

prietest™ Clinical Chemistry Reagents

HEMOGLOBIN - CMG

In vitro diagnostic test kit, for professional use only

INTENDED USE : Quantitative in vitro determination of Hemoglobin in whole blood on photometric systems.

ORDERING INFORMATION **Pack Size** **Cat No.**
 1 X 1000 ml HBCMG 1000

CLINICAL SIGNIFICANCE :

Hemoglobin conveys dissolved gases in plasma, especially O₂ and CO₂, and regulates cell gas exchanges. Hemoglobin also takes part in maintenance of the plasmatic buffer power. Increased levels are found in polycythaemia, congenital cyanotic heart disease, heat stroke and dehydration. Decreased levels are found in anemia resulting from deficiency of iron or folic acid, red blood hemolysis, defective globin synthesis and structural abnormalities.

METHOD :

Colorimetric, Cyanmethaemoglobin Method, End point.

PRINCIPLE :

Potassium ferricyanide converts hemoglobin to methaemoglobin. The methaemoglobin further reacts with potassium cyanide to produce a stable cyanmethaemoglobin complex. Intensity of the complex formed is directly proportional to the amount of hemoglobin present in the sample.



REAGENT COMPOSITION :

Potassium Ferricyanide 0.60 mmol/l
 Potassium Cyanide 0.75 mmol/l

Preservative & Stabilizer

STORAGE INSTRUCTIONS AND REAGENT STABILITY :

The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 20°C to 25°C, protected from light and contamination is avoided.

WARNINGS AND PRECAUTIONS :

This reagent is harmful by inhalation, contact with skin, if swallowed and in contact with acids liberates very toxic gas. Do not pipette the reagent with mouth as it is poisonous. Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT :

Please refer to local regulation requirements.

REAGENT PREPARATION :

The reagent is ready-to-use.

MATERIAL REQUIRED BUT NOT PROVIDED :

NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, pipettes etc.

SPECIMEN :

Fresh whole blood collected in EDTA.

Discard contaminated specimens.

ASSAY PROCEDURE :

Wavelength : Hg 546 nm
 Optical path : 1 cm
 Temperature : 37°C
 Mode : End Point

Bring all the contents of the kit to Room Temperature prior to use.

Read absorbance of sample against distilled water.

Label the test tube as blank, sample, control and pipette into respective test tube the reagent, sample, control sample as per the table given below :

	Blank	Sample / Control
Distilled Water	5000 µl	—
Reagent	—	5000 µl
Sample / Control	—	20 µl

Mix well and allow to stand at R. T. for 5 minutes then read absorbance within one hour.

CALCULATION :

Concentration of unknown Sample (g/dl) = (Abs of Sample – Abs of Blank) X Factor
Factor at 546 nm = 36.8

CONVERSION FACTOR : Hemoglobin [g/dl] X 10 = Hemoglobin [g/L]

CALIBRATION :

For the calibration of photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL :

To ensure adequate quality, use of the commercially available control is recommended.

PERFORMANCE CHARACTERISTICS :

MEASURING RANGE :

The test has been developed to determine Hemoglobin concentrations within a measuring range from 1 to 25 g/dl (10 to 250 g/L).

SPECIFICITY/INTERFERENCE :

No interference was observed by Ascorbic Acid up to 5 mg/dl (283.9 µmol/L), Glucose up to 500 mg/dl (27.75 mmol/L) and lipemia up to 800 mg/dl (9.12 mmol/L) Triglycerides. A list of drugs and other interfering substances with Hemoglobin determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :

The lower limit of detection is 1 g/dl (10 g/L).

