Practical Endocrinology
HB Estimation

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Endocrinology BIO

Spring semester

Week 4

18/ 02 /2024

Aim

To estimate the Hemoglobin levels in mg/dl using Sahli's Method.

- Easy to perform.
- Minimal cost.
- Less time consuming.

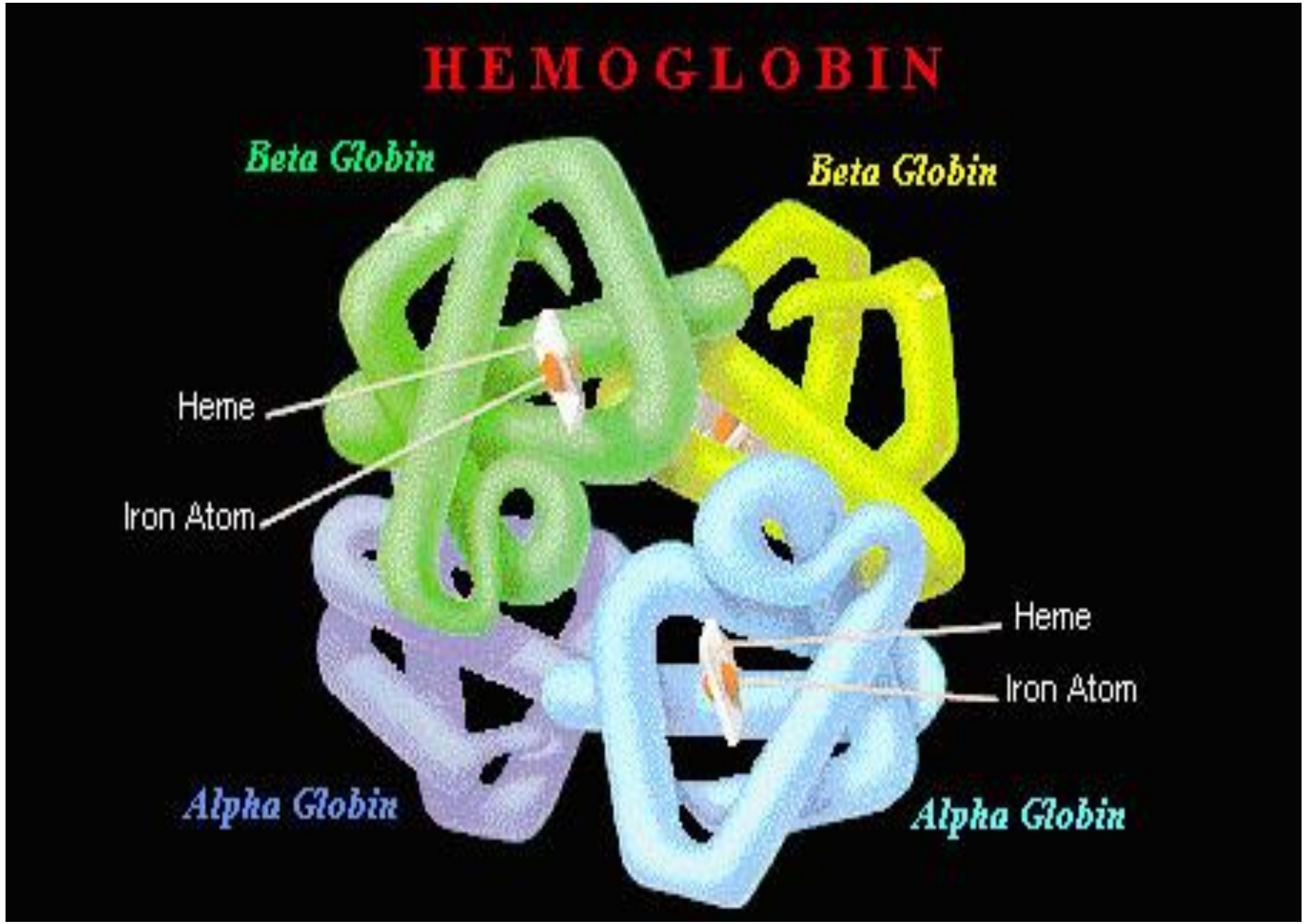
Outline:

- Introduction.
- HB Function.
- Condition of increasing and decreasing HB.
- Hemoglobinometry.
- Materials and method.
- Procedure.
- Normal Value.

Hb Estimation

A hemoglobin test measures the amount of hemoglobin in your blood. Hemoglobin is **a protein in your red blood cells** that carries oxygen to your body's organs and tissues and transports carbon dioxide from your organs and tissues back to your lungs.

