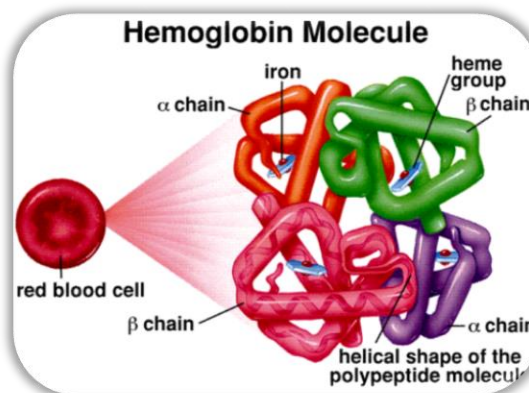




University of Diyala/ College of Medicine
Department of Physiology
Physiology Lab

Estimation of Hemoglobin (Hb)

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Outlines

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- Objectives of Hb estimation experiment
 - Introduction

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- Principle of the experiment
 - Materials and Methods

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- Procedure
 - Clinical implications

Objectives



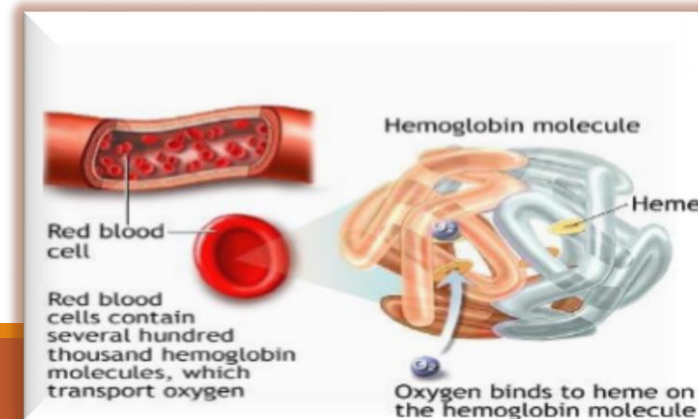
As part of a complete blood count (CBC), during a health checkup, or when a healthcare practitioner suspects that you have a condition such as anemia or polycythemia.

By the end of this lab, you should have useful information about the structure of Hb, its functions and types, methods for determination Hb value, and clinical importance of Hb .

To learn how to use different methods in the lab to get the value of Hb.

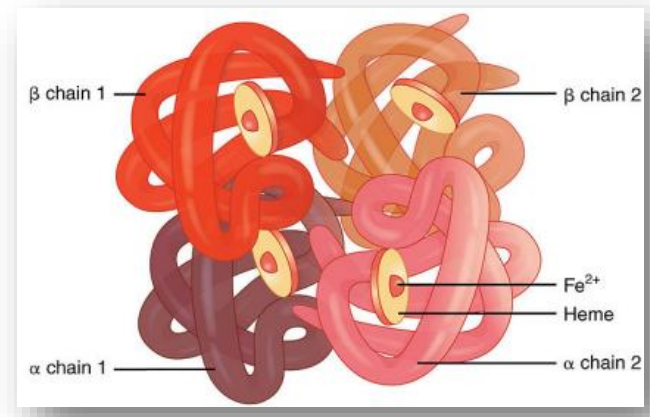
Introduction

- ✓ Hemoglobin is the iron-containing oxygen transporter (metalloprotein) in the red blood cells of humans. It gives the blood its red color.
- ✓ Hemoglobin carries oxygen from the lungs to the rest of the body (i.e the tissues) where it releases the O_2 to burn nutrients to provide energy to power the functions of the body & collects the resultant CO_2 to bring it back to the lungs to be released to the atmosphere .
- ✓ Hemoglobin is the major constituent of the red cell cytoplasm, accounting for approximately 90% of the dry weight of the mature cell.



Structure of Hb

- ✓ Hemoglobin molecule is a tetramer consisting of two pairs of similar polypeptide chains called *globin chains*.
- ✓ To each of the four chains *heme* is attached which is a complex of iron in ferrous form and protoporphyrin. It gives red color to the blood.
- ✓ The major (96%) type of hemoglobin present in adults is called *HbA* and it has 2 alpha globin chains & 2 beta globin chains ($\alpha_2\beta_2$)



Normal Hb Levels

The normal value of Hb varies according to the age and sex of the individuals. The normal ranges are:



Male

14 -18 g/dl



Female

12 – 16 g/dl



Neonate

13 – 20 g/dl

Keep this table as a reference , you don't have to memorize the values here.



