HEMOGLOBIN

Cyanide free.

INTENDED USE
Bioline Hemoglobin reagent is used for the quantitative determination of hemoglobin in human blood.

CLINICAL SIGNIFICANCE
Hemoglobin is a porphyrin-iron (II) protein compound that transports oxygen from the lungs to body tissues where it is utilized for energy metabolism. Measurements of hemoglobin from venous or capillary blood aid in the detection of a variety of conditions which alter the normal hemoglobin concentration of blood, e.g. anemia or polycythemia.

METHOD AND PRINCIPLE
The determination of iron content in whole blood is the most accurate method for assessing blood hemoglobin. Of the various methods used, cyanmet hemoglobin is the most widely accepted.It is this internationally adapted method that is employed in this procedure.
In the cyanmet hemoglobin method, erythrocytes are lysed by a stromatolytic agent in the presence of a surfactant and release their hemoglobin into solution. Hemoglobin is oxidized to methemoglobin by ferricyanide, and the methemoglobin is converted into the stable cyan methemoglobin . The absorbance of cyanmethemoglobin is measured at 540nm and color intensity is proportional to hemoglobin concentration.

REAGENT COMPOSITION
When reconstituted as directed, the reagent for hemoglobin contains the following:
1. Hemoglobin reagent: Potassium ferricyanide 0.5 Mm, Sodium Lauryl sulphate 0.7mM, buffers and stabilizers included.

WARNINGS AND PRECAUTIONS
1. For *invitro* diagnostic use only.
   CAUTION:Invitro diagnostic reagents may be hazardous .Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Specimens should be considered infectious and handled appropriately.
3. Use distilled or deionized water where indicated.

REAGENT PREPARATION
Reagent comes in a ready to use form.

REAGENT STORAGE AND STABILITY
Store the hemoglobin reagent and standard at room temperature (15 -30^{0}C).

REAGENT DETERIORATION
Do not use hemoglobin reagent if:
1. It has become a different color than yellow.
2. The reagent becomes turbid or a precipitation forms.

SPECIMEN COLLECTION AND STABILITY
1. Use whole blood with EDTA as an anticoagulant.
2. Oxalate, citrate or heparin may also be used as anticoagulants.
3. Capillary or venous blood maybe collected if used before clotting occurs.
4. Whole blood mixed well with an anticoagulant appears stable for one (1) week at room temperature (15 - 30^{0}C).

INTERFERENCES
1. Substances that cause turbidity will falsely elevate the hemoglobin value. These include lipids, abnormal plasma proteins (macroglobulinemia) or erythrocyte stroma.
2. A review by young *et al*. reveals the numerous drugs that exert an *in vivo* effect to decrease blood hemoglobin.

MANUAL PROCEDURE
Wavelength : 546 (520-550)nm
Temperature : RT

	Blank	**Sample**
Reagent	2.5mL	2.5mL
Distilled water	10µL	-
Sample	-	10µL

Mix and read the optical density (OD) of sample against the reagent blank after 5 minutes of incubation at RT.

CALCULATION
Hemoglobin g/dl= Abs x Factor (41).

LIMITATIONS
1. Specimens with values above 20.0 g/dl must be re-run using one half the sample volume. Multiply final results by two

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established hemoglobin values maybe routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration ,instrument malfunction, or procedural errors

EXPECTED 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

Factors such as age, race, exercise, season and altitude are reported to influence the values of normal ranges. The above range should serve only as a guideline. Each laboratory should establish its own range.

PERFORMANCE CHARACTERISTICS
1. *Linearity*: 20 g/dl.
2. *Sensitivity:* Based on an instrument resolution of 0.001 absorbance ,the present procedure has a sensitivity of 0.03 g/dl.
3. *Comparison*: Studies conducted against a similar procedure yielded a coefficient of correlation of 0.98 with a regression equation of y=1.03x-0.48 on samples with values from 7.2 to 17.9 g/dl (n= 20).
4. *Precision*:

Within Run: Two samples of human blood were assayed twenty (20) times and the following within run precision was obtained.

	Mean(g/dL)	S.D.	C.V.%
Normal	13.8	0.6	4.6
Abnormal	10.2	0.3	3.4

Run-to-Run: Two samples of human blood were assayed for five (5) consecutive and the following run to run precision was obtained.

	Mean(g/dL)	S.D.	C.V.%
Normal	14.3	12.3	0.5
Abnormal	12.3	0.5	4.3

GENERAL TECHNICAL PARAMETER

Mode	**End Point**
Wavelength (Filter)	**546(520-550)nm**
Reaction Direction	**Increasing**
Sample Vol.	**10µl**
Reagent Vol.	**2.5 ml**
Incubation Time	**5 Min**
Reagent Blank Abs.(Max)	**NMT 0.05**
Calibration Method	**Fix factor**
Factor	**41**
Linearity	**20 g/dL**
Decimal Places	**1**
Temp.	**RT**
Unit	**g/dL**
AdultMales	**13.0 - 18.0 g/dl**
AdultFemales	**11.0 - 16.0 g/dl**
Children	**10.0 - 14.0 g/dl**
Newborns	**14.0 - 23.0 g/dl**

REFERENCES

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