



Al-Rasheed University College Pharmacy Department
2nd Stage / 1st Semester
2021-2022



Determination of Hemoglobin

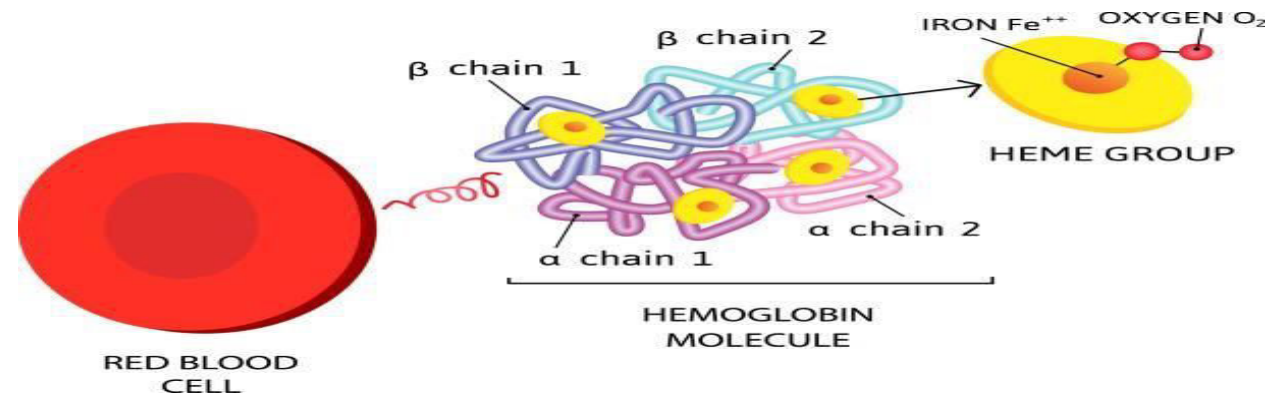
Physiology lab #5

Done by:

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Introduction

- Hemoglobin (Hb) is an iron-containing protein → Main constituent of RBCs
- Hb consist of two parts:
- Heme pigment: Displays iron in Fe⁺² form, constitute 4% (4 molecules)
- Globin protein: Constitute up to 96% (4 molecules)



Hemoglobin's functions

- **Hb** binds to the oxygen in the lungs >> Transport oxygen >> Release oxygen to tissues to permit aerobic respiration.
- Transport H⁺, Carbon dioxide from tissues to lungs
- **Hb** provides **blood's** distinctive “Red color”.
- Regulate **blood's PH** by working as a **buffer**.
- Contribute in formation of bile pigment.

Hemoglobin's forms

- **Oxyhaemoglobin:** Hb combined with O₂. Each of the 4 iron atoms in Hb molecule can bind reversibly to one O₂ molecule!

Iron stays in ferrous state (Fe⁺²) → Reaction is oxygenation not oxidation

- **Carbaminohaemoglobin:** Hb combined with CO₂.
- **Carboxyhaemoglobin:** is a complex Hb found mainly in smoker's blood

It can be produced and released in the following conditions:

1-Hb binds to CO.

2-During degradation of Hb

Hemoglobin's forms

- **Methaemoglobin (MHb):** A type of Hb in which the iron in heme group is ferric (fe+3) state.
 - *Unable to carry O₂*
 - *MetHb enzyme converts MHb → Hb*
 - *Lacking of MetHb → increase in Mhb levels → Methemoglobinemia*
- **Sulphaemoglobin:** Hb containing sulfar and is unable to transport O₂. It is usually formed by certain oxidizing drugs.

Hemoglobin's classifications

- **Adult hemoglobin (HbA)**: Consists of 2 types of polypeptide chains (Alpha, Beta).
(α, β) $\rightarrow \alpha_2\beta_2$
- **Adult hemoglobin (HbA2)**: Consists of 2 types of polypeptide chains (Alpha, Delta).
(α, δ) $\rightarrow \alpha_2\delta_2$
- **Fetal hemoglobin (Hbf)**: Consists of 2 types of polypeptide chain (Alpha, Gamma).
(α, γ) $\rightarrow \alpha_2\gamma_2$.

[This type of Hb is replaced after birth by adult Hb](#)

- **Glycosylated hemoglobin (HbA1C)**: Consists of 2 types of polypeptide chains (α, β) with glucose molecule attached to the terminal amino acid of each chain.

