

Heamatology module

Lab. 1

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1- Hematocrite value (H.V.) or packed cell volume:

Definition:-

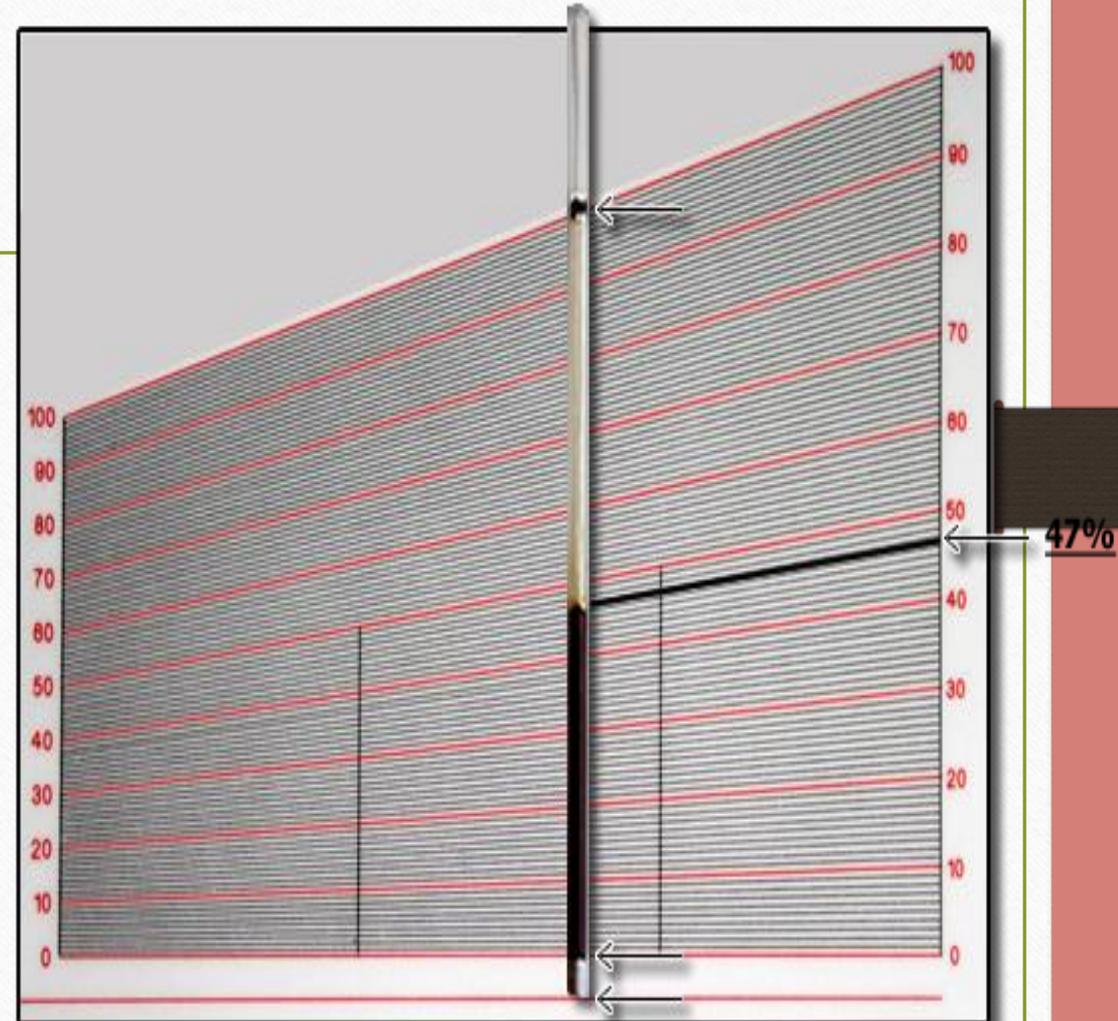
H.V. is the volume of RBCs in 100 ml blood or it is the percentage ratio of the volume of RBCs to the volume of whole blood.

$$= \frac{\text{Volume of RBCs}}{\text{Volume of blood}} \times 100$$

Materials:-

- Microhematocrite tube (75 mm long, 1 mm pore, heparinized)
- Microhematocrite centrifuge.
- Micro hematocrite tube reader.
- Sterile lancet, 70 % ethyl alcohol.

