Lab 04: HB Estimation

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HB Estimation

• Measurement of Hemoglobin (Hb) concentration in a whole blood sample is a basic screening test for anemia and polycythemia.

• The hemoglobin concentration of the solution may be estimated by measurement of its color, by its power of combining with oxygen or carbon monoxide, or by its iron content.

• The methods mostly used are color or light-intensity matching techniques. Ideally, for assessing clinical anemia, a functional estimation of hemoglobin should be carried out by measurement of oxygen capacity, but this is hardly practical in the routine hematology laboratory. It gives results that are at least 2% lower than those given by the other methods.

• The iron content of hemoglobin can be estimated accurately, but again the method is impractical for routine use. Estimations based on iron content are generally taken as authentic, iron bound to inactive pigment is included.
Haemiglobincyanide (Cyanmethaemoglobin) Method

• The haemiglobincyanide (Cyanmethaemoglobin) method is the internationally recommended method for determining the hemoglobin concentration of blood.

• **Principle**
  - Blood is diluted in Drabkin's solution, a solution containing potassium cyanide and potassium ferricyanide.
  - Potassium ferricyanide oxidizes hemoglobin to methaemoglobin.
  - Methaemoglobin then combines with potassium cyanide to form Cyanmethaemoglobin (HiCN).

\[
\text{Hemoglobin (Fe}^{+2}\text{)} + \text{K+ Ferricyanide} \rightarrow \text{Methemoglobin (Fe}^{+3}\text{)} + \text{K+ Cyanide}
\]
Haemiglobincyanide (Cyanmethaemoglobin) Method

• The absorbance of the solution is measured in a spectrophotometer at a wave length of 540 nm against Drabkin’s solution as a blank. The result is calculated using the formula provided below and it is expressed in gm/dl.

• Test Sample

• Venous or capillary blood collected into an EDTA tube. Alternatively, free flowing capillary blood may be added directly to the diluting fluid and measured.
Procedure

1. Add **5 ml** of **Drabkin** reagent to a test tube.
2. Add **20ul blood** to the test tube using **Hb pipette** or **micropipette**.
3. **Clean** outside of pipette and wash out the blood in the tube containing the diluent.
4. **Mix** and leave for **5-10 minutes** for the reaction to complete.
5. Measure the **absorbance** of the sample **against blank (drabkin’s reagent)** at **540 nm** by **spectrophotometer**.
Calculation

- Use the following formula:

\[ \text{Hb in g/dl} = \frac{A \times 64500 \times \text{DIL}}{44 \times D \times 1000 \times 10} = A \times 29.3 \]

- Where: \( A = \) Reading of absorbance of Hb solution
  - 64500 = Molecular wt. of Hb
  - 44 = Millimolar extinction coefficient
  - \( D = \) Thickness of cuvette
  - 1000 = Conversion factor of mg to gm
  - \( \text{DIL} = \) Dilution Factor = 200

Notes on Technique:

- The blood sample must be properly mixed before sampling and allowed to warm
- Care should be taken when handling potassium cyanide.
- Use clean tubes and pipettes.

* The cyanmethaemoglobin method is the reference method for Hb estimation because:
  a) all Hb forms except sulphhaemoglobin are estimated, b) highly reliable and stable reagents are available and c) the method can be easily standardized.
The normal ranges

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborns</td>
<td>17-22gmdl</td>
</tr>
<tr>
<td>One (1) week of age</td>
<td>15-20gmdl</td>
</tr>
<tr>
<td>One (1) month of age</td>
<td>11-15gm\dl</td>
</tr>
<tr>
<td>Children</td>
<td>11-13gm\dl</td>
</tr>
<tr>
<td>Adult men</td>
<td>14-18gm\dl</td>
</tr>
<tr>
<td>Adult women</td>
<td>12-16gm\dl</td>
</tr>
<tr>
<td>Men after middle age</td>
<td>12.4-14.9gm\dl</td>
</tr>
<tr>
<td>Women after middle age</td>
<td>11.7-13.8gm\dl</td>
</tr>
</tbody>
</table>

