

# Manual Procedure

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

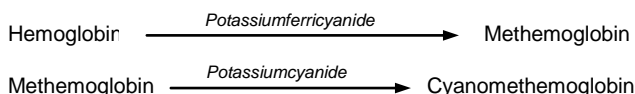
## Hemoglobin

Drabkin colorimetric method

### Liquid Reagent

#### Test Principle

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



#### 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

- Use whole blood with EDTA as an anticoagulant.
- Oxalate, citrate, or heparin may also be used as anticoagulants.
- Capillary or venous blood may be collected if used before clotting occurs.
- 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:

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

$$\text{Factor} = 36.8$$

##### Calculation using Standard:

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

#### 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.<sup>4</sup>

#### 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	moth.	9.2 – 15.0	g/dl
3 – 3.5	moth.	9.6 – 12.8	g/dl
5 – 7	moth.	10.1 – 12.9	g/dl
8 – 10	moth.	10.5 – 12.9	g/dl
11 – 13.5	moth.	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

- Drabkin D.L., et al J. Biol. Chem., 98, (1932), 719.
- 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.
- Tietz N.W Fundamentals of clinical chemistry ,2nd ed , W.B. Saunders Co, Philadelphia p. 411.1976.
- Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.