

DRABKIN'S REAGENT

CHEMPAK

Last update 02-2017

(CYANMETHEMOGLOBIN METHOD)

INTENDED USE

Reagent kit for quantitative estimation of hemoglobin in blood.

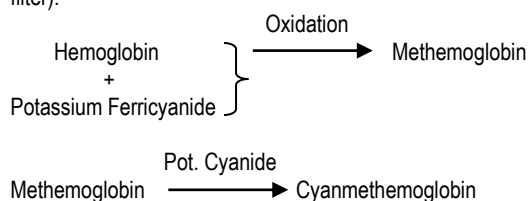
INTRODUCTION

Estimation of Hemoglobin in blood is commonly carried out by visual comparison of solutions containing acid or alkali hematin with coloured glass standard references. In 1967, the International Committee for Standardization in Hematology (ICSH) recommended the photometric estimation of one of the most stable hemoglobin derivative known, namely cyanmethemoglobin. CHEMPAK Hemoglobin is formulated on the principle recommended by International Committee for Standardization of Hematology.

Hemoglobin estimation is equivalent to oxygen carrying capacity of circulating blood and is an indicator of general health status.

PRINCIPLE

Hemoglobin is oxidized to methemoglobin. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which is measured photometrically. The concentration of hemoglobin in the sample is directly proportional to the intensity of the coloured complex which is measured at 540 nm (520-560 nm or with GREEN filter).



PRESENTATION

	Pack Size	
	1000 ml	5 Liters
• Drabkin's Reagent (Ready for use)	1	1
• Drabkin's Standard (60 mg/dL)	1	3

Store at 2-8°C

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent -1	
• Potassium Ferricyanide	0.5 mmol/L
• Buffer	100 mmol/L
• Detergent	5 mmol/L
• Sodium Chloride	10 mmol/L
• Potassium Chloride	-

pH: 7.2 ± 0.1 at 25°C.

Hemoglobin Standard (60 mg/dl):

Ready to use.

Also contains non-reactive fillers and stabilizers.

PRECAUTION

CHEMPAK Hemoglobin is for *IN-VITRO* diagnostics use only. Avoid contact of reagent with skin and eyes. Hemoglobin reagent contains potassium cyanide, hence use automated pipettes. Avoid ingestion.

PREPARATION OF WORKING REAGENT

For 1000ml & 5 Liters:

Drabkin's Reagent is Ready to use.

REAGENT STORAGE AND STABILITY

The reagent provided is stable at room temperature until the expiry date printed on the label.

Hemoglobin Standard (separately provided) is stable at 2-8°C until the expiry date indicated on the label.

SPECIMEN COLLECTION

The specimen required is whole blood either fresh capillary blood or venous blood collected with anticoagulant.

TEST PROCEDURE

PIPETTE INTO TEST TUBES	TEST
WORKING REAGENT (ml)	5.00
SAMPLE (ml)	0.02

Mix by inversion and read absorbance against distilled water after 3 minutes at 540 nm (520-560 nm or GREEN filter). The color stability of the reaction mixture is 24 hours **Read the standard directly without dilution.**

For a spectrophotometer or standard instrument (with regular calibrations) use of standard is not required. Use factor directly or read in the chart.

Details of calculation are as given below (for your reference).

TEST RESULTS

For Colorimeter:

$$\begin{aligned} \text{Hb gm/dL} &= \frac{\text{Abs. of test}}{\text{Abs. of Std}} \times \frac{\text{Dilution of Sample}}{1} \times \frac{\text{Strength of Std.}}{1000} \\ &= \frac{\text{Abs. of Test}}{\text{Abs. of Std.}} \times 15 \end{aligned}$$

For spectrophotometer or standard instrument:

$$\text{Hb gm/dl} = \text{Abs. of test} \times F \quad (F = 36.77)$$

$$\text{Where } F = \frac{\text{Mol. Wt. of Hb.}}{\text{mmol Ext. Coeff.}} \times \frac{\text{Dilution factor}}{1000} \times \frac{1}{10}$$

Mol Wt. of Hb = 64458

mmolar Extinction Coefficient of Hb = 44.0

Dilution Factor = 251

1000 to convert mg to gm

10 to convert gm/L to gm/dL

NORMAL VALUES

MEN : 13.5 - 18.0 gm/dL

WOMEN : 11.5 - 16.4 gm/dL

LINEARITY

This procedure is linear upto 25 gm/dL.

NOTE

Hemoglobin standard is stable at 2-8°C until the expiry date indicated on the label. The Hb Standard provided (60 mg/dL) is for a single use and needs no further dilution.

The absorbance of the standard may be noted and used in the calculation. The chart provided may be used to determine the hemoglobin concentration in the sample.

Any turbidity in the standard indicates contamination. Such standard should be discarded.

REFERENCES

Br.J.Haematology 13 (Suppl) 17, (1967).

International Committee for Standardization in Haematology recommendations for hemoglobinometry in human blood. HENRY R.J. CANNON D.C. and WINKLEMAN.

Clinical Chemistry Principles and Techniques. Harper and Row, 2nd Edition, 1974.