- Hemoglobin contains iron, which allows it to pick up Oxygen.
- Heme (the iron) , Globin (oxygen transport protein) found in red blood cells.

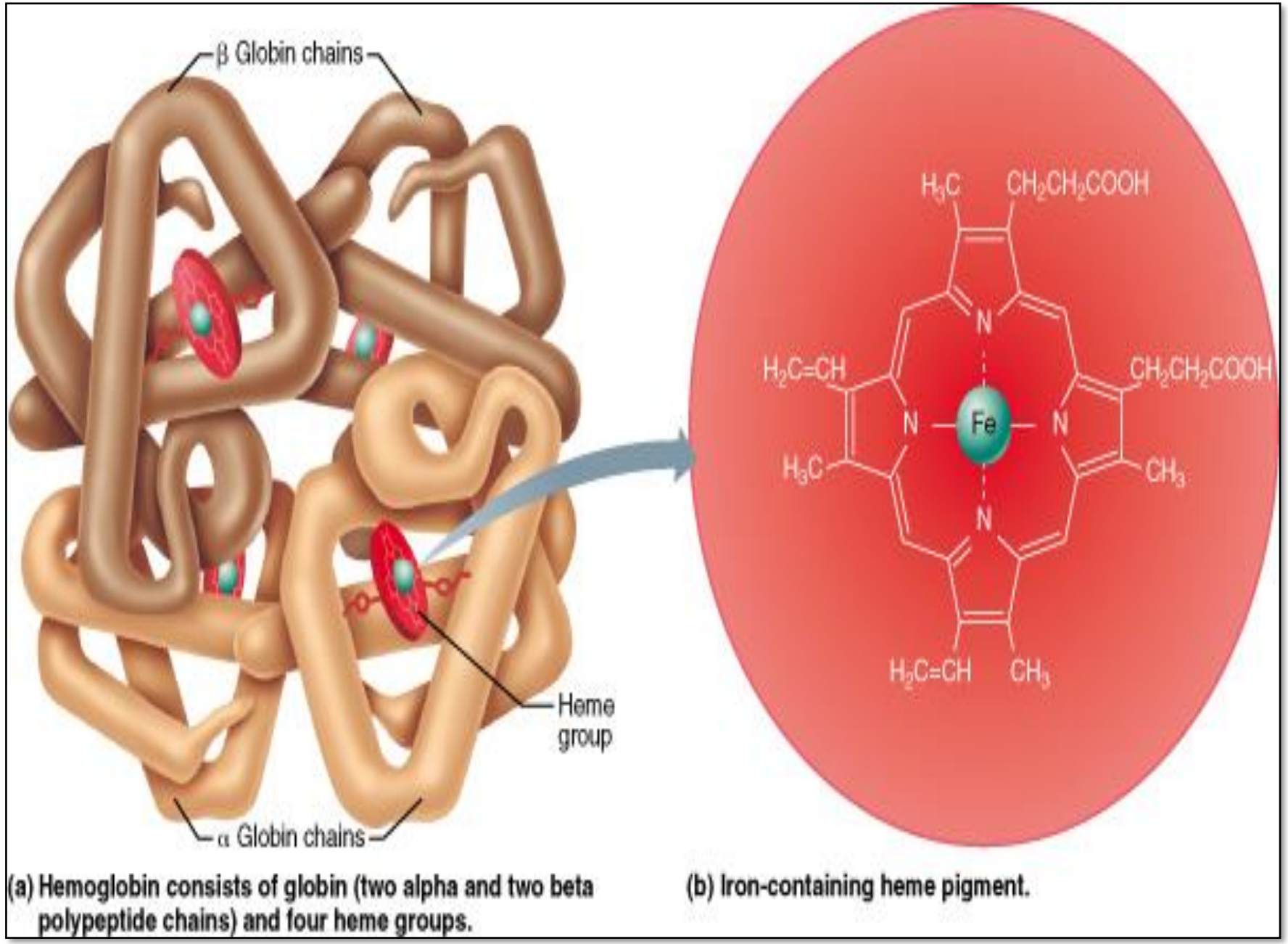

HEMOGLOBIN
Beta Globin
Beta Globin
Heme
Iron Atom
Heme
Iron Atom
Alpha Globin
Alpha Globin

HB consists of two parts:

1- Pigment heme (iron containing pigment), which constitutes (4%)

2- Protein globin (96%)

Hemoglobin consists of 4 heme groups, each is protein chain (2 pairs of polypeptides in each), 2 of those protein chains form hemoglobin molecule.



