

**Kit for the quantitative determination of Hemoglobin in Whole Blood.**

**INTRODUCTION :**

Hemoglobin (Hb) estimation is the primary method for anemia screening. Hb being most commonly advised test in laboratory, needs a most suitable and economical method of estimation. The various methods have been recommended for estimation of Hb for assessment of anemia like acid haematin method, Hemoglobinocynide (HiCN) method and automated cell counter method. The most widely used HiCN method contains potassium ferricyanide and potassium or sodium cyanide which are hazardous to environment and occasionally to laboratory technicians also. The present study was conducted to analyze the precision, accuracy, suitability, cost effectiveness and feasibility of HiCN method and non- cyanide alkali haematin method with AHD reagent for the estimation of hemoglobin.

**Principle :**

This method is based on the conversion of all heme, hemoglobin and hemoglobin derivatives in the presence of a non - ionic detergent, into a stable end product, which shows an absorption maximum at 578 nm. The reaction mixture converts chlorohemin, foetal hemoglobin, hemoglobin, cyano-hemoglobin, and sulfo- hemoglobin, quantitatively into alkaline hematin complex. Intensity of the complex formed is directly proportional to the amount of hemoglobin present in the sample.

**Determination of the coefficient of variation for the two methods of hemoglobin measurement.**

Method	Range	Mean	SD	CV
Cyanmethemoglobin	10.4-10.6	10.506	0.045	0.43%
AHD	10.4-10.6	10.486	0.051	0.49%

SD= Standard Deviation, CV= Coefficient of Variation

**CONCLUSION :**

The AHD method using AHD reagent is cheap, non toxic, non biohazardous, stable and gives rapid, accurate and precise comparable Hb estimation results with internationally accepted reference HiCN method. Also, this method overcomes constraints of HiCN method and hence clearly warrants its introduction into healthcare facilities and laboratories for Hb estimation.

**CLINICAL SIGNIFICANCE:**

Hemoglobin (Hb) is an intracorporeal pigment present in erythrocytes. It has the property of combining with oxygen in a reversible fashion that serve an important role as respiratory pigment.

Determination of hemoglobin content is critical step while screening for anemia, Anemia signifies a disease showing reduced oxygen carrying capacity of RBC that may be as a result of less number of RBC or decreased hemoglobin content in RBC.

**SPECIMEN COLLECTION & STORAGE:**

- Whole blood or blood collected with anticoagulants like EDTA, Heparin or Oxalate can be used

**PRECAUTION:**

- Estrom hemoglobin reagent is for In Vitro diagnostic use only.

**Reagent:**

The reagent is to be stored at Room Temperatures.

**No. of Bottles**      **Qty.**  
1 L                      1 no.

**Preparation of working reagent:**

Reagent is ready to use.

**GENERAL INSTRUMENT PARAMETERS:**

Reaction Type	:	End Point
Slope of Reaction	:	Increasing
Units	:	gms/dl
Wavelength	:	578nm
Incubation	:	3 Minutes (RT)
Flowcell Temperature	:	37° C
Zero Setting	:	Distilled Water
Reagent Volume	:	2.0 ml
Path length	:	1.0 cm
Sample Volume	:	20 µl (0.02 ml)
<b>Factor</b>	:	<b>25</b>

**PROCEDURE:**

Dispense	Test
Reagent (ml)	2 ml
Blood Sample (µl)	20 µl

Mix well & incubate at RT for 3 Min. Read the absorbance of test against Distilled water at 578 nm.

**Calculation :**

$$\text{Hemoglobin} = \text{Abs. of test} \times (25) \text{ Factor}$$

*Concentration (gms/dl)*

**LINEARITY:**

This method is linear for Hemoglobin up to 20 gm/dl.

**REFERENCE VALUE:**

<b>Men</b>	<b>: 13.6-18.0 gm/dl</b>
<b>Women</b>	<b>: 11.5 -16.4 gm/dl</b>

**REFERENCES :**

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2. Moharram NM, Aouad R, Busaidy S, Fabricius A, Heller S, Wood WG. International collaborative assessment study of the AHD method for the measurement of blood haemoglobin. Eastern Mediterranean Health Journal. 2006;12(6):722-34.
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	For in vitro diagnostic use only		Catalog #
	Store at RT		Lot Number
	Do not use if package is damaged		Date of Manufacturing