Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12452 R 4 x 250 ml
For 400 tests

Hemoglobin

Drabkin colorimetric method

Liquid Reagent

Test Principle

Hemoglobin (oxyhemoglobin, methemoglobin, carboxyhemoglobin) is converted to cyanomethemoglobin according to the following reactions :

Hemoglobin —*Potassiumferricyanide*→ Methemoglobin

Methemoglobin —*Potassiumcyanide*→ Cyanomethemoglobin

Concentrations in the test

Reagent R		
Phosphate buffer		
Potassium ferricyanid	0.65	mmol/L
Potassium cyanide	0.75	mmol/L
Detergent		

Stability of reagent

Reagent : *liquid, ready to use.*

Warning: This reagent is poisonous. Don't pipette by mouth.
The reagent is stable up to expiry date given on the label when stored at + 20 ➔ + 25 °C.

Note: Don't use the reagent if it has a different color than yellow, or if the reagent becomes cloudy.

Specimen collection and handling

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 is stable for 1 week at 20 – 25°C.

Quality control

Meditrol Hb1 Cat. No. 15251
Meditrol Hb2 Cat. No. 15261

Procedure

Wavelength	Hg 546 (520 - 560 nm)
Spectrophotometer	540 nm
Cuvette	1 cm light path
Temperature	20 - 25°C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Semi macro	Macro
Sample	--	10 µl	20 µl
Reagent	2.5 ml	2.5 ml	5.0 ml
Mix well, incubate for 5 min. at 20 – 25 °C, Read the absorbance (A). The final color is stable for 1 hour.			

Calculations

Calculation using Factor:

$$\text{Conc.}_{\text{Hemoglobin}}\ (\text{g/dl}) = A_{\text{Sample}} \times \text{Factor}$$

Factor = 36.8

Calculation using Standard:

$$\text{Conc.}_{\text{Hemoglobin}}\ (\text{g/dl}) = \frac{A_{\text{Sample}}}{A_{\text{STD.}}} \times \text{Conc.}_{\text{STD.}}(\text{g/dl})$$

Linearity

Up to 21 g/dl (13 mmol/L).
If the result exceeds 21 g/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interference

A review by Young et al reveals the numerous drugs that exert an in vitro effect on blood hemoglobin values.[4]

Precautions

The reagent contains cyanide.
The reagent poisonous is may be fatal if swallowed.

Reference range

1	d.	15.2 – 23.5	g/dl
2 – 6	d.	15.0 – 24.0	g/dl
14 – 23	d.	12.7 – 18.7	g/dl
24 – 37	d.	10.3 – 17.9	g/dl
40 – 50	d.	9.0 – 16.6	g/dl
2 – 2.5	mth.	9.2 – 15.0	g/dl
3 – 3.5	mth.	9.6 – 12.8	g/dl
5 – 7	mth.	10.1 – 12.9	g/dl
8 – 10	mth.	10.5 – 12.9	g/dl
11 – 13.5	mth.	10.7 – 13.1	g/dl
1.5 – 3	yr.	10.8 – 12.8	g/dl
5	yr.	11.1 – 14.3	g/dl
10	yr.	11.9 – 14.7	g/dl
12	yr	11.8 – 15.0	g/dl
15	yr.	12.8 – 16.8	g/dl
Adults	women	12.3 – 15.3	g/dl
	men	14.0 – 17.5	g/dl

References

1. Drabkin D.L., et al J. Biol. Chem., 98, (1932), 719.
2. Fundamentals Diagnostic Hematology Anemia, second edition, Bruce L. Evatt, M.D., William N. Gibbs, M.D., F.R.C. Path., S.M., Lewis, M.D., F.R.C. Path., James R. McArthur, M.D.
3. Tietz N.W Fundamentals of clinical chemistry ,2nd ed , W.B. Sounders Co, Philadelphia p. 411.1976.
4. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington,D.C.

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