HAEMOGLOBIN Colorimetric method (Cyanmethemoglobin)

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

TECHNICAL SUPPORT AND ORDERS
Tel : (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CLINICAL SIGNIFICANCE (1)
A sufficient concentration of haemoglobin in blood is essential for adequate transport of O₂ and CO₂ 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).

Fe²⁺ of hemoglobin is oxidised to the Fe³⁺ 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 concentration of cyanmethemoglobin, is measured at 546 nm (520-560).

REAGENT COMPOSITION

- Vial R1: REAGENT (50 x concentrated) Working reagent
  - Phosphate Buffer 50 mmol/L
  - Potassium cyanide 37.5 mmol/L
  - Potassium ferricyanide 30 mmol/L
  - Detergent 5 g/L
  - Preservative < 5 %

Before dilution:

T: R3, 22/24:5; 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.

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.

INTERFERENCES (2) (3)

Lipemia or leucocitar concentration > 25.10⁹/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.

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.

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

CALCULATION

Measure the absorbance of cyanmethemoglobin (Hb) assayed with the same method.

\[ \text{Hb (g/L)} = \frac{(A_{546} - A_{520}) \times 50 \times C_{\text{Hb}}}{28} \]

Where:
- \( A_{546} \): Absorbance of sample at 546 nm
- \( A_{520} \): Absorbance of reagent blank at 520 nm
- \( C_{\text{Hb}} \): Concentration of haemoglobin in blood

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

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Les Hautes Rives
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Made in France
Latest revision : www.biolabo.fr Revision : 26/07/2011
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

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

In cord blood

<table>
<thead>
<tr>
<th>g/dL</th>
<th>g/L</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5-20.5</td>
<td>135-205</td>
<td>8.37-12.7</td>
</tr>
</tbody>
</table>

In total blood

<table>
<thead>
<tr>
<th>g/dL</th>
<th>g/L</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 months</td>
<td>13.4-19.8</td>
<td>134-198</td>
</tr>
<tr>
<td>1 months</td>
<td>10.7-17.1</td>
<td>107-171</td>
</tr>
<tr>
<td>2 months</td>
<td>9.4-13.0</td>
<td>94-130</td>
</tr>
<tr>
<td>4 months</td>
<td>10.3-14.1</td>
<td>103-141</td>
</tr>
<tr>
<td>6 months</td>
<td>11.1-14.1</td>
<td>111-141</td>
</tr>
<tr>
<td>9 months</td>
<td>11.4-14.0</td>
<td>114-140</td>
</tr>
<tr>
<td>12 months</td>
<td>11.3-14.1</td>
<td>113-141</td>
</tr>
<tr>
<td>1-2 years</td>
<td>11.0-14.0</td>
<td>110-140</td>
</tr>
<tr>
<td>2-5 years</td>
<td>11.0-14.0</td>
<td>110-140</td>
</tr>
<tr>
<td>5-9 years</td>
<td>11.5-14.5</td>
<td>115-145</td>
</tr>
<tr>
<td>9-12 years</td>
<td>12.0-15.0</td>
<td>120-150</td>
</tr>
<tr>
<td>12-14 years M</td>
<td>12.0-16.0</td>
<td>120-160</td>
</tr>
<tr>
<td>12-14 years F</td>
<td>11.5-15.0</td>
<td>115-150</td>
</tr>
<tr>
<td>15-17 years M</td>
<td>11.7-16.6</td>
<td>117-166</td>
</tr>
<tr>
<td>15-17 years F</td>
<td>11.7-15.3</td>
<td>117-153</td>
</tr>
<tr>
<td>18-44 years M</td>
<td>13.2-17.3</td>
<td>132-173</td>
</tr>
<tr>
<td>18-44 years F</td>
<td>11.7-15.5</td>
<td>117-155</td>
</tr>
<tr>
<td>45-64 years M</td>
<td>13.1-17.2</td>
<td>131-172</td>
</tr>
<tr>
<td>45-64 years F</td>
<td>11.7-16.0</td>
<td>117-160</td>
</tr>
<tr>
<td>65-74 years M</td>
<td>12.6-17.4</td>
<td>126-174</td>
</tr>
<tr>
<td>65-74 years F</td>
<td>11.7-16.1</td>
<td>117-161</td>
</tr>
</tbody>
</table>

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

PERFORMANCES

<table>
<thead>
<tr>
<th>Within run</th>
<th>Low level</th>
<th>High level</th>
<th>Between run</th>
<th>Low level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean g/dL</td>
<td>6.7</td>
<td>18.9</td>
<td>Mean g/dL</td>
<td>6.3</td>
<td>17.1</td>
</tr>
<tr>
<td>S.D. g/dL</td>
<td>0.05</td>
<td>0.1</td>
<td>S.D.g/dL</td>
<td>0.29</td>
<td>0.42</td>
</tr>
<tr>
<td>C.V. %</td>
<td>0.7</td>
<td>0.6</td>
<td>C.V. %</td>
<td>4.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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
- Demineralised water: 20 µL
- Homogenized 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 at least for 1 hour.

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

CALCULATION

\[ \lambda = 530 \text{ nm} \]
\[ \lambda = 546 \text{ nm} \]
\[ \lambda = 550 \text{ nm} \]

\[ \text{Hb (g/L)} \, \text{Abs} \times 386.1 \]
\[ \text{Abs} \times 367.7 \]
\[ \text{Abs} \times 376.2 \]

\[ \text{Hb (g/dL)} \, \text{Abs} \times 38.61 \]
\[ \text{Abs} \times 36.77 \]
\[ \text{Abs} \times 37.62 \]

\[ \text{Hb mmol/L (Hb/4)} \, \text{Abs} \times 23.96 \]
\[ \text{Abs} \times 22.82 \]
\[ \text{Abs} \times 23.34 \]

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

REFERENCES

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

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

Made in France Latest revision : www.biolabo.fr Revision : 26/07/2011

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