

LifechemTM HEMOGLOBIN

(CYANMETHEMOGLOBIN METHOD)

CLINICAL SIGNIFICANCE:

Hemoglobin (Hb) has the major function of supplying oxygen to the tissue cells. Hb estimation is one of the commonest screening tests for the diagnosis of Anemia. Decreased levels of hemoglobin concentrations are observed in all varieties of anemia, resulting from hemorrhage or from deficiency of iron, Vitamin B12 or Folic acid. Increased levels of hemoglobin concentration is observed in polycythemia vera, congenital cyanotic heart disease and in hemoconcentration due to various clinical causes like heat stroke and dehydration.

The International Committee for Standardisation in Hematology (ICSH) recommends the Cyanmethemoglobin (CMG) method as a standard method for the estimation of Hemoglobin. This method is simple, rapid and reliable and measures all types of hemoglobin except sulfhemoglobin.

TEST PRINCIPLE:

The Cyanmethemoglobin standard complies to the specifications defined by ICSH which is based on the molecular weight of Hb (64,458 daltons) and a millimolar extinction coefficient of 44. Cyanmethemoglobin Standard is used for direct comparison with blood.

Drabkin's solution on mixing with whole blood converts Hemoglobin to Cyanmethemoglobin. The absorbance of Cyanmethemoglobin is proportional to the Hemoglobin concentration.

NORMAL RANGE:

Male : 13.5 – 18 gm/dl
Female : 11.5 – 16.4 gm/dl

It is recommended that laboratories establish their own normal range.

KIT CONTENTS:

| | Code No. KHB1 (1000 ml) | Code No. KHB2 (5000ml) |
|---|-------------------------------|------------------------------|
| Reagent 1 Drabkin's Solution | 1000 ml | 5000 ml |
| Reagent 2 Cyanmethemoglobin Standard (CMG - mg/dl) (Stamped on the label) | 1x5ml | 2x5 ml |

SPECIMEN:

Whole Blood/Blood with anticoagulants like EDTA, Heparin or Oxalate.

WORKING REAGENT PREPARATION:

Drabkin's Solution is Ready-to-use and is to be stored at room temperature. CMG Standard has a storage condition of 2-8°C. Both reagents are stable till the expiry date mentioned on the labels.

PRECAUTION: Drabkin's solution is poisonous. Do not pipette by mouth.

PROCEDURE:

Pipette in a clean dry test tube labelled Test (T)

| | |
|------------------------|---------|
| Drabkin's solution (1) | 5.0 ml |
| Blood | 0.02 ml |

Mix well and keep it at RT for 5 minutes. Read the absorbance of test (T) and CMG standard (S) against distilled water at 540nm.

CALCULATIONS:

$$\text{Hemoglobin in gm/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times \frac{251}{1000} \times \text{Value of CMG Standard stamped on the label in mg\%}$$

251 = dilution factor
1000 = factor to convert mg/dl to gm/dl (OR)

$$\text{Hemoglobin conc. in gm/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 16.31$$

CALIBRATION CURVE: Use Result referral table for calculation

| Dilute CMG Standard as shown below using Drabkin's Solution | | | | |
|---|---------|--------------------|---------------|-----------------------|
| Tube No: | CMG Std | Drabkin's Solution | Dilution (df) | Factor (F) df x 0.251 |
| 1. | 5.0 ml | — | 1.00 | 0.251 |
| 2. | 3.75ml | 1.25ml | 0.75 | 0.188 |
| 3. | 2.50ml | 2.50ml | 0.50 | 0.126 |
| 4. | 1.25ml | 3.75ml | 0.25 | 0.063 |

Mix well and read the absorbance of the tubes labelled 1, 2, 3 and 4 respectively against distilled water at 540nm. Multiply the CMG concentration (mg/dl) by Factor (F) to get a corresponding hemoglobin concentration in gm/dl for all test tubes respectively. Plot hemoglobin concentration on X-axis (horizontal) and corresponding absorbance on Y-axis (vertical axis). It will be straight line passing through the origin. Read the Hemoglobin concentration of Test (T) on the X-axis corresponding to its absorbance on the Y-axis.

QUALITY CONTROL:

It is recommended to include Assayed Quality Control Serum (Level I & II) with each assay batch to verify the performance of the procedure. Failure to obtain the proper range of values in the assay of control sera may indicate either reagent deterioration, instrument malfunction or procedural errors.

SYSTEM PARAMETERS:

| | | | |
|----------------|-------------|----------------|----------|
| Reaction Type | : End Point | Wave length | : 540 nm |
| Flow Cell temp | : RT | Sample volume | : 20 µl |
| Reagent volume | : 5000µl | Standard Conc. | : mg/dl |
| Units | : mg/dl | Blank | : DW |
| Incubation | : 5 minutes | Linearity | : 20 |
| Low Normal | : 11 | High Normal | : 18 |

NOTES:

- Care should be taken not to pipette Drabkin's Solution by mouth as it is highly poisonous.
- Read absorbance of CMG Standard directly against distilled water at 540 nm
- CMG Standard taken for reading absorbance should be discarded after use.
- As with all diagnostic procedures the physician should evaluate data obtained by use of this kit along with other clinical information.

LINEARITY:

Linearity is 20 mg/dl.

BIBLIOGRAPHY

- ICSH Committee (1978) J.of Clin. Path. 31 ; 139
- Drabkin,D.L. (1932), J.Biol.Chem, 98:719
- Alan,H.Gowenlock,(1988), Varley's Practical Clinical Biochemistry, 6th edition, 664



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