Age Group	Hemoglobin Count (gm/dl)
Normal Hemoglobin Levels in Newborn Babies	17 – 22
Normal Hemoglobin Levels in Children	11 – 13
Normal Hemoglobin Levels in Adults (Male)	14 – 18
Normal Hemoglobin Levels in Adults (Female)	12 – 16
Normal Hemoglobin Levels in Elderly (Male)	12.4 – 14.9
Normal Hemoglobin Levels in Elderly (Female)	11.7 – 13.8

Types of Normal Hemoglobin

- **HbA (2 α 2 β):** It is the normal adult Hb. The two types of polypeptide in HbA are called **α chains** (each has 141 amino acids) and **β chains**, (each has 146 amino acids). HbA is predominant type in adult (95-97%).
- **HbA2 (2 α 2 δ):** In normal adult, about 2.5% of the total Hb is HbA2. In HbA2, β chains are replaced by δ chains. Each δ chain contains 146 amino acids but 10 of them are different from those of β chain.
- **HbF(2 α 2 γ):** The main Hb in fetus. γ chain also has 146 amino acids but 37 are different from those in β chain. HbF is replaced gradually by HbA soon after birth (usually about 6 months to one year of age, HbA predominates).
- In normal adult, HbF may be found in a level of less than 2% of the total Hb. It has a greater affinity for O₂ than HbA which facilitates the movement of O₂ from maternal to fetal circulation.

Types of Normal Hemoglobin

- During embryonic and fetal life, other different types of hemoglobin predominate.
- ***Gower I, Gower II and Hb Portland*** present in early embryonic life.
- After the ***8th week*** of development, embryonic hemoglobin is replaced by ***Fetal hemoglobin HbF ($\alpha_2 \gamma_2$)***
 - This remains the predominant hemoglobin until after birth and constitutes 50-90% of the total hemoglobin.
 - After birth, it's concentration decreases to less than 2% by 30 weeks of age.

Heterogeneity of Human Hemoglobin

Hemoglobin Type	Structure	Developmental Stage
Hb Gower 1	$\zeta_2 \epsilon_2$	embryo
Hb Gower 2	$\alpha_2 \epsilon_2$	embryo
Hb Portland	$\zeta_2 \gamma_2$	embryo
HbF	$\alpha_2 \gamma_2$	fetal and adult
HbA₂	$\alpha_2 \delta_2$	adult
HbA	$\alpha_2 \beta_2$	adult

Forms of Hemoglobin

1

Oxyhemoglobin

- Hb combined with O₂. Each of the 4 iron atoms in Hb molecule can bind reversibly to one O₂ molecule. The iron stays in the ferrous state, so that the reaction is oxygenation not oxidation.

2

Carbaminohemoglobin

- Hb combined with CO₂.

3

Carboxyhemoglobin

- Hb combined with CO. A concentration of about 0.5% carboxyhemoglobin is produced by the normal degradation of Hb. Slightly increased level can be found in the smoker's blood and due to environmental pollution. Hb becomes useless to transport O₂.

