bilirubin up to 38 mg/dL and triglycerides up to 250 mg/dL do not interfere significantly.

Triglycerides values over 250 mg/dL provide false increased results.

Materials required not provided

1. Photometer capable of measuring absorbance at 520 - 550 nm.
2. Pipettes to measure reagents and samples.
3. Timer.
4. Hemoglobin Standard Ref. 47.

Preparing the working color reagent . Add the content of a bottle (10 mL) to 990 mL of distilled or deionized water. It is stable 6 months in a dark bottle at 15 - 25 ºC.

The water must have resistivity ≥1 megaohm, or conductivity ≤1 microsiems and silicates concentration must be <0.1mg/L.
Do not refrigerate and do not store in a plastic bottle.

Manual procedure

	Unknown
Working Color Reagent	5.0 mL
Total Blood	0.02 mL

Homogenize and wait 5 minutes. Measure the absorbance at 540 nm or green filter (520 - 550 nm) against distilled water. The color is stable for several hours. Get the value in g/dL using the calibration factor obtained with the Standard Hemoglobin Labtest (Ref.: 47).

Labtest

The suggested measurement procedure is appropriated to photometer of which the minimal volume of solution for reading is equal or lower than 5.0 mL. It should be done a verification of the necessity of volume adjustment for the photometer to be used. Sample and reagent volume may be modified proportionally without affecting the test performance and the calculation procedure. In case of volume reduction is important to observe the minimum volume needed to the photometric reading. Volume of sample lower than 0.01 mL is critical in manual applications and should be avoided because it increases the measurement imprecision.

Calibration

Traceability . The milimolar absorptivity of hemoglobin is used as reference system for the assay calibration. The calibration of the photometer is traceable to the Standard Reference Material (SRM) 931 of the National Institute of Standards and Technology (NIST).

Manual calibrations
Two points calibration;
After reagent lot change;
When the internal quality control indicates or the bottle is changed.

Measurement / reportable range

Measurement result is linear up to 25 g/dL.

Quality control . The limits and control interval must be adapted to the laboratory requirements. Each laboratory should establish corrective actions to be taken if values fall outside the control limits.

Calculations

Calculation must be performed using the Standard Hemoglobin Labtest (Ref.: 47).

$$\text{Hemoglobin (g/dL)} = \frac{\text{A Unknown}}{\text{A Standard}} \times 10$$

Due the great reproductive results of the assays system, it is possible to use the factor method:

Calibration factor = 10/A standard

Hemoglobin (g/dL) = A Unkown x Factor

Expected values . Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Men . 12.5 - 17.5 g/dL

Women . 11.5 - 15.5 g/dL

Conversion factor . Conventional Unit (g/dL) x 0.621 = IS Unit (mmol/L).

Performance characteristics

Recovery studies . In two samples with hemoglobin concentrations of 7.3 and 8.0 g/dL were added different quantities of the analyte. Subsequent analyses provided recoveries ranging from 99 to 103%. The mean proportional systematic error at 10 g/dL decision level was 0.1 g/dL or 1 %.

Method comparison . A group of 80 samples were assayed by the proposed method and a similar technique. Sample hemoglobin values ranged from 5.0 - 24.9 g/dL. The comparisons yielded a correlation coefficient of 0.986 and regression equation was y = 1.03x - 0.23. The total systematic error (constant and proportional) verified at the decision level (10 g/dL) was equal to 0.07 g/dL or 0.7 %.

Imprecision - Within Run

	N	Mean (g/dL)	SD (g/dL)	(%) CV
Sample 1	20	9.6	0.12	1.2
Sample 2	20	17.1	0.23	1.4

Imprecision - Run-to-Run

	N	Mean (g/dL)	SD (g/dL)	(%) CV
Sample 1	20	9.1	0.15	1.5
Sample 2	20	16.5	0.23	1.4

Analytical sensitivity . Detection limit: 0.39 g/dL. The detection limit represents the lowest measurable hemoglobin concentration that can be distinguished from zero. It is calculated as two standard deviations of 20 replicates of one sample without hemoglobin. Using the Standard Absorbance as parameter, the limit of photometric detection is 0.04 g/dL, corresponding to an absorbance equal to 0.001.

Matrix dilution effects . Two sample with values equal of 27.4 and 24.0 g/dL were used to evaluate the system response on the matrix dilutions with 150 mmol/L NaCl (0.85%). Recoveries were found a range of 100 and 109 %, using dilution factors that vary from 2 to 4.

NOTES

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiems/cm and silicates concentration must be <0.1mg/L.

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References

1. Drabkin DL., Austin JH. J Biol Chem 1935;112:51.

2. Henry RJ, Cannon DC, Winkelman JW. Clinical Chemistry. Principles and Technics, 2nd Ed. New York, Harper & Row, 1974.

3. Westgard JO, Groth T. Clin Chem 1981; 27:493-501.

4. Labtest: data on file.

Presentation

Product	Reference	Contents
Hemoglobin	43	R 1 2 X 10 mL

Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

Labtest Diagnóstica S.A.

CNPJ: 16.516.296 / 0001 - 38

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Periodo post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		

Ref.: 140214

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