BIOLABO
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HAEMOGLOBIN Colorimetric method (Cyanmethemoglobin)

Concentrated reagent (to be diluted 1/50) for quantitative determination of haemoglobin (Hb) in whole blood

REF 82250 **R1** 1 x 50 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD **IN VITRO DIAGNOSTIC USE**

CLINICAL SIGNIFICANCE (1)

A sufficient concentration of 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 hemoglobin is oxidised to the Fe^{3+} of methemoglobin by ferricyanide, and the methemoglobin 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).

REAGENT COMPOSITION

Vial R1	REAGENT (50 x concentrated)		Working reagent	
Phosphate Buffer	50	mmol/L	1	mmol/L
Potassium cyanide	37.5	mmol/L	0,75	mmol/L
Potassium ferricyanide	30	mmol/L	0,6	mmol/L
Detergent	5	g/L	0.1	g/L
Preservative	< 5	%	< 0.1	%

Before dilution:

T , R23/24/25: Toxic by inhalation, in contact with skin and if swallowed.
N: Harmful for environment, R51-53: Toxic to aquatic organisms, may cause long–term adverse effects in the aquatic environment.

S28, S29, S45, S60-61: After contact with skin, rinse immediately with plenty of water. Do not empty into drains. In case of accident or if you feel unwell, seek medical advice immediately (if possible, show him the label). This material and its container must be disposed as an hazardous waste. Avoid release to the environment. Refer to special instruction/safety data sheet.

Once diluted: None

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- Once diluted, content of vial R1 is not classified as dangerous preparation according to criteria defined in directive 1999/45/CE as well as his updates.
- In case of contact with skin and eyes. Thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENT PREPARATION

Dilute the content of vial R1 (1 + 49) with demineralised water.
Mix gently in order to homogenize the solution.
See § CAUTIONS.

STABILITY AND STORAGE

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

- Without contamination, reagent is stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Without contamination, diluted reagent is stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Discard diluted reagent if cloudy or if absorbance at 546 nm is > 0.010.
- Don't use diluted reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

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

Swirl gently to homogenise before assay.

Hemoglobin is stable in specimen for:

- 48 h at 2-8° C.
- 24 h at room temperature (< 25° C).

INTERFERENCES (2) (3)

Lipemia or leucocitar concentration > 25.10^9/L involve overestimated results. Overestimation has 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. Normal and pathological control blood.

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

QUALITY CONTROL

- Assayed control blood referring to the same method.
- 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.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

In fetal 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

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

Detection limit: approximately 0.3 g/dL

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

LINEARITY

The assay is linear up to 250 g/L, 25 g/dL, 15.5 mmol/L (Hb/4).

MANUAL PROCEDURE

Pipette into test tubes.	**Blank**	**Assay**
Diluted working reagent R1	5 mL	5 mL
Demineralised water	20 µL	
Homogeneized 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

This 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

(4) DRABKIN, D.L., and AUSTIN, J.H., J Biol, Chem., (1935), 112, p.51

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

(6) VAN KAMPEN, E.J. and ZIJLSTRA W.G., International Committee for standardization in haematology, British journal of haematology (1967), 13 [Suppl] 7

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with