

Hemoglobin

(Cyanmethemoglobin Method)

Code : 10012 (100ml)

(For the analyser/Colorimetric estimation of Hemoglobin in whole blood)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Hemoglobin (Hb) has the major function of supplying oxygen to the tissue cells. Hb estimation is one of the commonest screening test for the diagnosis of anemia. Decreased levels of HEMOGLOBIN are observed in all varieties of anemia, resulting from hemorrhage or from deficiency of Iron, Vitamin B₁₂ or Folic acid. Red cells hemolysis resulting from autoimmune process or due to enzyme abnormality (G-6-PD) may result in anemia. The defective globin chain synthesis as in thalasaemias or structural abnormalities of the hemoglobin molecule as in abnormal hemoglobins may result in severe anemia. Increased levels of Hb concentrations are observed in polycythemia vera, congenital cyanotic heart disease and in hemoconcentration due to various clinical causes e.g., heatstroke and dehydration. Exercise has been reported to lead to a reversible increase of about 3-10%.

Techniques used to determine HEMOGLOBIN have measured it as oxyhemoglobin, carboxyhemoglobin, cyanmethemoglobin, acid and alkaline haemin, by its oxygen capacity and by iron content. The International Committee for Standardisation in Hematology (ICSH) recommended the cyanmethemoglobin method as a Standard method. This method is simple, rapid and reliable. It measures all types of HEMOGLOBINS except Sulfhemoglobin (Hbs). HEMOGLOBIN KIT incorporates the recommendations of ICSH. Cyanmethemoglobin Standard complies to the specifications defined by ICSH which are based on the molecular weight of Hb 64, 458 and a millimolar extinction coefficient of 44. The Drabkin's solution & Cyanmethemoglobin Standard are specially stabilized.

PRINCIPLE:

The HEMOGLOBIN is treated with a reagent containing Potassium Ferricyanide, potassium cyanide and potassium dihydrogen phosphate. The ferricyanide forms methemoglobin which is converted to cyanmethemoglobin by the cyanide. The cyanmethemoglobin has an absorbance which is proportional to the Hemoglobin concentration.

REAGENTS:

1. Drabkin's Solution (stock) 100 ml
2. Cyanmethemoglobin Standard 10 ml

The reagents are ready to use and usable to the expiration date when stored at 2-8°C, if contamination is avoided.

WORKING REAGENT PREPARATION :

To prepare working reagent, dilute 10ml of Drabkin's solution upto 200 ml with distilled water (1 : 20)

SAMPLE :

Whole blood, E D T A, Heparin or Oxalate blood.

EXPECTED RANGE:

- * Males 13.5 – 18.0 gm%
- * Females 11.5 – 16.4 gm%

LINEARITY:

Hemoglobin kit is linear upto 20 gm%

Samples exceeding 20 gm% should be diluted and reassayed. The result has to be multiplied by the dilution factor.

DIRECTIONS FOR USE ON ANALYSERS:

Reaction Type : End point with std
Wave Length : 540nm (green filter)
Incubation Temp : Room Temperature
Incubation Time : 5 mins.
Linearity : 20 gm%
Unit : gm%

Cyanmethemoglobin Standard is used for direct comparison with blood, which when treated with Drabkin's Solution is also converted to Cyanmethemoglobin.

PROCEDURE:

Pipette in a clean dry test tube labelled (T)

Test (T)

Working Drabkin's solution 5.0 ml

Blood 0.02 ml

Rinse the pipette well with the reaction mixture. Mix well and keep at R.T. for 5 minutes. Measure the absorbance of Test (T) and Cyanmethemoglobin Standard (S) against distilled water on photocolormeter using green filter or on a spectrophotometer at 540 nm (Hg 546 nm).

CALCULATIONS:

$$\text{Hemoglobin in gm\%} = \frac{\text{A of T} \times 251 \times}{\text{A of S} \times 1000}$$

251 = Blood dilution factor

1000 = Factor to convert milligrams to grams

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a correlation of test results with other clinical observations / Diagnostic labels. Value of Std. stamped on test labels. mg%

BIBLIOGRAPHY:

1. ICSH Committee (1978) J. Clin. Path. 31,139.
2. Dace, J.V. (1985) Practical Hematology, 6th edition, P 28.
3. Drabkin, DL. (1932) J. Biol. Chem 98,719.

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