PRECISION :

Intra-assay precision n = 20	Mean [g/dl]	SD [g/dl]	CV [%]
Sample 1	7.46	0.07	0.94
Sample 2	11.6	0.10	0.86
Sample 3	19.1	0.07	0.37
Inter-assay precision n = 20	Mean [g/dl]	SD [g/dl]	CV [%]
Sample 1	7.67	0.11	1.43
Sample 2	14.4	0.17	1.18
Sample 3	19.10	0.32	1.67

METHOD COMPARISON :

A comparison between Robonik Prietest Hemoglobin (y) and a commercially available test (x) using 20 samples gave following results:

Linear Regression : $y = 0.9628x + 0.2367$ g/dl

Correlation Coefficient : $r = 0.9983$

REFERENCE RANGE :

Newborns : 16 to 25 g/dl (160 to 250 g/L)
 Infants : 11 to 14 g/dl (110 to 140 g/L)
 Male : 13.9 to 16.3 g/dl (139 to 163 g/L)
 Female : 12 to 15 g/dl (120 to 150 g/L)

It is recommended that each laboratory should assign its own normal range.

LITERATURE :

1. Van Kampen E.J. And Zijlstra W.G., Clinica. Chim. Acta., 6:538 (1961).
2. Sir John V. Dacie and S.M. Lewis., Practical Haematology., 8th edition.
3. Bauer, J.D., Hemoglobin. Clinical Chemistry, Theory, Analysis, and Correlation (Mosby ed.) 33 (1989), 513 - 517.

MEASUREMENTS ON COLORIMETERS :

On colorimeters where the exact wavelength of 540 nm (hg 546 nm) is not available the absorbances have to be taken on a yellow green filter.

A cyanmethaemoglobin standard (Hemoglobin Standard) available separately to be used. The absorbance of Standard (Abs. Std) is taken against distilled water. The test procedure remains the same as given before.

CALCULATIONS ON COLORIMETERS :

$$\text{Hemoglobin in g/dl} = \frac{\text{Abs. Sample} - \text{Abs. Bl.}}{\text{Abs. Std} - \text{Abs. Bl.}} \times \frac{251}{1000} \times 60$$

Where:

- 251 is the Dilution Factor i.e. Total Reaction Vol. (5.02 ml) / Sample Vol. (0.02 ml).
- 1000 is the Multiplication Factor to convert mg To Gram
- 60 is the Concentration of the Haemoglobin Standard in mg/dl.

PLOTTING A CALIBRATION CURVE:

If plotting of a calibration curve is desired then pipette into five clean dry test tubes

Dilution Factor	0.0	0.25	0.5	0.75	1.0
Addition Sequence	1 (ml)	2 (ml)	3 (ml)	4 (ml)	5 (ml)
Reagent	5.00	3.75	2.50	1.25	0.00
Standard	0.00	1.25	2.50	3.75	5.00

Mix well and measure the absorbance of the tube Nos. 2, 3, 4 and 5 against tube No. 1 which serves as a blank. Multiply the cyanmethaemoglobin concentration of the Standard (60 mg/dl) by 0.251 and dilution factor to get the corresponding Hemoglobin Concentration in g/dl for the tube Nos. 2, 3, 4 & 5 respectively. Plot these concentrations on the horizontal (X-axis) and corresponding absorbances on the vertical axis (Y-axis). It will be a straight line passing through the origin. Read the Hemoglobin Concentration of the test on X-axis corresponding to its absorbance (Abs.S) on the Y-axis of the calibration curve.

A 3 point calibration curve if desired can also be made with only tubes No. 1, 2 & 5 the rest of the procedure remains the same.

INSTRUMENT APPLICATION prietest TOUCH	
Name : HB,	Mod : END-P
Pri.: 546 , Sec.: 0	
Temp: 37C , KF : 1.000	
Vol : 500ul , Unit : g/dl	
Lag : 5 , Read : NA	
Blk : Y, QC : Y, Norm : Y	
Std : N , Factor : 36.8	
Normal HI = 16.3	
Normal LO = 12	
QCNH : *	
QCNL : *	
QCABH = *	
QCABL = *	
Rgnt. Linearity : 25	
NOTE :	
* Indicates user definable parameter.	
NA Implies Not Applicable	

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	HEMOGLOBIN-CMG
Reaction	End Point
Wavelength 1	546 nm
Temperature	37°C
Zero Setting	Distilled Water
Factor	36.8
Units	g/dl
Sample Volume	20 µl
Reagent Volume	5000 µl
Incubation Time	5 minutes
Reference Range	12 to 16.3
Reagent Linearity	25

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