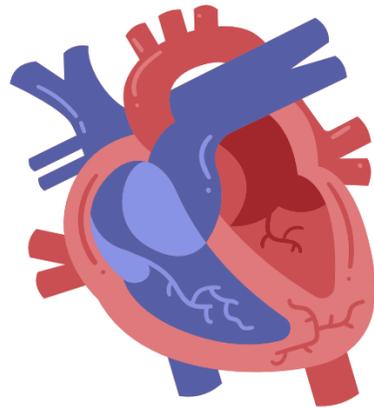
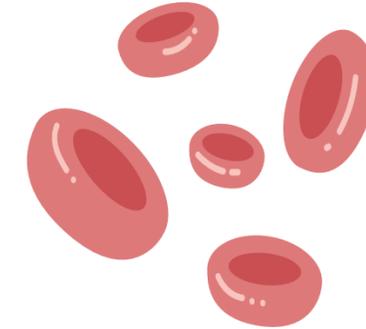




جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة المستقبل
كلية التقنية الطبية والصحية
قسم تقنيات المختبرات الطبية



Practical Hematology / 3rd stage ology



Rusul Mushtaq Talib Al-Muhammadawi

[Classroom](#)

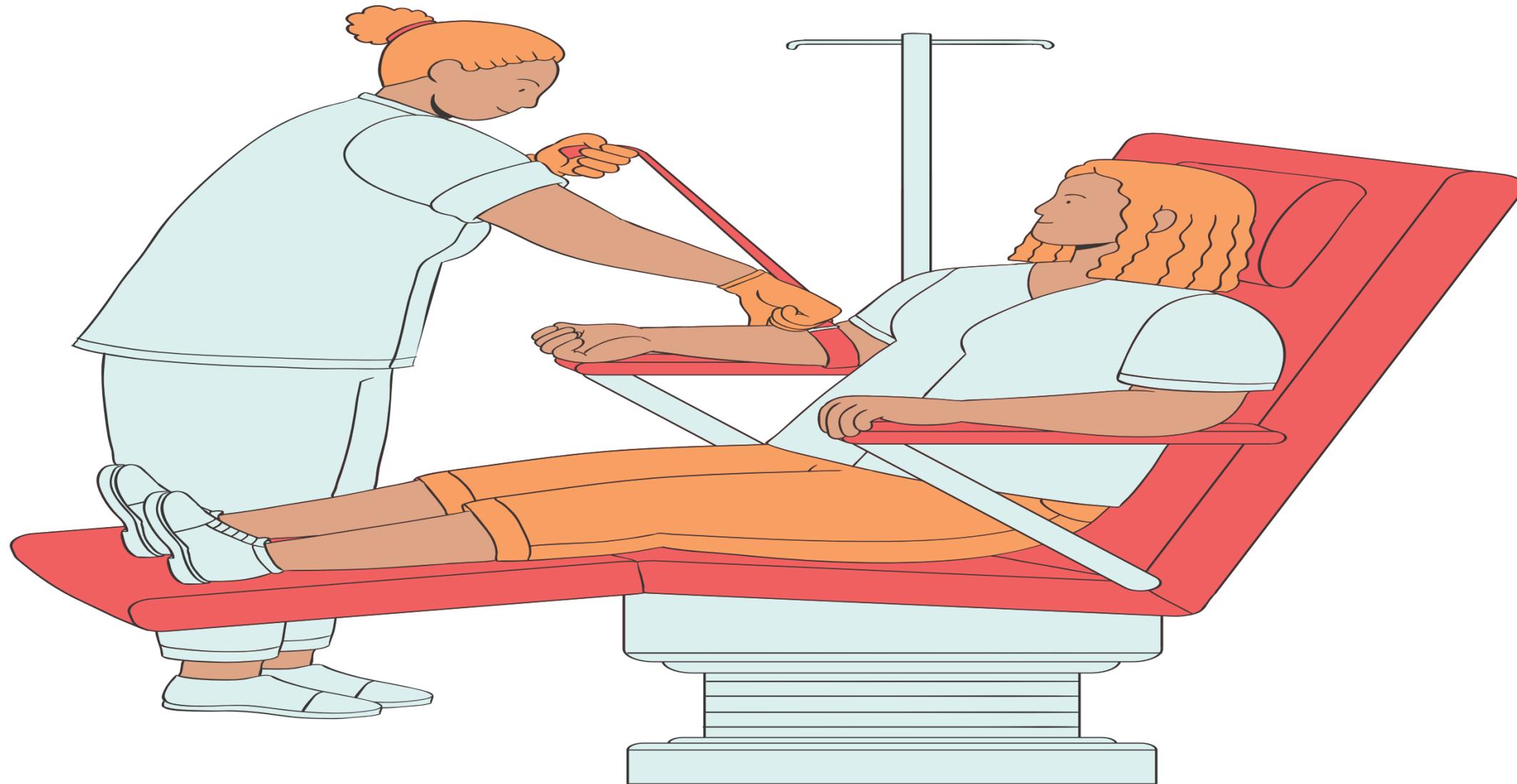
<https://classroom.google.com/c/ODA3NDIxNzE5>

