

كلية الرشيد الجامعة / قسم الصيدلة مختبر الفسلجة والامراض المرحلة الثانية (2020 – 2021)



Determination of <u>Hemoglobin (Hb)</u> Concentration

Physiology Lab. 2nd stage / 1st course Lab. (5)

Done by:

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Introduction:

- Hemoglobin is the main constituent of RBCs
- It gives the blood its characteristic red color
- Hemoglobin consists of two main parts:
 - Heme pigment; contains iron in the form of Fe+2 and constitutes 4%
 - Protein globin; which constitutes 96%



Types of Hemoglobin:

- Adult hemoglobin (HbA): consists of 2 types of polypeptide chains, α chain and β chain, so HbA is designed as $\alpha 2\beta 2$
- Adult hemoglobin (HbA2): consists of 2 types of polypeptide chains, α chain and δ (delta) chain. HbA2 is designed as $\alpha 2\delta 2$
- Glycosylated hemoglobin (HbA1C): also consists of 2 types of polypeptide chains, α chain and β chain with the addition of glucose molecule to the terminal amino acid of each chain, so it is important in diabetes mellitus
- Fetal hemoglobin (Hbf): consists of 2 types of polypeptide chain, α chain and γ (gamma) chain. Hbf is designed as α2 γ2, this type of Hb is replaced after birth by adult Hb

Function of Hemoglobin:

- Act as O2 carriers from the lungs to tissues and back transport of CO2 from tissues to lungs
- Acts as buffer that helps regulate the PH of the blood
- When it is broken down, it forms a bile pigment (which has a role in the digestion of lipids)

Abnormalities of Hemoglobin:

There are two major types of inherited disorders of Hb in humans:

- 1. Hemoglobinopathies: a group of inherited blood disorders that affect RBCs, which result in abnormalities in the structure of globin protein leading to premature destruction of RBCs and anemia (like sickle cell anemia), in this anemia the type of hemoglobin is usually (Hbs)
- 2. Thalassemia: results from decreased production of globin protein, it is of two types:
 - Alpha thalassemia
 - Beta thalassemia

Both types result from either reduction or absence of alpha or beta polypeptides



Determination of Hb Concentration:

1. Cyanmethemoglobin Method (accurate):

Principle: based on reacting the hemoglobin in blood sample with a reagent solution containing (potassium ferricyanide) to form cyanmethemoglobin (colored compound), then measured using a spectrophotometer (an instrument that measures the absorbance of light by a colored sample at certain wavelength)



Determination of Hb Concentration:

2. Hemoglobin Color Scale (Qualitative):

Principle: this method is based on having a drop of blood on a strip paper, then compare the color of the blood drop to a standard colored chart



INSTRUCTIONS FOR USE

 Clean the skin of a finger with alcohol, when of the excess and let it dry.

2 Prick the finger with a sterile lancet so that blood flows without squeezing

3. Take up a drop of blood at one end of the test paper, and as soon as the sheen chappears match it against the colour scale, holding the booket open in the left hand at 45° with the light coming from befind.

4 Side the blood stan beford the colour scale operanes until the best match is found and also try to match it against the side of the scale.

 Record the holomogicbin value of the best match, or 4 this lies between two values (e.g., 4.6 Hb).
 Use broad daylight but not dred sunight or actional light.

 Do not let light enter hom behod as this makes matching unreliable.

 Use only approved test papers because others do not give satistactory results.

Close the booklet and replace it in its case because light may cause colours to face.





Haemoglobin Strip

Determination of Hb Concentration:

3. Sahli Method (estimate):

Principle:

- Blood hemoglobin is converted to acid hematic (brown color) by the action of HCl
- The intensity of color is measured by comparing the sample to a standard colored bars

Aim of this Method:

- Measure the concentration of hemoglobin in blood sample
- Find the percentage of error in this method

Normal Values: Male: 13.6 – 17.2 gm/dl of blood Female: 11.5 – 15 gm/dl of blood

Sahli Hemoglobinometer



Procedure Requirements:

- 70% alcohol & cotton
- Sterile blood lancet
- 0.1 N hydrochloric acid
- Distilled water
- Sahli hemoglobinometer





Sahli Method (percentage of error):

- Sahli experiment is associated with percentage of error
- The percentage of error is calculated from the readings obtained from (hemoglobin graduated cylinder)
- Hemoglobin graduated cylinder has two readings:
 - Red (as percentage of Hb)
 - Yellow (as conc. gm/dl of Hb)
- If the percentage of error is ≤ (less than)
 10%, the results are accepted



Calculation the percentage of error:

Example: calculate the percentage of error in Sahli hemoglobin method, if the results obtained are 80% and 10.2 gm/dl for a female patient?

- Calculate the percentage from the <u>red reading (% of Hb)</u>:
- % of Hb (estimated) = 80%
- Normal Hb conc. for female is (11.5 15 gm/dl) <<< take the average (13.5 gm/dl)
- 13.5gm/dl 100% X 80%
- X = 10.8 gm/dl (calculated Hb)
- % of error = (calculated estimated)/average * 100
 - = 10.8 10.2 / 13.5 * 100
 - = 4.4 %

- Calculate the percentage from the <u>yellow</u> reading (gm/dl):
- Gm/dl of Hb (estimated) = 10.2
- Normal Hb. Conc. For female is (11.5 15 gm/dl) <<< take the average (13.5 gm/dl)
- 13.5gm/dl 100% 10.2gm/dl X X = 75.6 %
- % of error = calculated estimated
 - = 75.6% 80%
 - = 4.4 %