[Clinically important in diabetes mellitus.](#)

Hemoglobin's abnormalities

Hemoglobinopathies:

Group of **inherited blood disorders** that affects **RBC** → Abnormalities in structure of globin protein → Premature destruction of **RBCs and anemia**.

Sickle cell anemia: an inherited disease causes a mutation in **B chain** → Sickle shaped RBC.

Thalassemia: Results from decreased production of globin proteins, it have two types:

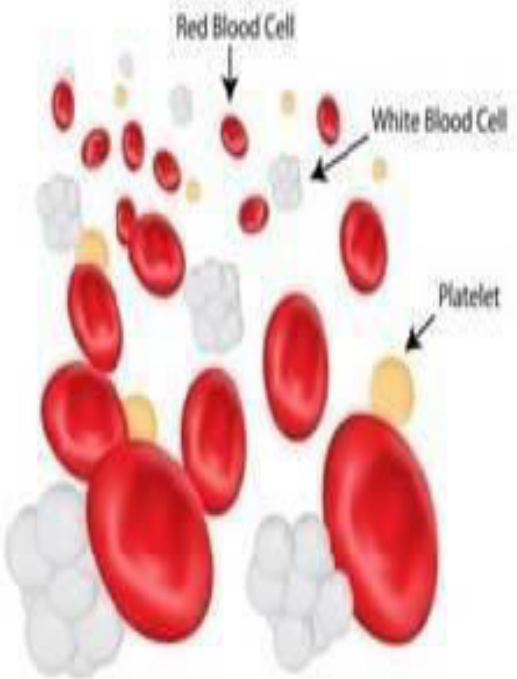
Alpha – thalassemia

Beta – thalassemia

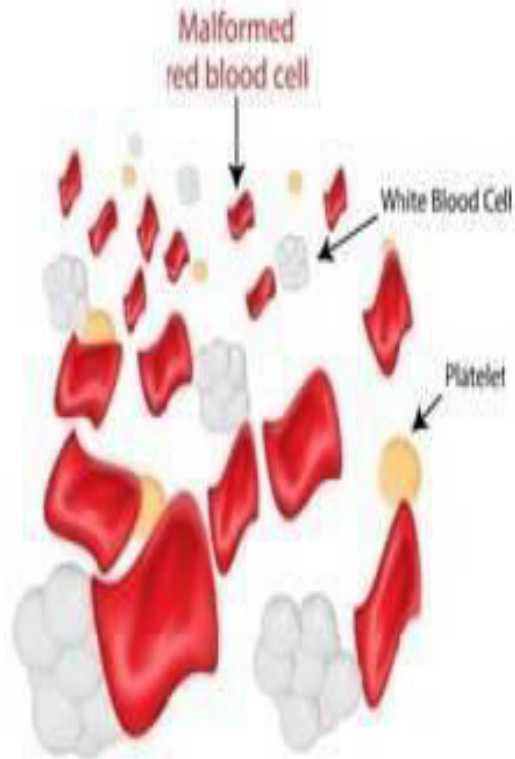
Both types results from either reduction or absence of alpha or beta polypeptides

