**HEMOGLOBIN**

**PRINCIPLE**
The Fe (II) of all forms of hemoglobin, with the exception of sulfohemoglobin, is oxidized to the Fe(III) of methemoglobin which, in turn, reacts with ionized cyanide (CN⁻) to form cyanmethemoglobin, a stable derivate which absorbs at 540 nm. The intensity of the color produced is proportional to the concentration of total hemoglobin in the sample.¹

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>R1</th>
<th>Drabkin’s reagent (50x). Modified.² Potassium ferricyanide 30 mmol/L, potassium cyanide 38 mmol/L, potassium hydrogen phosphate 50 mmol/L, surfactant 2.5% (w/v). Xn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL</td>
<td>Hemoglobin standard. Hemoglobin 12 g/dL (7.5 mmol/L). Bovine origin. Concentration value is traceable to Standard Reference Material CRM 522.</td>
</tr>
</tbody>
</table>

**STORAGE AND STABILITY**
Store at 2-30°C. All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the vials tightly closed, protected from light and prevented contaminations during the use.

Discard if appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 540 nm > 0.010 in 1cm cuvette.

**REAGENT PREPARATION**

**Working reagent**
By automatic pipette withdraw 5 mL of R1 and deliver the contents into a 250 mL volumetric calibrated flask letting the flow to slide along the neck to minimize foaming. Add distilled water to the mark, cap, and mix by inversion. Stable for at least 6 months at 15-25°C when stored in a tightly closed brown borosilicate glass bottle. Discard if reagent becomes darkened or discolored.

**PROCEDURE**

1. Pipette into labelled tubes:
   - Working reagent
   - Sample
   - CAL. Standard

2. Mix and let the tubes stand 3 minutes at room temperature.
3. Read the absorbance (A) of the samples and the standard at 540 nm against the reagent blank.

**CALCULATIONS**

With Standard:

\[
\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{g/dL total hemoglobin}
\]

With Factor: \( A_{\text{Sample}} \times 36.8 \times C_{\text{Sample}} \) (g/dL total hemoglobin)

If results are to be expressed as SI units apply:

\[
g/dL \times 0.621 = \text{mmol/L}
\]

**SAMPLES**
Capillary or venous blood. Venous blood should be anticoagulated with 1.5-1.8 mg Na₂EDTA per mL of blood and mixed immediately. Hemoglobin is stable for 1 week at 2-8°C.

**INTERFERENCES**
- Gross lipemia or large amounts of lipoproteins may falsely elevate the hemoglobin value up to 3 g/dL, because of turbidity.
- Bilirubin does not interfere.
- When liquid K₃EDTA is used as anticoagulant the hemoglobin content may be up to 0.5% low because of dilution of the blood sample.
- Other drugs and substances may interfere.⁴

**MATERIALS REQUIRED**
- Photometer or colorimeter capable of measuring absorbance at 540 ± 20 nm.
- 250 mL volumetric, calibrated, glass flask.
- Pipettes to measure reagent and samples.

**REFERENCES**
REFERENCE VALUES

### Whole blood

<table>
<thead>
<tr>
<th>Group</th>
<th>Range in g/dL</th>
<th>Range in mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>13.5 - 18.0</td>
<td>8.4 - 11.2</td>
</tr>
<tr>
<td>Women</td>
<td>11.5 - 16.5</td>
<td>7.1 - 10.2</td>
</tr>
<tr>
<td>Newborns (cor blood)</td>
<td>13.6 - 19.6</td>
<td>8.4 - 12.2</td>
</tr>
<tr>
<td>Infants, 6 months</td>
<td>12.8 - 16.0</td>
<td>8.0 - 10.0</td>
</tr>
<tr>
<td>Infants, 1 year</td>
<td>11.0 - 13.0</td>
<td>6.8 - 8.1</td>
</tr>
<tr>
<td>Children, 14 years</td>
<td>11.5 - 14.8</td>
<td>7.1 - 9.2</td>
</tr>
</tbody>
</table>

Age, race, exercise, seasons and altitude can influence the values of the normal range. It is recommended that each laboratory establishes its own reference range.

### QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used. To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns. If the values are found outside of the defined range, check the instrument, reagents and procedure. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

### CLINICAL SIGNIFICANCE

The determination of the total concentration of hemoglobins is indicative of the oxygen and carbon dioxide-carrying capacity between lungs and other tissues, being also important as an initial step in the detection of anemia and erythrocytosis. Blood hemoglobin concentration may be diminished as a consequence of hemorrhage or hemolysis or as a result of impaired blood formation in the bone marrow. Conversely, blood hemoglobin concentration may be increased when gas exchange through the lungs is impaired or in various other diseases.

### NOTES

1. The concentration of potassium cyanide in solution is so low that it does not constitute any significant hazard to personnel. The amount present in one liter of reagent is appreciably less than the minimum lethal dose for a 70 Kg human.
2. Since hydrogen cyanide is liberated by acidification of the working reagent discard spent reagents and samples into running water in the sink and never allow them to come in contact with acid.
3. Homogenize the content of the vial by swirling gently, avoiding formation of foam. Do not shake.
4. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

### ANALYTICAL PERFORMANCE

- **Detection Limit:** 0.02 g/dL
- **Linearity:** Up to 20 g/dL
- **Precision:**

<table>
<thead>
<tr>
<th>g/dL</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>SD</td>
<td>0.85</td>
<td>0.54</td>
</tr>
<tr>
<td>CV%</td>
<td>5.70</td>
<td>3.63</td>
</tr>
</tbody>
</table>

### REFERENCES