Quantitative determination of Hemoglobin in Whole Blood
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
HB 500	1 X 500 ml
HB 1000	1 X 1000 ml

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.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Method

Cyanomethemoglobin Photometric Test.

PRINCIPLE

In the cyanomethemoglobin method, erythrocytes are lysed by a stromatolytic reagent in the presence of a surfactant and release their hemoglobin in to the solution. Hemoglobin is oxidised to methemoglobin by ferricyanide, and the methemoglobin is converted into the stable cyanmethemoglobin by addition of KCN. The absorbance of cyanmethemoglobin is measured at 540 nm and the color intensity is directly proportional to hemoglobin concentration.

REAGENT

Reagent I : Cyanomethemoglobin reagent

REAGENT PREPARATION

The reagents are ready to use.

REAGENT STORAGE AND STABILITY

The reagent stability is till expiry, if stored at R.T. (away from sunlight).

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

Whole blood with EDTA using aseptic technique.
Whole blood collected with EDTA is stable for one week at 2 - 8°C.

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, standard and samples to room temperature 18 - 28°C, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	540 nm
Reaction Type	End point
Cuvette	1 cm light path
Reaction Temperature	R.T.
Measurement	Against reagent blank
Sample Volume	20 µl
Reagent Volume	5000 µl
Incubation	5 minutes
Factor	36.7
Low Normal	10.0 g/dl
High Normal	18.0 g/dl
Linearity	20.0 g/dl

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

	BLANK	SAMPLE
Sample	-	20 µl
Standard	-	-
Reagent	5000 µl	5000 µl

Mix, Incubate at R.T. for 5 min. Measure final absorbance of the sample (Ac) against the reagent blank. at 540 nm (520 - 550 nm)

SAMPLE DILUTIONS

- This method is linear upto a concentration of 20 g/dL.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

CALCULATION

Results are calculated, usually automatically by the instrument, as follows:

Ac x 36.7 = g/dl HEMOGLOBIN

CALIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Low	11.16	0.35	3.15%
Normal	12.14	0.18	1.44%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Low	11.24	0.34	3.05%
Normal	12.21	0.17	1.43%

LINEARITY

The method is linear upto a concentration of 20g/dL. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

Limit of detection: The limit of detection for Hemoglobin is 1 g/dL.

METHOD COMPARISON

A comparison of Accucare Hemoglobin with a commercially available assay (x) using 20 samples gave following results: $R^2 = 0.9900$

REFERENCE VALUES

Adult Males	13.0 - 18.0 g/dl
Adult Females	11.0 - 16.0 g/dl
Children	10.0 - 14.0 g/dl
Newborns	14.0 - 23.0 g/dl

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

BIBLIOGRAPHY

1. Eilers R.J., Am. J. Clin. Path ., 47 :212 (1967).
2. Tietz N.W., Fundamentals of Clinical Chemistry. 2nd ed. W.B. saunders Co., Philadelphia p 411 (1976)

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
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