(a) Hemoglobin consists of globin (two alpha and two beta polypeptide chains) and four heme groups.

(b) Iron-containing heme pigment.

human hemoglobin (Hb_A)

- In normal adults, human hemoglobin (Hb_A) the 2 types of polypeptides are called α-chains, each of which contains (141 amino acids) and the β-chains, each of which contains (146 amino acids), so the HbA is designated ($\alpha_2\beta_2$).
- Not all the hemoglobin in the normal adult is Hb_A; about 2.5% of hemoglobin is Hb_{A2}, in which the beta chains are replaced by delta (δ) chains ($\alpha_2\delta_2$), where delta contains (146 amino acids) residues, but the last 10 contain individual residues differ from those in the β chain.

Hemoglobin of the fetus

the blood of the human fetus contains fetus Hb (Hb_f), its structure similar to that of HbA except that the beta chains are replaced by gamma (ɣ) chains, so Hb_f is ($\alpha_2\gamma_2$), and fetal Hb is replaced by adult Hb soon after birth.

HB Function:

- Hema could bind Oxygen reversibly and carry it to tissues.
- It impart red color to the blood.
- It deliver Oxygen to the tissue and carbon dioxide from tissue to the lungs.

Decrease in Hb content

Physiological

- Pregnancy
- Children
- Females

Pathological

- Anemia.

Increase in Hb content

Physiological

- High altitude
- Newborns and infants
- Males

Pathological

- Polycythemia.
- Severe diarrhea, vomiting .
- Chronic hypoxia.

Methods of HB estimation

1. Visual Method.
- Sahil's method
- Tallquist chart (Obsolete)
- WHO Hb color scale

2. photoelectric Method.

- Cyanmethemoglobin method
- Oxyhemoglobin method
- Electronic method
- Direct reading Electronic haemoglobinometer

Sahli's, acid hematin method

- Blood is **mixed** with an **acid** solution so that Hb is converted to brown colored acid hematin

- Diluted with water till **brown color** matches that of brown glass standard.

- Hb value is read directly from the scale

Normal Values of HB

- Males - 14 -18 g/dl
- Females - 12-16 g/dl
- Newborns - 17- 22 g/dl

Materials and Method

- Materials for finger prick.
- Comparator box
- N/0.1 HCL
- Hemoglobin tub or Graduated tube
- Hemoglobin pipette, or Pipette marked to contain 20 microliter of blood.
- Stirrer
- Distil water

Blood can be collected from 3 different sources:

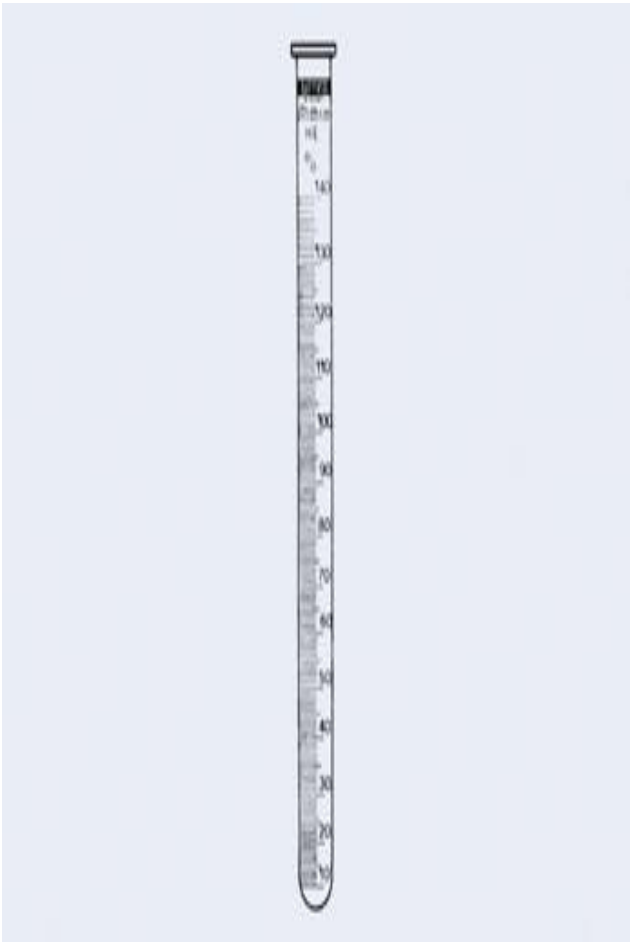
- Capillary blood
- Venous blood
- Arterial blood

