HAEMOGLOBIN
Ready For Use

INTENDED USE

Vitro haemoglobin reagent is intended for the in vitro quantitative determination of haemoglobin in whole blood on manual systems.

METHOD

Colorimetric endpoint cyanmethaemoglobin method[1].

BACKGROUND

Haemoglobin the main component of the red blood cells, functions in the transportation of oxygen and CO_2. Haemoglobin consists of one molecule of globin and four molecules of haeme (each containing one molecule of iron in the ferrous state). Globin consists of two pairs of polypeptide chains. In the haemoglobin molecule, each polypeptide chain is associated with one haeme group; each haeme group can combine with one molecule of oxygen or CO_2. Haemoglobin carries oxygen from places of high oxygen pressure (lung) to places of low oxygen pressure (tissues), where it readily releases the oxygen. Haemoglobin also returns CO_2 from the tissues to the lungs. Haemoglobin in circulating blood is a mixture of haemoglobin, oxyhaemoglobin, carboxyhaemoglobin and minor amounts of other forms of this pigment. Measurements of haemoglobin from venous or capillary blood aids in the detection of a variety of conditions that alter the normal haemoglobin concentration of the blood, e.g. anemia or polycythemia[2,3].

ASSAY PRINCIPLE

The methods used for estimating haemoglobin can be either visual, or photo-electric. In visual methods changes of colour are judged by eye, whereas in photo-electric methods they are estimated by a photo-electric colorimeter. The visual color comparison methods include the use of the Tallqvist haemoglobin chart, Sahli haemoglobinometer, and Lovibond comparator. Previous methods used for the determination of blood haemoglobin were based on estimation of oxygen or carbon monoxide capacity or iron content. Of all methods, only the cyanmethemoglobin has gained popular acceptance. The original cyanmethemoglobin technique was proposed by Stadie[4] in 1920. This method used separated alkaline ferricyanide and cyanide reagents. A single reagent was introduced by Drabkin and Austin[5] in 1935. In 1958 the National Research Council (NRC) recommended adoption of the cyanmethemoglobin procedure based on field trials conducted by the Army Medical Department.[6,7] In 1966 the International Committee on Standardization in Hematology approved the proposal that all clinical laboratories should adopt this method exclusively[8].
It is necessary to make a stable derivative involving all forms of haemoglobin in the blood in order to measure this compound accurately. The cyanmethemoglobin derivative can be conveniently and reproducibly prepared and is widely used for haemoglobin determinations. All forms of circulating haemoglobin are readily converted to cyanmethemoglobin except for sulfhemoglobin which is rarely present in significant amounts.

1. In a reagent solution the ferrous ions of haemoglobin are oxidized to the ferric state by potassium ferricyanide to form methemoglobin.
2. Methemoglobin subsequently reacts with the cyanide ions provided by potassium cyanide to form cyanmethemoglobin.

Haemoglobin + Cyanide + Ferricyanide ⟶ **Cyanmethemoglobin**

The amount of cyanmethemoglobin can be measured spectrophotometrically at a wavelength of 546 nm. The intensity of color is directly proportional to haemoglobin concentration in the specimen[9].

EXPECTED VALUES

Adults [10,11]	
Males	13.0 – 18.0 g/dl
Females	11.0 – 16.0 g/dl
Children	
At birth	13.6 – 19.6 g/dl
At one year	11.3 – 13.0 g/dl
10 – 12 years	11.5 – 14.8 g/dl

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

REAGENTS

R_1	**Drabkin's Reagent**		
	Potassium ferricyanide	62	mmol/l
	Potassium cyanide	76	mmol/l
	Potassium phosphate	60	mmol/l
	Non ionic surfactant	5	ml/l
R_2	**Cyanmethemoglobin standard**		
	Equivalent to 15 g/dl hemoglobin		

- **Reagent Preparation & Stability**

All reagents are stable up to the expiry date given on label when stored at room temperature protected from light.

The cyanmethemoglobin standard is stable up to expiry date provided that contamination is avoided. Store at 2 - 8 ^{0}C
Never return standard to original bottle after use.

SPECIMEN

- 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.
- **Specimen Preparation & Stability**

Whole blood mixed well with an anticoagulant appears stable for one week at room temperature.

PROCEDURE

Wavelength	520 - 560 nm
Cuvette	1 cm light path
Temperature	20 - 25 ^{0}C
Zero adjustment	against reagent blank

Label test tubes: blank, and specimens.

Pipette into test tube or cuvette	
Working solution	2.5 ml
Sample	10 µl
Rinse the pipette used for the blood specimen few times with the reaction mixture.	

Shake reagent solution slightly after adding the blood to avoid clumping of the erythrocytes.
Incubate for 5 minutes at room temperature.
Measure the absorbance of specimen ($A_{specimen}$) against reagent blank.
The color is stable for 60 minutes. Don't expose to strong light. The test tubes must be kept stoppered when stored for several hours.

CALCULATION

Method (1)

Calculate the haemoglobin concentration by using the following formulae:
Haemoglobin Concentration (g/dl)= Specimen absorbance x 36.77

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Method (2)

Hemoglobin Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \text{ X Standard value*}$$

* Hemoglobin standard concentration given on label.

LINEARITY

When run as recommended, the assay is linear up to 20 g/dl.
Specimens with values above 20.0 g/dl must be re-run using one half the sample volumes. Multiply final result by 2.

SENSITIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 2.0 g/dl.

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established haemoglobin values may be routinely used for quality control.
Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control blood and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Substances that cause turbidity[10]:**
  These substances will falsely elevate the haemoglobin value. These include lipids, abnormal plasma proteins (macroglobulinemia) or erythrocyte stroma.
- **Drugs:**
  A review by Young et al[12] reveals the numerous drugs that exert an in vitro effect to decrease blood haemoglobin values.
- **Others:**
  Extremely high leukocyte counts or platelets.

WARNING & PRECAUTION

- Vitro haemoglobin reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Reagent contains cyanide. Poison may be fatal if swallowed. *DON'T PIPETTE BY MOUTH*.

Don't mix reagent with acids. Discard by flushing with large volumes of water.

- Don't use haemoglobin reagent if it has a different color than yellow, or reagent becomes cloudy.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
12701	1 X 500 ml
12702	1 x 1000 ml
12703	2 x 500 ml

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
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+202 26439698
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