DIRECT HEMOGLOBIN REAGENT

FOR THE COLORIMETRIC DETERMINATION OF HEMOGLOBIN IN BLOOD

SUMMARY AND EXPLANATION:

The cyanomethemoglobin procedure has become internationally adopted as the reference method for hemoglobin assay. The present method is based on the original procedure described by Drabkin in which cyanide and ferricyanide in an alkaline medium convert hemoglobin to the highly colored cyanomethemoglobin derivative, which may be measured spectrophotometrically at 540 nm. The method is essentially a measure of total hemoglobin because reduced hemoglobin, oxhemoglobin, methemoglobin and carboxyhemoglobin react to give the cyanomethemoglobin derivative.

The cyanomethemoglobin standard is a stabilized preparation of human hemoglobin with an accurately assigned value and is used as a calibrator for the hemoglobin procedure.

Reagents for in vitro diagnostic use:

1. Drabkin's reagent powders - sodium bicarbonate, reagents for in vitro diagnostic use for the hemoglobin procedure. The cyanomethemoglobin standard is a stabilized preparation of human hemoglobin with an accurately assigned value and is used as a calibrator for the hemoglobin procedure.

PROCEDURE:

Cuvettes

<table>
<thead>
<tr>
<th>DRABKIN’S REAGENT (mL)</th>
<th>5.0</th>
<th>3.0</th>
<th>2.0</th>
<th>1.0</th>
<th>—-</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD (mL)</td>
<td>5.0</td>
<td>—-</td>
<td>—-</td>
<td>—-</td>
<td>—-</td>
</tr>
<tr>
<td>UNKNOWN (L)</td>
<td>—-</td>
<td>—-</td>
<td>—-</td>
<td>0.02</td>
<td>—-</td>
</tr>
</tbody>
</table>

Mix and allow to stand 5 minutes at room temperature.

Zero the instrument with the blank cuvette at 540 nm. Read and record the absorbance of the standard and unknown cuvettes.

Calculations:

The following equation is used to determine unknown concentration:

\[ \text{Unknown Absorbance} = \frac{20 \times \text{Standard Absorbance}}{\text{Hemoglobin in standard absorbance (g/dL)}} \]

Example of calculations:

\[ \text{Unknown Abs} = \frac{0.48 \times 15.8 \text{ g/dL}}{0.61} \]

0.61

NOTE: For direct readout instrument, set readout to concentration of standard and read the unknown concentrations directly.

Method using calibration curve:

An alternate method for standardization of the hemoglobin procedure is the use of a calibration curve. By this method, a straight line is generated and the results may be read directly from a graph.

\[ \text{Hemoglobin (g/dL)} = \frac{10 \times \text{Absorbance}}{20} \]

Example:

\[ \text{Hemoglobin (g/dL)} = \frac{10 \times 0.340}{20} \]

0.17

QUALITY CONTROL:

The regular use of control blood is recommended as part of a quality control program.

LIMITATIONS OF THE METHOD:

The extremely low concentrations of hemoglobin in plasma preclude the use of this specimen in this procedure. Blood collected without an anticoagulant (such as that obtained from a finger puncture) must be employed expeditiously in order to avoid clotting of the specimen.

If condensation appears on the inner wall of the bottle, mix well before use.

Performance characteristic of the test:

Linear regression analysis of 40 samples of whole blood with hemoglobin concentration ranging from 6.8 and 17.8 g/dL was performed comparing the present method with other commercially available reagent. Based also on the cyanomethemoglobin method, with the following results:

\[ y (\text{curtiss}) = 1.002 + 0.1r \]

\[ r (\text{CORRELATION COEFFICIENT}) = 0.998 \]
PRECISION BETWEEN RUN PRECISION WAS DETERMINED BY ASSAYING TWO WHOLE BLOOD SAMPLES, 7 TIMES DAILY OVER A 3 DAY PERIOD (N=21):

<table>
<thead>
<tr>
<th>SAMPLE #1</th>
<th>SAMPLE #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN (g/dL)</td>
<td>11.2</td>
</tr>
<tr>
<td>STANDARD DEVIATION (g/dL)</td>
<td>0.31</td>
</tr>
<tr>
<td>COEFFICIENT OF VARIATION (%)</td>
<td>2.77</td>
</tr>
</tbody>
</table>

SENSITIVITY UNDER THE CONDITIONS DESCRIBED ON PROCEDURES A CHANGE OF 0.001 ABSORBANCE UNITS, WHEN MEASURED IN 1 CM LIGHT-PATH CUVEIT, IS EQUIVALENT TO APPROXIMATELY 0.27 g/dL.

REFERENCES

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