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HEMOGLOBIN REAGENT
(DRABKIN'S REAGENT)

REF 042A

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE
For the quantitative determination of hemoglobin in blood using cyanmethemoglobin color/end point method.

DIAGNOSTIC 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 aids in the detection of a variety of conditions that alter the normal hemoglobin concentration of blood, e.g. anemia or polycythemia. Decreased blood levels are associated with anemia and increased levels found in patients with polycythemia or dehydration. The determination of iron content in whole blood is the most accurate method for assessing blood hemoglobin. Of the various methods used, cyanmethemoglobin method is the most widely accepted. It is the internationally adapted method that is employed in this procedure[1].

EXPECTED VALUES[2,3]

Adult males	13.0 - 18.0 g/dL or 8.1-11.2 mmol/L (¼ Hb4)
Adult females	11.0 - 16.0 g/dL or 6.8- 9.9 mmol/L (¼ Hb4)
Children	10.0 - 14.0 g/dL or 6.2- 8.7 mmol/L (¼ Hb4)
New borns	14.0 - 23.0 g/dL or 8.7-14.3 mmol/L (¼ Hb4)

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 normal range.

METHOD PRINCIPLE
In the cyanmethemoglobin method, erythocytes 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 cyanmethemoglobin by KCN. The absorbance of cyanmethemoglobin is measured at 540 nm which is proportional to hemoglobin concentration[4].

REAGENT COMPOSITION
1. ***HEMOGLOBIN REAGENT (DRABKIN'S REAGENT):***
   Potassium ferricyanide 0.6 mM, Potassium cyanide 0.7 mM, Surfactant, Buffer and Stabilizers included.

REAGENT STORAGE & STABILITY
Store reagent at room temperature protected from light. Stable upto expiration date indicated on individual bottle label.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use.
   CAUTION: In vitro diagnostic reagent 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. Discard with large volumes of water.
4. Specimens should be considered as infectious and handled appropriately.
5. Use distilled or deionized water where indicated.

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.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate Pipetting devices.
2. Timer.
3. Test tubes/rack
4. spectrophotometer with ability to read at 540 nm (530-550 nm).

PROCEDURE (MANUAL)
Pipette into clean dry test tubes:

	TEST
Hemoglobin Reagent	2.0 ml
Sample	0.01 ml

Mix, allow to stand at room temperature for 3 minutes. Read the absorbance at 540 nm against Hemoglobin Reagent in a 1 cm cuvette.

NOTES
1. For spectrophotometers requiring greater volumes for proper reading, use 4.0 ml reagent and 0.02 ml (20 µl) sample. Follow above instructions.
2. Final color appears quite stable but should be read within one (1) hour to avoid evaporation.
3. Do not use the given standard with spectrophotometers having fixed wave length of 546 nm. Instead, use a factor of 29.4 to calculate the results provided that spectrophotometer is well calibrated.

CALIBRATION
See procedure notes.

CALCULATION OF RESULTS (Refer procedure notes 1 & 2)
Abs. = Absorbance
Conc. = Concentration
Hb = Hemoglobin

Abs. of Test x 29.4 = Hb. Conc. in Test (g/dL)
(540 nm)

OR

Abs. of Test x 18.2 = Hb. Conc. in Test mmol/L(1/4Hb4)
(540 nm)

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Example: If absorbance of Test is 0.480 then the concentration of hemoglobin in test will be

$$0.480 \times 29.4 = 14.1 \text{ g/dL.}$$

OR

$$0.480 \times 18.2 = 8.7 \text{ mmol/L (¼ Hb4).}$$

PROCEDURE NOTES

1. The factor given above is based on A^{540} in a 1 cm cuvette and based on 1 in 200 dilution. Any change in these parameters will change factor and appropriate correction be made by using following formula.

$$\frac{A^{540} \times 64500 \times \text{dilution factor}}{44 \times d \times 10^4} = \text{g/dL Hemoglobin}$$

Where A^{540} = Absorbance of solution at 540 nm.
64500 = Molecular weight of hemoglobin (derived from 64458).
Dilution factor = 201 when 10 µl of sample is diluted in 2 ml of Drabkin's reagent.
44.0 = Millimolar extinction coefficient.
d = Layer thickness in cm (Light Path).
10^4 = Conversion factor for mg/L to g/dL.

2. Calibration of spectrophotometer should be checked from time to time by verifying that it gives an accurate value for cyanmethemoglobin standard. Slight deviations from the expected A^{540} HiCN value for the standard may be used to correct the results of test samples for a bias in measurement [5]
3. To convert g/dL Hb to mmol/L Hb (¼ Hb4), multiply by 0.62.
4. This procedure measures hemoglobin and its derivatives except sulfhemoglobin.
5. Specimen with values above 20.0 g/dL must be re-run using one-half the sample volume. Multiply final results by two (2).
6. Substances that cause turbidity will falsely elevate the hemoglobin value. These include lipids, abnormal plasma proteins (macroglobulinemia) or erythrocyte stroma.
7. A review by Young et al. reveals the numerous drugs that exert an "in vivo" effect to decrease blood hemoglobin[6].

PERFORMANCE CHARACTERISTICS

1. LINEARITY : 20 g/dL
2. SENSITIVITY : Based on an instrument resolution of A = 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 = 1.03 x -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 (g/dL)	Std.Dev.	CV
Normal	13.8	0.6	4.6%
Abnormal	9.0	0.165	1.83%

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 (g/dL)	Std.Dev.	CV
Normal	14.3	0.3	2.7%
Abnormal	9.1	0.3	3.3%

QUALITY CONTROL

For accuracy and precision check, we recommend use of

UDI § "HEMOGLOBIN CONTROL SET" (Level I, II, III).

ORDERING INFORMATION

HEMOGLOBIN CONTROL SET (LEVEL I, II, III)

CAT # 042-SET **3 x 1.5 ml**

REFERENCES

1. Eilers R.J., Am. J. Clin. Pathol. 47:212 (1967).
2. Henry R.F., et al. Principles and Techniques in Clinical Chemistry, 2nd ed., Harper and Row, Hagerstown, MD p 1128-1135 (1974).
3. Wolf, P.L., Practical Clinical Hematology, Johy Wiley and Sons, NY, p 144 (1973).
4. Tietz, N.w., Fundamentals of Clinical Chemistry, 2nd ed., W.B. Saunders Co., Philadelphia p 411 (1976).
5. International Committee for Standardization in Haematology: Recommendations for reference method for haemoglobinometry in human blood and specifications for international. haemoglobincyanide reference preparation. Journal of Clinical Pathology, 31, 139.
6. Young, DS et al. Clin Chem. 21:10 (1975).

PRODUCT AVAILABILITY

HEMOGLOBIN REAGENT (DRABKIN'S REAGENT)

REF # 042AT-500	**1 x I L**
REF # 042A - 500	**4 x 250 ml**

EC REP
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