Thalassemia

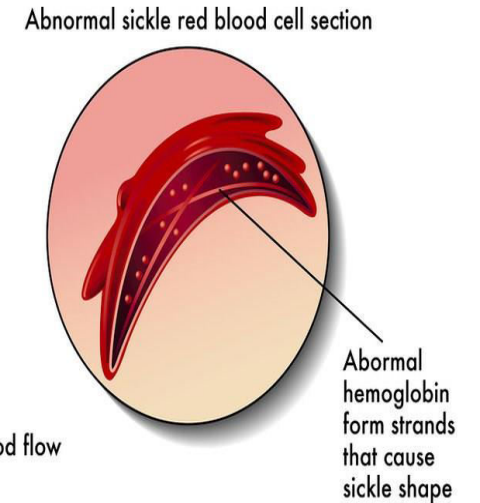
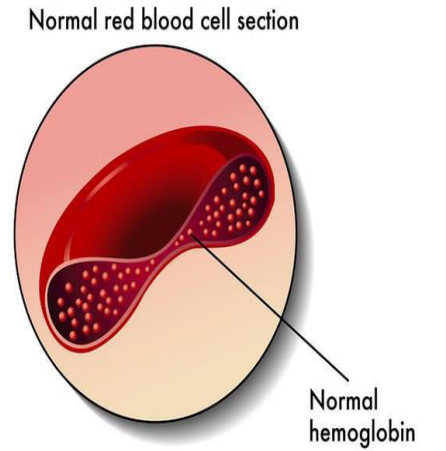
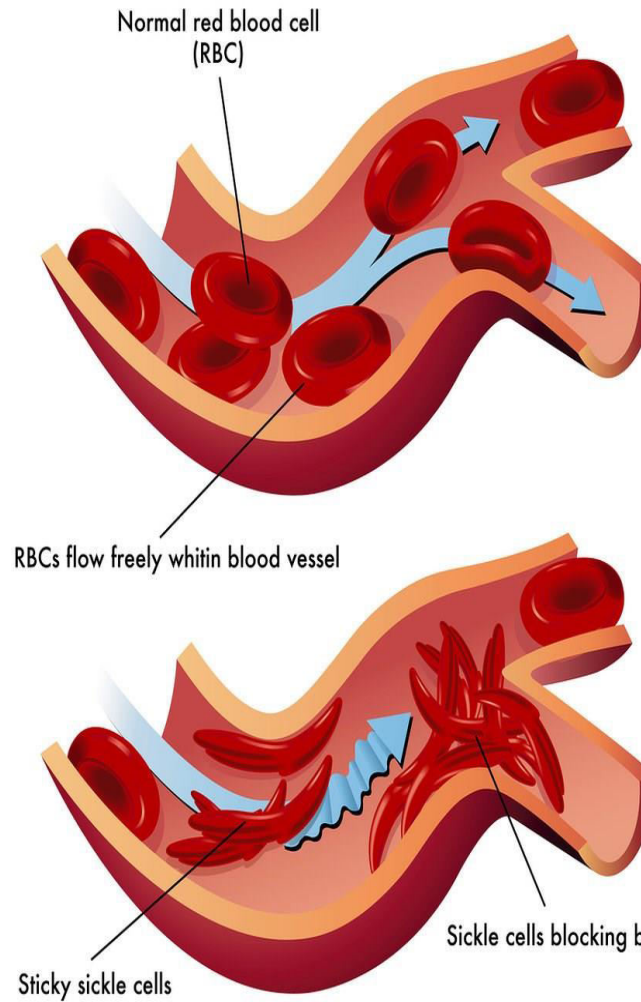
Normal