[Mzkz](#)

Class code
cfldolyf

Hemoglobin and Hematocrit

Laboratory Tools for Assessing Blood Status



General Objective



🎯 To enable students to acquire the practical skills and theoretical knowledge necessary for measuring hemoglobin concentration and determining hematocrit (PCV) in blood samples using standard laboratory methods, and to interpret the normal values and diagnostic significance of the results.

behavioral Objectives



By the end of the lecture, the student should be able to:

Cognitive:

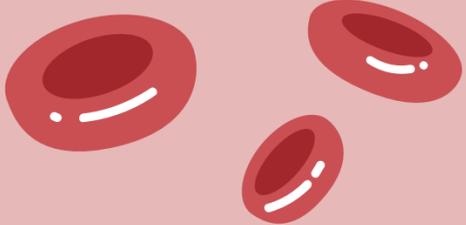
- **Define the principles of Hb (Cyanmethaemoglobin) and PCV tests.**
- **Explain their clinical significance and normal ranges.**
- **Identify causes of abnormal Hb and PCV values.**

Psychomotor:

- **Prepare reagents and handle samples safely.**
- **Perform Hb and PCV tests accurately.**
- **Calculate and interpret test results correctly.**

Hb estimation by different methods





Introduction:

Measurement of Hb concentration in whole blood is a basic screen for **anaemia or for polycythemia**. There are many methods for the Hb estimation, but the **best** recommended method is the **Cyanmethaemoglobin** method.



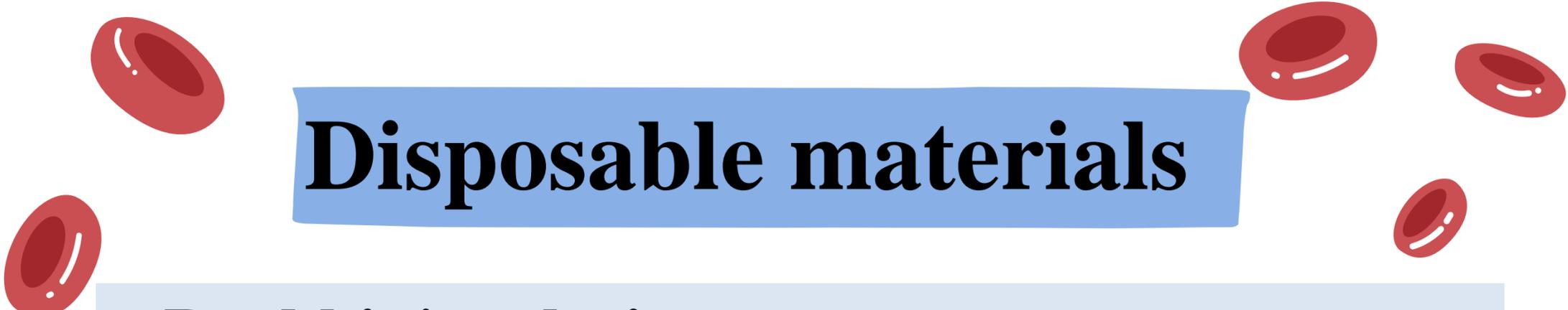
The advantage

standardized and possessing stable solutions. **Venous** or capillary blood collected in **EDTA**. Alternatively, free flowing capillary **blood may be added directly to the diluting fluid and measured.**



