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HEMOGLOBIN REAGENT SET

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HEMOGLOBIN REAGENT SET
For the quantitative determination of hemoglobin in blood.

INTRODUCTION
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 rods in the detection of a variety of conditions that alter the normal hemoglobin concentration of blood, eg. anemia or polycythemia. The determination of iron content in whole blood is the most accurate method for assessing blood hemoglobin. Of the various methods used, cyanmethemoglobin is the most widely accepted. It is this internationally adapted method that is employed in this procedure.[1]

PRINCIPLE
In the cyanmethemoglobin method, erythrocytes are lysed by a stromatolytic agent in the present of a surfactant and release their hemoglobin into solution. Hemoglobin is oxidized to methemoglobin by ferricyanide, and the methemoglobin is converted into the stable cyanmethemoglobin by addition of KCN. The absorbance of cyanmethemoglobin is measured at 540 nm and color intensity is proportional to hemoglobin concentration.[2]

REAGENT COMPOSITION
When reconstituted as directed, the reagent for hemoglobin contains the following:
1. Hemoglobin reagent: Potassium ferricyanide 0.5 mM, potassium cyanide 0.7 mM, buffers and stabilizers included.
2. Standard: Methemoglobin (60 mg/dl) dissolved in cyanmethemoglobin reagent. This amount is equivalent to 15.0 g/ul hemoglobin. This standard has been referenced against a CAP (College of American Pathologists) certified standard to its concentration and further checked by using the known molar absorptivity of cyanmethemoglobin.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use.
   CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.
2. Contains cyanide. Poison - may be fatal if swallowed. **DO NOT PIPETTE BY MOUTH.**
3. Do not mix with acids. Discarding with large volumes of water.
4. Specimens should be considered infectious and handled appropriately.
5. Use distilled or deionized water where indicated.

REAGENT PREPARATION
Reagent comes in a ready to use form.

STORAGE and STABILITY
1. Hemoglobin reagent is stored at room temperature.
2. Hemoglobin standard is stored at room temperature.

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
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 may be collected if used before clotting occurs.
4. Whole blood mixed well with an anticoagulant appears stable for one (1) week at room temperature.

INTERFERING SUBSTANCES
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.[3]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Timer.
3. Test tubes/rack.
4. Spectrophotometer with ability to read at 540 nm.

GENERAL INSTRUCTIONS
The reagent for Hemoglobin is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

PROCEDURE (AUTOMATED)
Refer to appropriate instrument application instructions available from us.

PROCEDURE (MANUAL)
1. Dispense 2.0 ml of hemoglobin reagent into test tubes labeled "blank", "control ", "patient", etc.
2. Place 0.01 ml (10 ul) of sample into respective tubes. Mix.
3. Allow all tubes to stand for three (3) minutes at room temperature.
4. To a tube labeled standard, place 2.0 ml of standard.
5. Set spectrophotometer to 540 nm and zero with the reagent blank.(Wavelength range:520-550).
6. Read and record absorbance values of all tubes.
7. See "**CALCULATIONS**" to obtain values.

NOTE:
1. For spectrophotometers requiring greater volumes for proper reading, use 4.0 ml reagent and 0.02 ml (20 ul) sample. Follow above instructions.
2. Final color appears quite stable but should be read within one (1) hour to avoid evaporation.

PROCEDURAL LIMITATIONS

1. This procedure measures hemoglobin and its derivatives except sulfhemoglobin.
2. Specimens with values above 20.0 g/dl must be re-run using onen half the sample volume. Multiply final results by two (2).

CALIBRATION

Use hemoglobin standard provided.

CALCULATION OF RESULTS

Abs. = Absorbance

$$\frac{\text{Abs. of unknown}}{\text{Abs. of standard}} \times \text{conc. of standard (g/dl)} = \text{Value (g/dl)}$$

Example: If a 15 g/dl standard has an absorbance of 0.602 and the absorbance of the unknown is 0.480 then:

$$\frac{0.480}{0.602} \times 15.0 = 11.9 \text{ g/d1}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established hemoglobin values may be 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 VALUE[4,5]

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

	Mean(IU/L)	S.D.	C.V.%
Normal	13.8	0. 6	4.6
Abnormal	10. 2	0. 3	3.4

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

	Mean(IU/L)	S.D.	C.V.%
Normal	14.3	0. 3	2.7
Abnormal	12.3	0. 5	4.3

REFERENCES

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