Sahli´s Kit (Haemometer)

Dropper

Sahli's paipette

Hemometer comparative tubes with double scales (g%) & (percent of normal)



Procedure

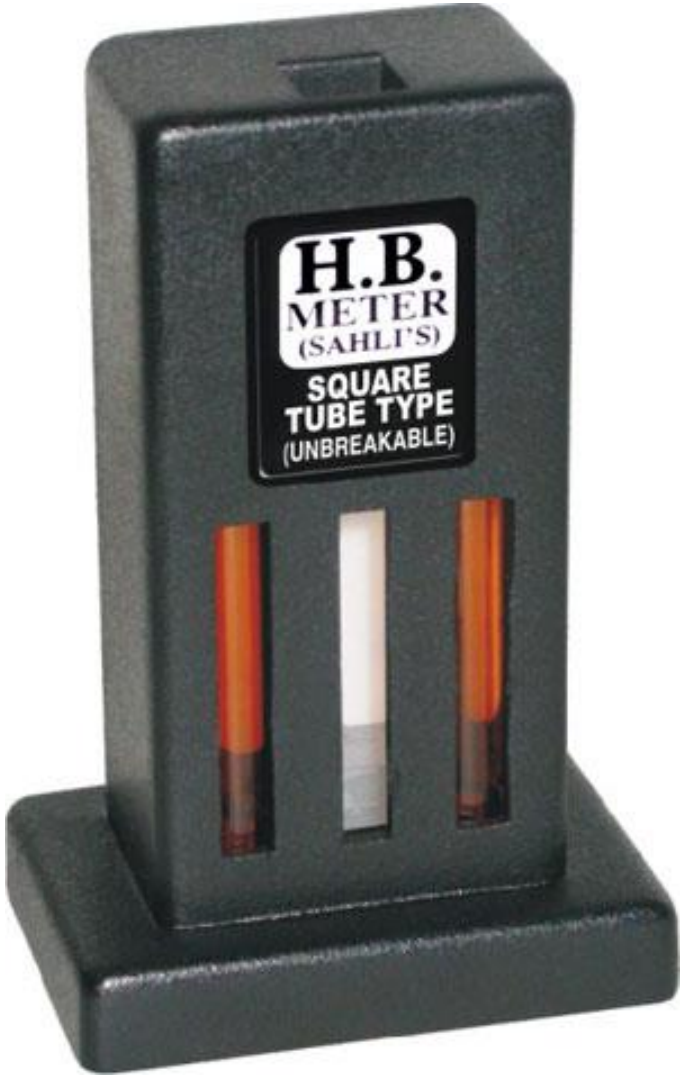
1. Place 0.1 N HCl into Hb tube up to 2 grams.
2. Blood sample in Sahli's Hb pipette up to 20 micro liter.

