Experiment no.: 7.

Experiment name: Determination of HEMOGLOBIN in the blood serum.

The aim of the Experiment:

Determination of HEMOGLOBIN TOTAL using Colorimetric method ENDPOINT

Equipment and martials used in the Experiment:

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

R1 Drabkin’s reagent (50x). Modified. Potassium ferricyanide 30 mmol/L, potassium cyanide 38 mmol/L, potassium hydrogen phosphate 50 mmol/L, surfactant 2.5% (w/v). Xn

Hemoglobin standard. Hemoglobin 12 g/dL (7.5 mmol/L). Bovine origin. Concentration value is traceable to Standard Reference Material CRM 522.

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.

Property of the machine:

Normal UV-Vis spectrophotometer:

Machine usage:

7- Wavelength set up step.
8- Blank against the solvent solution using a proper cuvette.
9- Reach O.D.
Experiment procedure or protocol:

1. **PROCEDURE**

1. Pipette into labelled tubes:

<table>
<thead>
<tr>
<th>TUBES</th>
<th>Blank</th>
<th>Sample</th>
<th>CAL. Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>2.5 mL</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sample CAL.</td>
<td>2.5 mL</td>
<td>2.5 mL</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Standard</td>
<td>–</td>
<td>10 μL</td>
<td>–</td>
</tr>
<tr>
<td>Working reagent</td>
<td>2.5 mL</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sample CAL.</td>
<td>2.5 mL</td>
<td>2.5 mL</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Standard</td>
<td>–</td>
<td>10 μL</td>
<td>–</td>
</tr>
</tbody>
</table>

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.

The color is stable for several hours. Storage for periods over 6 hours should be kept at 2-8 °C.

**CALCULATIONS**

With Standard:

\[
\text{A Sample / A Standard } \times \text{C Standard} = \text{g/dL total haemoglobin}
\]

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

If results are to be expressed as SI units apply: \(\text{g/dL} \times 0.621 = \text{mmol/L}\)

**Experiment data and results:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (g/dL)</th>
<th>Range (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>en</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 (cord 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>

**Conclusion:**

- What is the role of this experiment?
- How does its deficiency affect the health?
- What are the normal level values?
- How can you determine its quantity on the blood?
- Discuss the methodology?