Equipments

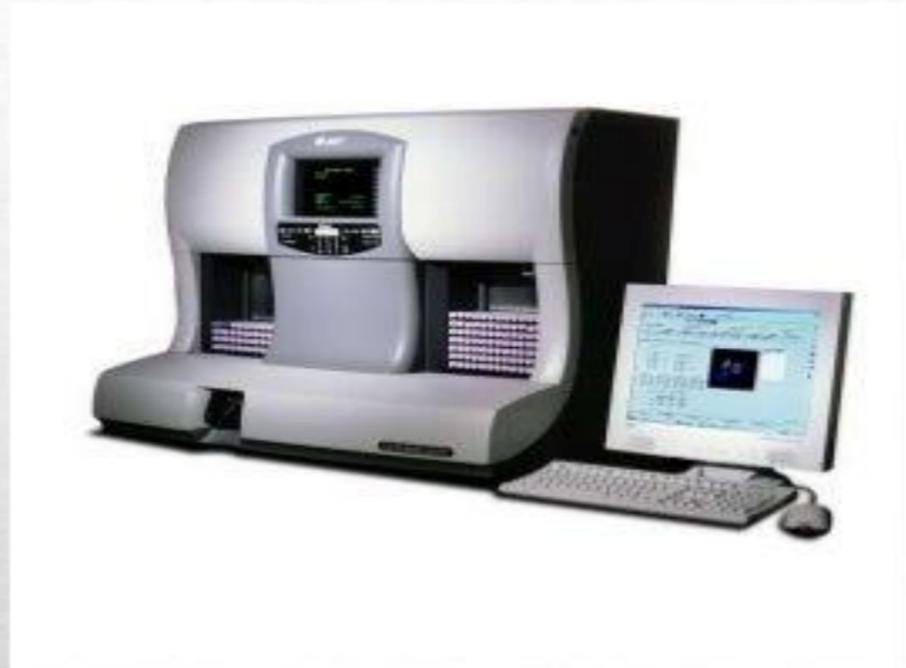
Spectrophotometer.
–Automatic pipettes.
- Racks.

The slide features several red blood cells scattered around the top and sides. The title 'Disposable materials' is centered in a blue box.

Disposable materials

- **Drabkin's solution.**
 - **Plastic or glass tubes.**
 - **Blue tips.**
 - **Yellow tips.**
-
- **Drabkin's solution** is a chemical reagent containing **potassium cyanide** and **potassium ferricyanide**, used to convert hemoglobin into **cyanmethemoglobin** for accurate measurement by spectrophotometer at 540 nm.

Lecture 3: Hb estimation by different methods



The image features several red blood cells, depicted as red biconcave discs, scattered around the central text. There are three cells on the left and four on the right, all rendered in a simple, illustrative style with a slight shadow to give them a three-dimensional appearance.

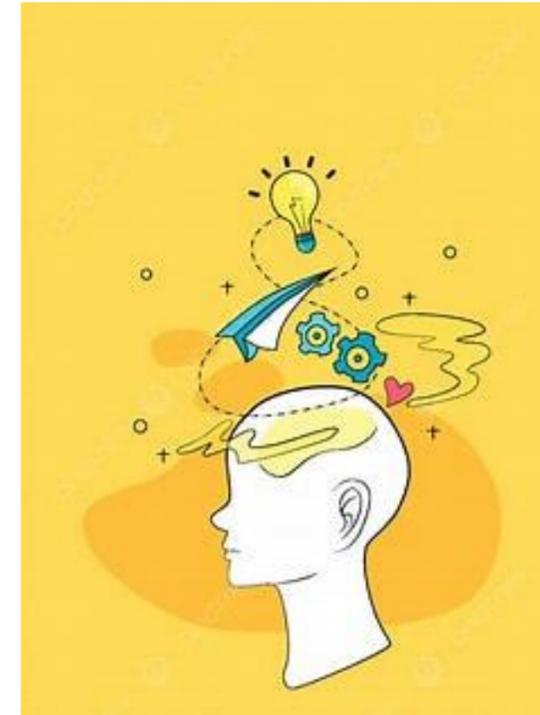
Introduction:

Measurement of Hb concentration in whole blood is a basic screen for anaemia or for polycythemia. There are many methods for the Hb estimation, but the best recommended method is the Cyanmethaemoglobin method.

Question (Individual Activity):

2 minutes.

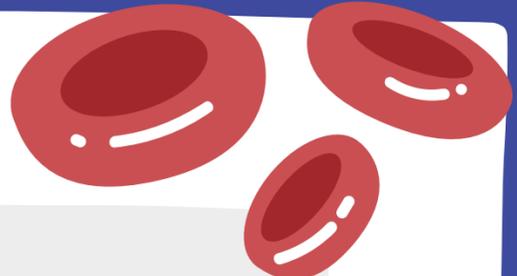
What is the basic principle on which the **Cyanmethaemoglobin method** for hemoglobin estimation is based?





The advantage

standardized and possessing stable solutions. Venous or capillary blood collected in **EDTA**. Alternatively, free flowing capillary blood may be added directly to the diluting fluid and measured.



Equipments

Spectrophotometer.
– Automatic pipettes.
- Racks.

Disposable materials

- Drabkin's solution.
- Plastic or glass tubes.
- Blue tips.
- Yellow tips.



Principle

Blood is diluted in a solution containing potassium cyanide potassium ferri-cyanide (Drabkin's solution), Hb is oxidized to methaemoglobin by potassium ferri-cyanide, methaemoglobin in turn combines with potassium cyanide to form cyanmethaemoglobin . The absorbance of the solution is measured in a spectrophotometer at wave length 540 nm against Drabkin's solution as a blank. The result is (calculated from formula provided below) expressed in gm/liter or mg/dl.