Forms of Hemoglobin

4

Methemoglobin

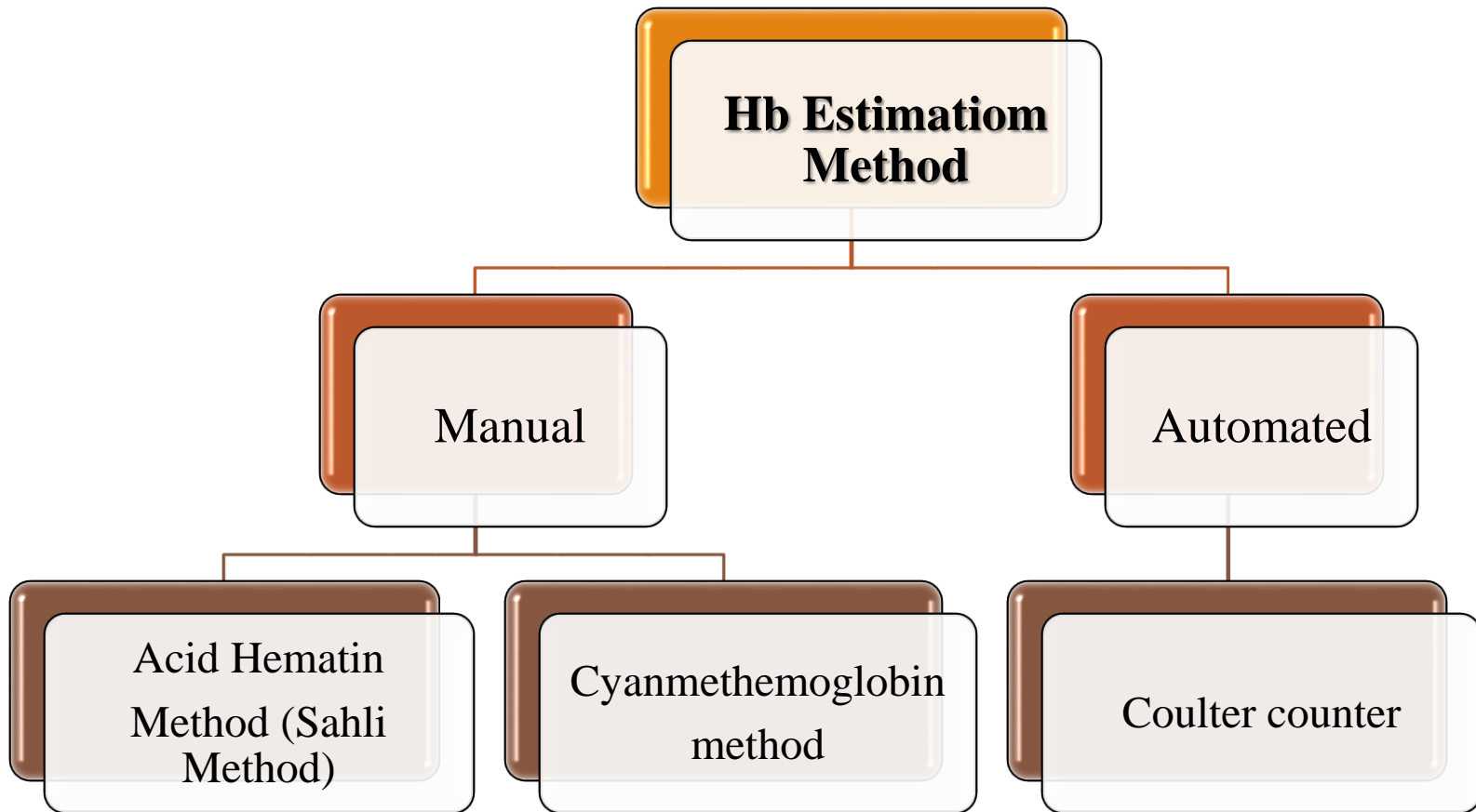
- A type of Hb in which the iron in the heme group is in ferric (Fe^{+3}) state , not ferrous (Fe^{+2}) of normal Hb . Some oxidation of Hb to MetHb occurs normally but an enzyme called MetHb reductase in RBC converts MetHb back to Hb. The congenital absence of that enzyme is one cause of hereditary Methemoglobinemia.

5

Sulfhemoglobin

- Hb containing sulfur and is unable to transport O_2 . It is usually formed by certain oxidizing drugs.

Methods of Hb Estimation



Principle of Acid Hematin (Sahli) Method

- ✓ Blood is mixed with an acid solution so that all the Hb is converted into brown colored acid hematin and the intensity of the color is measured by comparing it with a standard solution. This is done visually and the concentration of Hb is measured in grams per deciliter (g / dl) .



Materials of Sahli Method

- ✓ Sahli hemoglobinometer comparator
- ✓ Pipette marked to contain 20 micro liters of blood .
- ✓ Graduated tube .
- ✓ Glass dropper
- ✓ Glass rod for stirring
- ✓ 0.1 normal HCL and Distilled water (D.W.)



Procedure

- ✓ Fill the graduated tube to mark (2 or 10) with 0.1 normal HCL .
- ✓ Draw blood by hemoglobin pipette to mark 20 μ l (micro-liters).
- ✓ Dip the tip of the pipette in the graduated tube to blow the blood into the tube , 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 the result in g / dl .



Advantages and Disadvantages of Sahli Method

Advantages

- Quick, Inexpensive, and Easy to perform.
- Can be used as a bedside procedure.
- Does not require technical expertise.

Disadvantages

- There can be a visual error.
- Color of acid hematin is not stable . It fades after reaching its peak.
- Comparator can fade over years.
- Carboxy , met and sulfhemoglobin cannot be converted to acid hematin.
- This method is not suitable for infants as fetal hemoglobin is also not converted to acid hematin.
- Source of light influences the comparison of colors.