- Normal values in an adult are 12 to 18 grams per deciliter (100 milliliters) of blood.
Oxyhaemoglobin Method

• The HbO2 method is the simplest and quickest method for general use with a photometer. Its disadvantage is that it is not possible to prepare a stable HbO2 standard, so the calibration of these instruments should be checked regularly using HiCN reference.
Sahli’s Method or Acid Hematin method

• Principle
  • Blood is mixed with an acid solution so that Hb is converted to brown colored acid hematin
  • Diluted with water till brown colour matches that of brown glass standard
  • Hb value is read directly from the scale
Hb estimation by Sahli method

Specific Equipments

- Sahli hemoglobinometer
- Sahli pipette (marked at 20 microliter or 0.02 ml)
- Test tube (graduated)
- HCl 0.1N
- Distilled water
- Stirrer
- Comparator block
- Droppers
Procedure

1. Place N/10 HCl into Hb tube upto 2 grams.
3. Add **blood sample** to **acid solution**.
4. **Mix** with a stirrer.
5. **Allow** to stand for 10 minutes.
6. Add **distilled water** drop by drop till the colour of the solution matches to **brown glass standard**.
7. Take the reading of the lower **meniscus** from the graduated tube in grams.
Direct Reading Portable Haemoglobinometers

• **Color Comparators**

• These are simple clinical devices that compare the color of blood against a range of colors representing hemoglobin concentrations. They are intended for anemia screening in the absence of laboratory facilities.

• **Portable Hemoglobinometers**

• Portable hemoglobinometers have a built-in filter and a scale calibrated for direct reading of hemoglobin in g/dl or g/l. They are generally based on the HbO2 method. Some hemoglobinometers require dilutions of blood and others do not require dilutions because blood is drawn into cuvettes containing certain chemicals.
Direct Reading Portable Haemoglobinometers

• Noninvasive Screening Tests

• Methods are being developed for using infrared spectroscopy at body sites, mainly a finger, to identify the spectral pattern of haemoglobin in an underlying blood vessel and derive a measurement of haemoglobin concentration.
Automated Haemoglobin measurement

Most automated counters measure haemoglobin by a modification of the manual HiCN method with cyanide reagent or with a nonhazardous chemical such as sodium lauryl sulphate, which avoids possible environmental hazards from disposal of large volumes of cyanide containing waste. Modifications include alterations in the concentration of reagents and in the temperature and pH of the reaction. A nonionic detergent is included to ensure rapid cell lysis and to reduce turbidity caused by cell membranes and plasma lipids. Measurements of absorbance are made at a set time interval after mixing of blood and the active reagents, but before the reaction are complete.
SIGNIFICANT OF THE TEST:

• Physiological Variation:
  • HB values are affected by **age, sex, pregnancy, and altitude**.

• Below-normal hemoglobin levels may lead to anemia
  1. **Iron deficiency** or deficiencies in essential **vitamins** of other elements, such as **B12, folate, B6**.
  2. Inherited hemoglobin defects, such as **sickle cell anemia** or **Thalassemia**.
  3. Excessive **bleeding**.
  4. Excessive **destruction** of red blood cells.
  5. **Kidney** disease.
  6. Bone marrow failure or **aplastic anemia**.
  7. **Cancers** that affect the bone marrow.
• Above-normal hemoglobin levels is called polycythemia:

1. Primary polycythemia which is due to malignant variation in blood cells production in bone marrow

2. Secondary polycythemia which is may be due to:
   a) **Dehydration** (sever burns, diarrhea, vomiting, …etc.).
   b) **Severe lung** or **heart disease**.
   c) Living at high **altitudes**.
   d) **Heavy smoking**.
MECHANICAL SOURCES OF ERROR:

- Pipetting error.
- Use of dirty or scratched cuvettes.
- Use of expired reagent.
- Patient with Lipemia → ????