BIOLABO
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HAEMOGLOBIN

Colorimetric Method (Cyanmethemoglobin)

Reagent for quantitative determination of haemoglobin (Hb) in human whole blood.

REF 3502200 **R1** 2 x 200 mL

TECHNICAL SUPPORT AND ORDERS
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IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
It allows the quantitative determination of haemoglobin (Hb) in whole blood to screen its level.

I GENERALITIES (1)

Haemoglobin in blood is essential for adequate transport of O_2 and CO_2 between lungs and other tissues. Blood haemoglobin concentration may be diminished as a consequence of haemorrhage or haemolysis or as a result of impaired blood formation in bone marrow. Conversely, blood haemoglobin concentration may be increased when gas exchange through the lungs is impaired or in various other disorders. Measurement of the blood haemoglobin concentration is important as an initial step in the detection of anaemia (diminished haemoglobin concentration) or erythrocytosis (increased red blood cells count and haemoglobin concentration).

PRINCIPLE (4) (5)

Method recognised as reference method by ICSH (International Committee of Standardisation in Haematology).

Fe^{2+} of haemoglobin is oxidised to the Fe^{3+} of methaemoglobin by ferricyanide, and the methaemoglobin is converted into stable cyanmethemoglobin by addition of potassium cyanide (KCN).

The absorbance of cyanmethemoglobin, directly proportional to the haemoglobin concentration, is measured at 546 nm (520-560).

REAGENTS

R1 HAEMOGLOBIN Working Reagent

Phosphate Buffer	1	mmol/L
Potassium cyanide	0,75	mmol/L
Potassium ferricyanide	0,6	mmol/L
Detergent	0.1	g/L
Preservative	< 0.1	%

This reagent is not classified as hazardous according to CLP Regulation (EC) No 1272/2008

I SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENT PREPARATION

Reagent is ready for use.

STABILITY AND STORAGE

Store at 18-25°C, away from light, well cap in the original vial.

- Unopened:

  Reagent is stable until expiry date stated on the label

- Once opened:

  Reagent is table at least 6 months when free from contamination

  Discard any reagent if cloudy or if reagent blank at 546 nm is > 0.010.

SPECIMEN COLLECTION AND HANDLING (2)

Whole blood (EDTA).
Foetal blood: collect by percutaneous umbilical blood sampling.

Swirl gently to homogenise before assay.

Haemoglobin is stable in specimen for:
- 48 h at 2-8°C.
- 24 h at room temperature (< 25°C).

LIMITS (2) (3)

Lipemia or leucocytes concentration > 25.10^9/L involve overestimated results. Overestimation have been detected in the presence of HbC or HbS, in serious liver disorders or in globulin precipitation (ex: multiple myeloma or Waldenström macroglobulinemia).

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer
3. Assayed Blood controls

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

QUALITY CONTROL

- Internal Quality Control
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, verify analysis parameters: Wavelength, specimen/reagent ratio, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (2)

In foetal blood	g/dL	g/L	mmol/L
18-20 weeks	11.5 ± 0.78	115 ± 7.8	7.13 ± 0.48
21-22 weeks	12.3± 0.89	123± 8.9	7.63 ± 0.55
23-25 weeks	12.4 ± 0.77	124 ± 7.7	7.69 ± 0.48
26-30 weeks	13.4 ± 1.17	134 ± 12	8.31 ± 0.75

In cord blood	g/dL	g/L	mmol/L
	13.5-20.5	135-205	8.37-12.7

In total blood		g/dL	g/L	mmol/L
0.5 months		13.4-19.8	134-198	8.31-12.28
1 months		10.7-17.1	107-171	6.63-10.6
2 months		9.4-13.0	94-130	5.83-8.06
4 months		10.3-14.1	103-141	6.39-8.74
6 months		11.1-14.1	111-141	6.88-8.74
9 months		11.4-14.0	114-140	7.07-8.68
12 months		11.3-14.1	113-141	7.01-8.74
1-2 years		11.0-14.0	110-140	6.82-8.68
2-5 years		11.0-14.0	110-140	6.82-8.68
5-9 years		11.5-14.5	115-145	7.13-8.99
9-12 years		12.0-15-0	120-150	7.44-9.3
12-14 years	M	12.0-16.0	120-160	7.44-9.92
	F	11.5-15.0	115-150	7.13-9.3
15-17 years	M	11.7-16.6	117-166	7.25-10.29
	F	11.7-15.3	117-153	7.25-9.49
18-44 years	M	13.2-17.3	132-173	8.18-10.73
	F	11.7-15.5	117-155	7.25-9.61
45-64 years	M	13.1-17.2	131-172	8.12-10.66
	F	11.7-16.0	117-160	7.25-9.92
65-74 years	M	12.6-17.4	126-174	7.81-10.79
	F	11.7-16.1	117-161	7.25-9.98

It is recommended that each laboratory establish its own normal ranges for the population that it serves.

PERFORMANCES

Manual procedure at 546 nm:

Detection limit: approximately 0.3 g/dL

Measuring range: between 4,5 and 36 g/dL

Within run N = 20	*Low level*	*High level*
Mean g/dL	6.7	18.9
S.D. g/dL	0.05	0.1
C.V. %	0.7	0.6

Between run N = 20	*Low level*	*High level*
Mean g/dL	6.3	17 .1
S.D.g/dL	0.29	0.42
C.V. %	4.6	2.5

Sensitivity for 10 g/dL: approximately 0.272 Abs at 546 nm.

Comparison study with commercially available reagent:

y = 0.9999 x + 0.08 r = 0.9962

Interferences :

Turbidity : Positive interference from 0.275 abs.
Ascorbic acid : No interference up to 25 mg/dL.
Bilirubin : No interference up to 330 µmol/L.
Glucose : No interference up to 1000 mg/dL

CALIBRATION (6)

Use the calibration factor indicated in § **CALCULATION** or a calibrator (cyanmethemoglobin) assayed with the same method.

The calibration frequency depends on proper instrument functions and on preservation of the reagent.

It is recommended to calibrate in the following cases :

1. When changing vial of reagent.
2. After maintenance operations on the instrument.

If control values are out of range, even after using a new vial of fresh blood

PROCEDURE

Manual method

Pipette into test tubes.	Blank	Assay
Reagent	5 mL	5 mL
Demineralised water	20 µL	
Homogenised blood		20 µL

It is recommended to use a positive moved pipette to dispense blood. Rinse pipette several times into the reagent. Mix well and incubate at least for 3 minutes at room temperature. Read absorbance at 546 nm (520-560) against reagent blank.

Away from light, reaction is stable a least for 1 hour.

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

	λ = 530 nm	λ = 546 nm	λ = 550 nm
Hb (g/L)	Abs x 386,1	Abs x 367,7	Abs x 376,2
Hb (g/dL)	Abs x 38,61	Abs x 36,77	Abs x 37,62
Hb mmol/L (Hb/4)	Abs x 23,96	Abs x 22,82	Abs x 23,34

These factors were designated as a guide only and may slightly vary. It is recommended to verify with control blood.

REFERENCES

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1657-1688.

(2) Clinical Guide to Laboratory Test, 3rd Ed., N.W. TIETZ (1995) p. 312-314.

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-325 to 3-330

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(5) VAN KAMPEN, E.J. and ZIJLSTRA W.G., Determination of haemoglobin and its derivatives advances in clinical chemistry (1965), 8, 141-187

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