Let's Go to the Second Method for Hb Estimation :Cyanmethemoglobin Method

- ❖ Most accurate method for estimation of Hb.
- ❖ It is Recommended by International Committee for Standardization in hematology because:
 - ✓ All forms of Hb are converted to cyanmethemoglobin (except sulfhemoglobin).
 - ✓ Stable and reliable standard is available.

Principle of Cyanmethemoglobin Method

- Blood is mixed with Drabkin's solution that contains :
 - ✓ Potassium ferricyanide
 - ✓ Potassium cyanide
 - ✓ Potassium dihydrogen phosphate
- When blood is added to Drabkin's reagent, Potassium ferricyanide converts Hb to methemoglobin (Hb iron from ferrous to ferric state).
- Methemoglobin combines with potassium cyanide to form stable pigment(cyanmethemoglobin).
- Absorbance is measured in spectrophotometer at 540 nm.
- To obtain amount of unknown Hb sample, we use Beer's Law to get the concentration

Concentration of Hb (g/dL)=

(Absorbance of sample * Concentration of standard)/ Absorbance of standard

Materials of Cyanmethemoglobin Method

- ✓ Diluent (Drabkin's solution)
- ✓ 20 micro liter pipette (Sahli pipette)
- ✓ 5 ml pipette
- ✓ Cuvettes
- ✓ Spectrophotometer



Procedure

- ✓ Take 5 ml of Drabkins solution and add to it 20 microlitres of blood.
- ✓ Mix them and leave the sample for 15 min to allow a complete lyses of RBCs.
- ✓ Measure the absorbance of the standard in the spectrophotometer at 540 nm.
- ✓ Transfer the sample to cuvette and read the absorbance in the spectrophotometer at 540 nm
- ✓ Calculate Hb concentration using Beer's Law as in the previous slide.

Advantages and Disadvantages of Cyanmethemoglobin Method


Advantages

- All forms of Hb except sulphhemoglobin are converted to cyanmethemoglobin .
- Visual error is not there as no color matching is required.
- Cyanmethemoglobin solution is stable and it's color does not fade with time so readings may not be taken immediately.
- A reliable and stable reference standard is available from World Health Organization for direct comparison.

Disadvantages

- Diluted blood has to stand for a period of time to ensure complete conversion of Hb.
- Potassium cyanide is a poisonous substance and that is why Drabkin solution must never be pipetted by mouth.
- The rate of conversion of blood containing carboxyhemoglobin is slowed considerably. Prolonging the reaction time to 30min can overcome the problem.

Clinical Implications: Anemia

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- **Anemia** : A condition of decreased level of hemoglobin below 14 g/dl (male) or below 12 g/dl (female) . Hb level falls as RBCs count decreases as in case of severe hemorrhage . Hb level also decreases in case of hemodilution , pregnancy is an example .
 - Causes of anemia can be **physiological** as in pregnancy .The total volume of plasma is increased causing hemodilution (diluted blood) In this case the level of Hb is reduced but total RBC count remains unchanged.
 - **Pathological** causes of anemia include : severe bleeding, massive hemolysis of any cause , dietary deficiency of nutrients or vitamins , e.g. iron deficiency anemia (IDA) , megaloblastic anemia , chronic diseases, and bone marrow diseases e.g. aplastic anemia

Clinical Implications: Polycythemia

- ***Polycythemia*** : it literally means increased RBC count , as RBC count raises , the total level of hemoglobin is also increased .
- ***Physiological polycythemia*** occurs in people living at high altitudes , this is because the air density (hence oxygen concentration) is reduced , thus the body will compensate for this by increasing RBC count to fulfill the deficient in oxygen supply caused by low atmospheric air density . This compensatory mechanism is termed reactive polycythemia . Smoking also causes reactive polycythemia via the same way .
- ***Pathological polycythemia*** occurs in dehydration .The dehydration causes hemoconcentration (concentrated blood) but RBC count is unchanged . Polycythemia also occurs in bone marrow disease : e.g. polycythemia rubra vera (PRV), this a specific bone marrow pathology causing abnormally high RBC count due to increased RBC synthesis.



**Each
Red Blood Cell
Has 280 Million
Hemoglobin
Molecules**



Health Fun Facts
www.healthconscious.club

Thank you