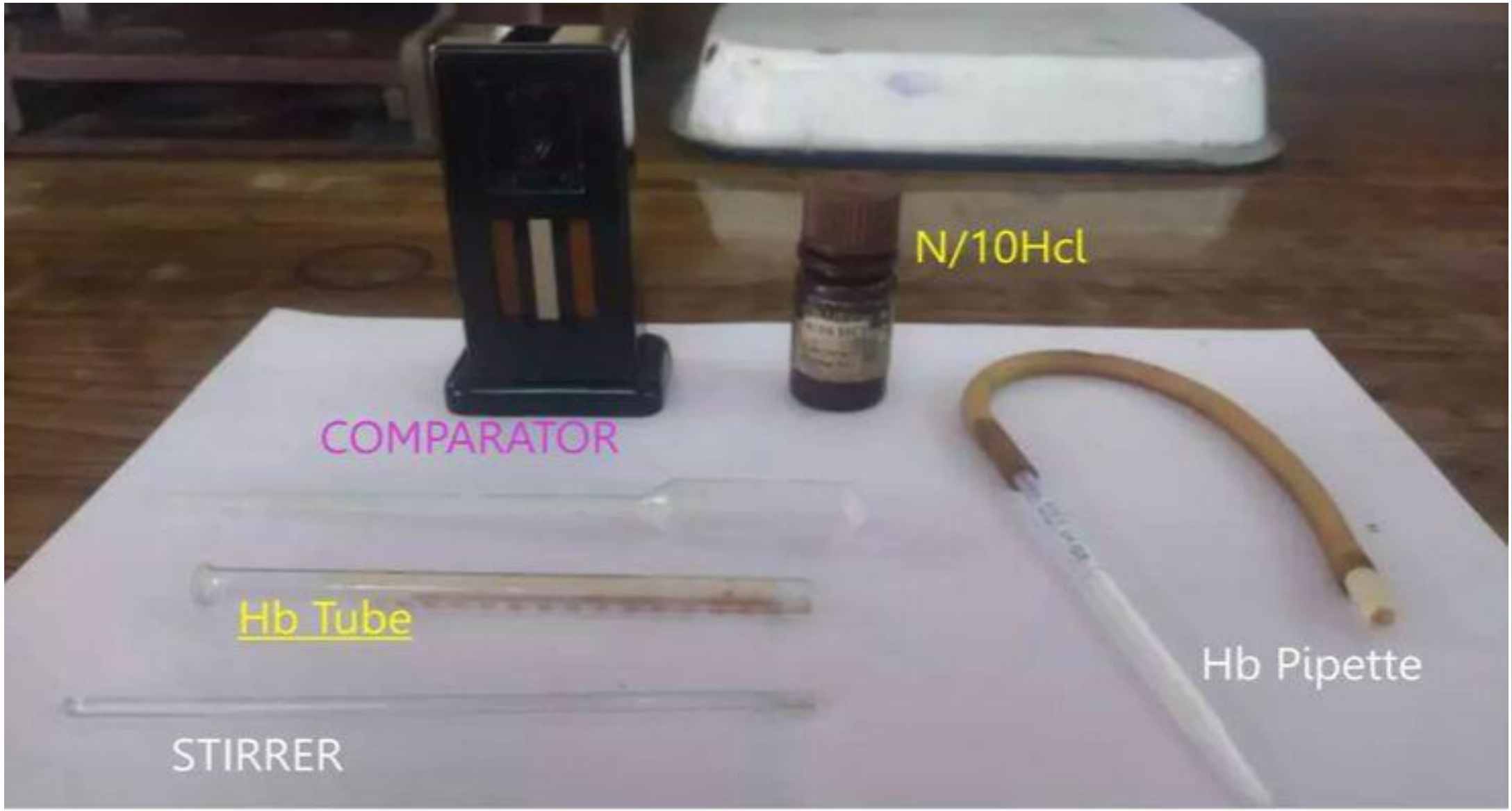
3. Add **blood sample** to **acid solution**.
4. **Mix** with a stirrer.

5. Allow to stand for 10 minutes.
6. Add **distilled water** drop by drop till the color of the solution matches to **brown glass standard.**

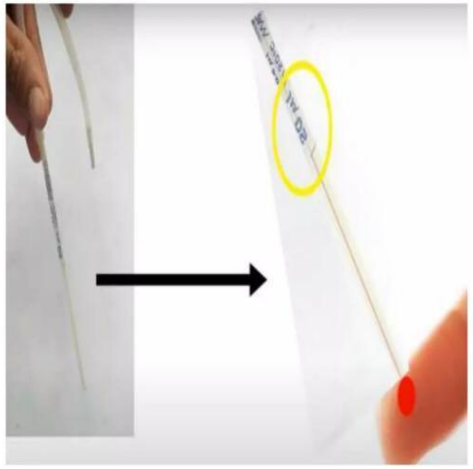
7. Take the reading of the lower **meniscus** from the graduated tube in grams.

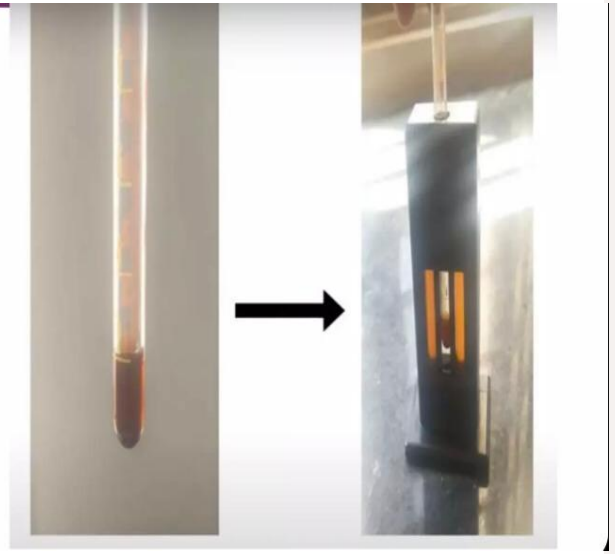
Color comparator




N/10Hcl
COMPARATOR
Hb Tube
Hb Pipette
STIRRER


DROPPER
N/10 HCL





Thank you
Any Question?!!