Micro-hematocrit Capillary Tube Reader



- **Procedure:-** - Obtain blood drop by pricking the thumb.
 - Micro hematocrite tube is filled up to its 2/3 with blood sample by touch the drop of blood by one end of the tube.
 - Close the empty end of the tube by plasticine .
 - Centrifuge the tube at 12000 per minute for 5 minutes .
 - Remove the tube and read H.V. by putting the tube on special micro hematocrite scale.
- **Normal value:-**
 - About 47 % in adult male , 42 % in adult female, In children = 36-44 % and in new born = 50-58%
- **Increases by:**
 - increased RBCs (polycythaemia)
 - decreased plasma (dehydration or hemo-concentration as after burn)
- **Decreases by:**
 - decreased RBCs (anaemia) -increased plasma (overhydration)
- **Used in :** Calculation of blood volume, Renal blood flow and blood indices.

-Physiologically Ht. value is More:

1- In **venous blood** than in arterial blood as RBCs volume is large in venous blood due to chloride shift phenomenon.

2- In **large vessels** due to skimming phenomenon as RBCs prefer to pass in large vessels than in small blood vessels.

3- And in **newborn** as he contains more RBCs due to relative ischemia during intra-uterine life.

2- Hemoglobin content of the blood (HB %)

Definition:- Hemoglobin is the principal constituent of RBCs. It is a red pigment, which gives the blood its red colour.

Materials:-

- Sahli Adams Haemometer (**Sahli' Haemometer** made up of plastic frame with two standard brown coloured glass and graduated tube and pipette)
- 0.1 HCl.
- Dropper.
- Ethyl alcohol, cotton, sterile lancets.



Procedure:-

- Fill the graduated tube of Sahli haemoeter to mark 5 with 0.1 N HCl. -
- 20UL blood is drawn from a thumb puncture, into a pipette then transfer it to the graduated tube and mix well until formation of a brown yellow solution (**acid hematin**). -
- Place the graduated tube beside the standard and allow to stand for 15 minutes. -
- Add distilled water drop by drop and mixing until the colour in graduated tube matches the standard. -
- Read the concentration of HB in gram / 100 ml

Normal value:-

- 14 – 17 gm /100 ml in **adult male**.
- 12 -15 gm / 100 al in **adult female** .
- more in **new born**.

Function of Hb:

- Carriage of O₂ & CO₂
- Strong buffer system.

- **Reactions of Hb:**

1-**Oxyhemoglobin:** O₂ bind with iron in ferrous state so it is called oxygenation not oxidation. This binding affected by pH, temperature and 2,3-diphosphoglycerate in RBCs.

2-**Met Hb:** strong oxidation by certain drugs or oxidizing agents → ferric state which not carry O₂ → dusky colouration of skin like cyanosis (normally, MetHb doesn't exceed 0.5% due to the activity of NADH-MetHb-reductase enzyme in the RBCs which converts it back to normal Hb).

3-**Carboxy Hb:** carbon monoxide is a toxic gas and attached to Fe⁺⁺ in high affinity (210 times as O₂). This part attached to CO doesn't carry O₂ and the remaining part of Hb, which carries O₂ doesn't give its O₂ to the tissue.

4-**Carbamino Hb:** normally Co[∇]2 attached to the globin part of Hb.

- **Types of Hb:**

1-**Adult (HbA):** contain 2 **α** chain (each is consisted of 141 amino acids) and 2**β** chain (146 amino acids).(97.5% of adult Hb.)

2-**HbA2:** contain 2**α** chains and 2 **delta** (146 amino acids) chains which differ from **β**-chains in the terminal 10 A.A.

3-**Fetal Hb (HbF):** It is the type of Hb in the human **fetus** then it is usually replaced by adult Hb after birth, It contain 2**α** and 2 **gamma** (146 amino acids) chains which differ from **β**-chains in 37 A.A.

-It has high affinity for O₂ and less to 2,3 diphosphoglycerate ,So this facilitate movement of O₂ from maternal circulation to the fetus.