Thalassemia



Sickle-Cell Anemia

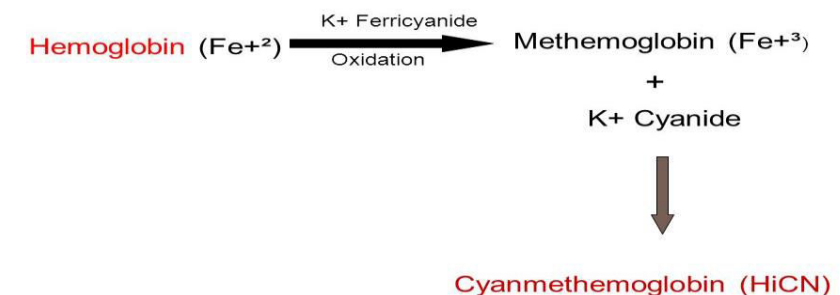


Determination of Hb's concentration

- **Cyanmethemoglobin Method (Accurate):**

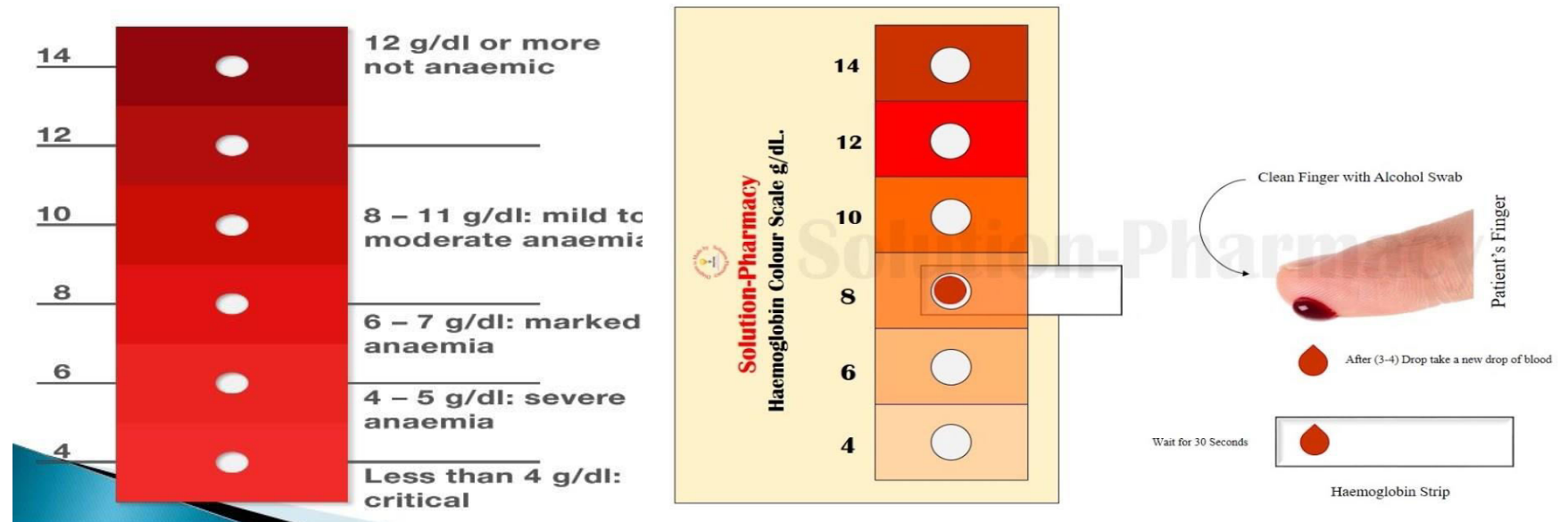
Principle: based on reacting the **hemoglobin** in blood sample with a reagent solution containing (**potassium ferricyanide**) to form **cyanmethemoglobin** (Colored compound).

The sample should then be assessed by using spectrophotometer (an instrument that measures the absorbance of light by a colored sample at certain wavelength)



Determination of Hb's concentration

- **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



Determination of Hb's conc

- **Sahli Method (Estimate):**

- **Principle:**

Blood hemoglobin is converted to hematic acid (brown color) by HCl.

- **Interpretation:**

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:**

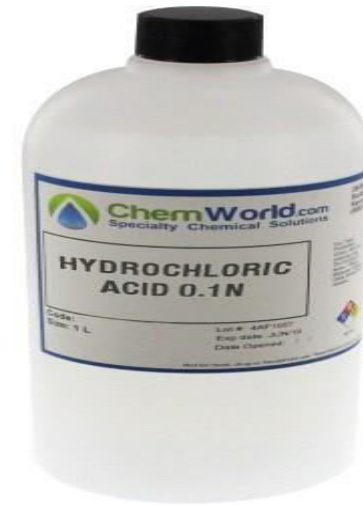
Male 13.6 – 17.2 gm/dl of blood .. **Female** 11.5 – 15 gm/dl of blood

Sahli Hemoglobinometer



Procedure requirements

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



Procedure

- Fill the graduated tube to **mark (2)** with **0.1 normal HCL** .
- Draw **blood** by **hemoglobin pipette** to **mark 20 μ l (Micro-litters)**.
- Dip the tip of the pipette in the graduated tube to blow the blood into the tube and mix content with a stirrer.
- Place the tube in the **hemoglobinometer** for **10 min** to **ensure complete reaction**.
- Add drop by drop **D.W** until the color in graduated tube is identical to the color of the standard .
- Read and assess the results in **g/dl**.

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 **red reading (% of Hb):**
- % of Hb (estimated) = 80%
- Normal Hb conc. for female is (11.5 – 15 gm/dl) <<< take the average (13.5 gm/dl)
- Calculate the percentage from the **yellow 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)

$$\begin{array}{rcl} 13.5\text{gm/dl} & 100\% & \\ X & 80\% & \\ X = 10.8 \text{ gm/dl} & \text{(calculated Hb)} & \end{array}$$

$$\begin{aligned} \text{\% of error} &= (\text{calculated} - \text{estimated}) / \text{average} * 100 \\ &= 10.8 - 10.2 / 13.5 * 100 \\ &= 4.4 \% \end{aligned}$$

$$\begin{array}{rcl} 13.5\text{gm/dl} & 100\% & \\ 10.2\text{gm/dl} & X & \\ X = 75.6 \% & & \end{array}$$

$$\begin{aligned} \text{\% of error} &= \text{calculated} - \text{estimated} \\ &= 75.6\% - 80\% \\ &= 4.4 \% \end{aligned}$$

**THANK YOU FOR LISTENING TO OUR
PRESENTATION**