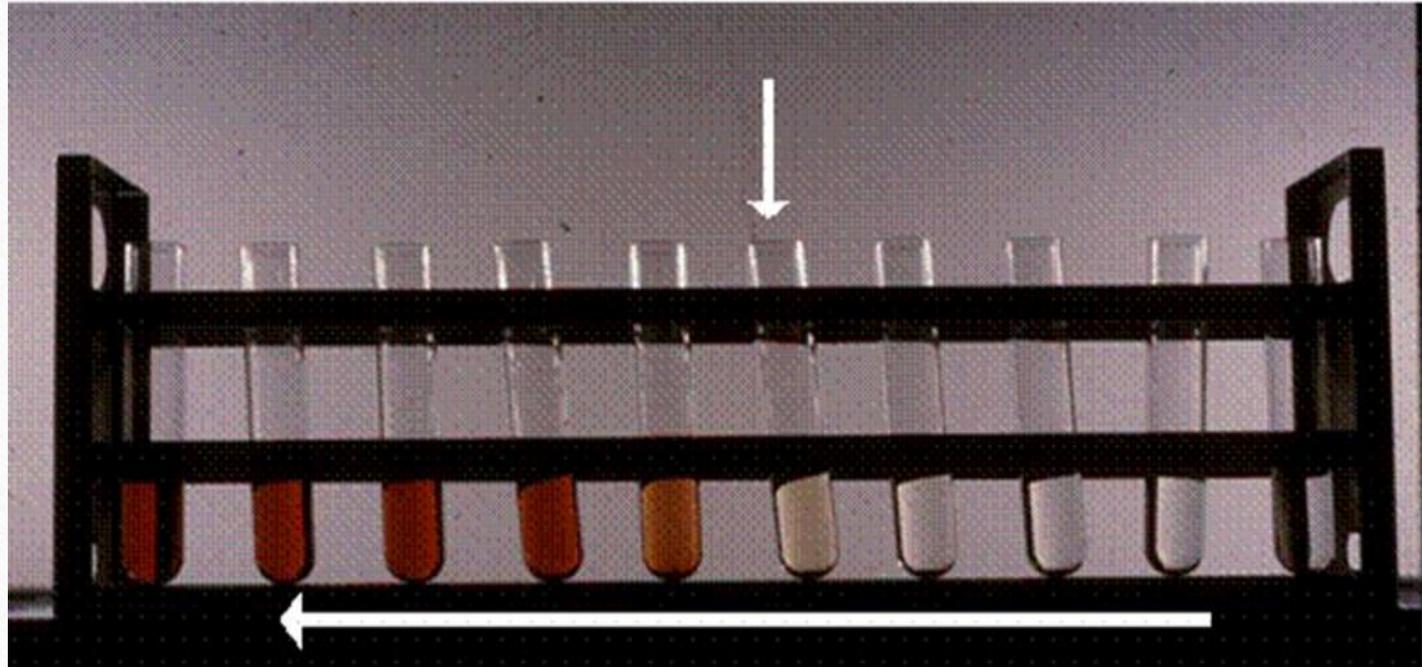
4-**Glycosylated Hb:** (3-7% of Hb) glucose is attached to terminal valine amino acid in **β**-chain. This value increases in cases of uncontrolled diabetes mellitus.

5-**HbS :** It is abnormal type of Hb due to congenital abnormality of **β**-globin in which valine amino acid present instead of normal glutamic acid at position 6 of **β**-chain → hemoglobin-S which causes sickle cell anaemia .

3- Osmotic Fragility test

- **Definition :** it's the ability of RBCs to withstand osmosis of hypotonic solution if placed in it.

- **Materials :**
 - . Rack of **11** test tubes
 - . Nacl 1% solution
 - . Dropper
 - . Distilled H₂O



Procedure

- Prepare tests tubes in the rack as follow

Test Tube	1	2	3	4	5	6	7	8	9	10	11
Drops of NaCl 1%	5	6	7	8	9	10	11	12	13	14	15
Drop of H ₂ O	15	14	13	12	11	10	9	8	7	6	5
Conc. of solution	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75

- Add one drop of blood to each tube
- Shake the tube gently to mix blood with the fluid
- After 15 minutes examine for the degree of hemolysis .

N.B : - **Red clear** transparent solution → **complete hemolysis**

- **Red opaque** fluid → **partial hemolysis**

-**Supernatant clear** fluid and RBCs **sedimented** in the bottom of the tube → **No hemolysis.**

-**Normal** : Partial hemolysis starts at a concentration of NaCl **0.45 %** and complete hemolysis occurs at a concentration of NaCl **0.3%**

- **More fragility occurs in:**

(1) Infant RBCs (2) venous RBCs (have big size) (3) ↑ Co₂ and acidity (4) old RBCs (5) Spherocytosis.

- **Less fragility occurs in :** sickle cell anaemia and iron deficiency anaemia.

Thank You