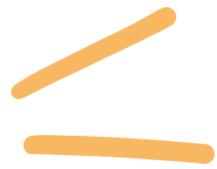
Individual Activity

- Why is the **wavelength 540 nm** chosen for measuring absorbance?
- What could happen if the **blank (Drabkin's solution)** is not used during spectrophotometer reading?





Method:



- 1. Pipette 4 ml of Drabkin's solution in a tube.**
- 2. Pipette exactly 0.02 ml (20 μ l) of well mixed blood using a pipette.**
- 3. Clean outside of the pipette and wash out the blood in the tube containing diluent (dilution=1/200).**
- 4. Mix and leave for 5-10 minutes for reaction to be completed.**
- 5. Read absorbance in the spectrophotometer at wavelength 540nm.**



Notes on this technique:



- 1. The blood sample must be properly mixed before taking the sample, and if refrigerated, allow to warm.**
- 2. Care must be taken when handling potassium cyanide.**
- 3. Using clean tubes and pipettes.**



Discussion Game



Why is the Cyanmethaemoglobin method considered the reference method for hemoglobin estimation?



Comments

The cyanmethaemoglobin is a reference method for Hb estimation: because:

1. All types and compounds of Hb except sulphaemoglobin are estimated.
2. Highly reliable, and stable standard are available.

estimation

Normal Range:

- Adult males: 14-18 g/dl
- Adult females: 12- 16 g/dl
- Children: 11-14 g/dl.
- Newborn infants: 13.0-20 g/dl

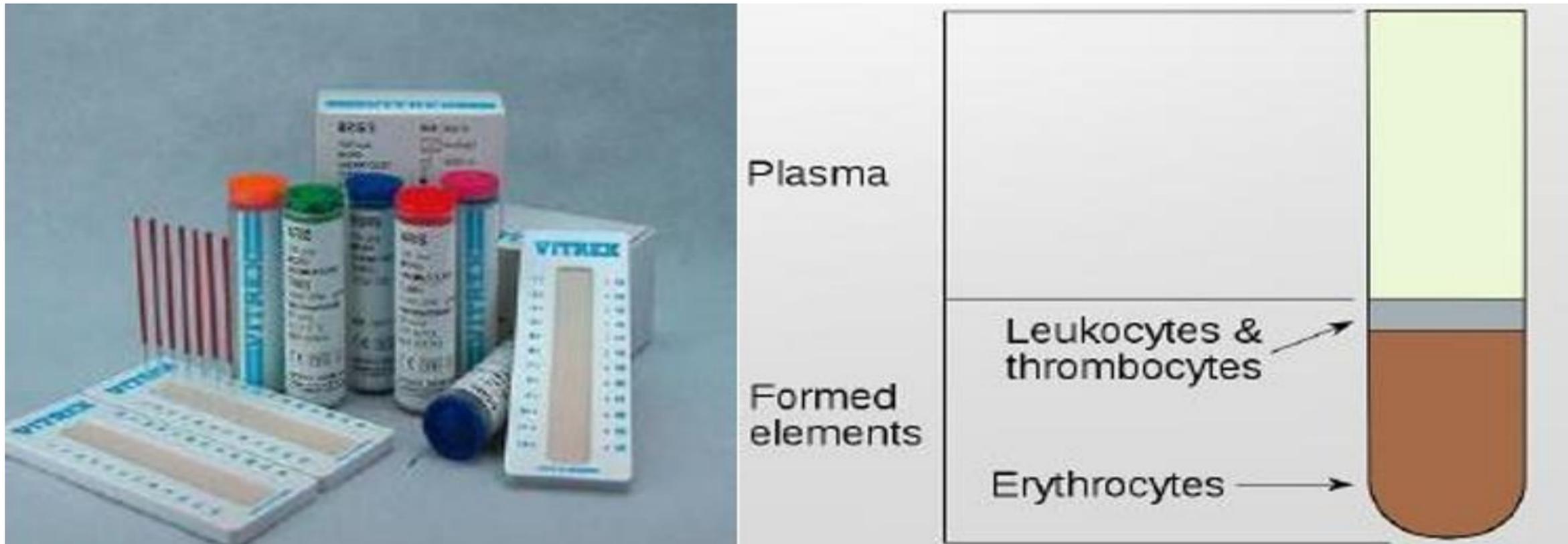
Calculation



Absorption of sample Hb(g/dl)= ----- X
concentration of standard Absorption of standard
Haematocrit (Packed Cell Volume – PCV) Determination

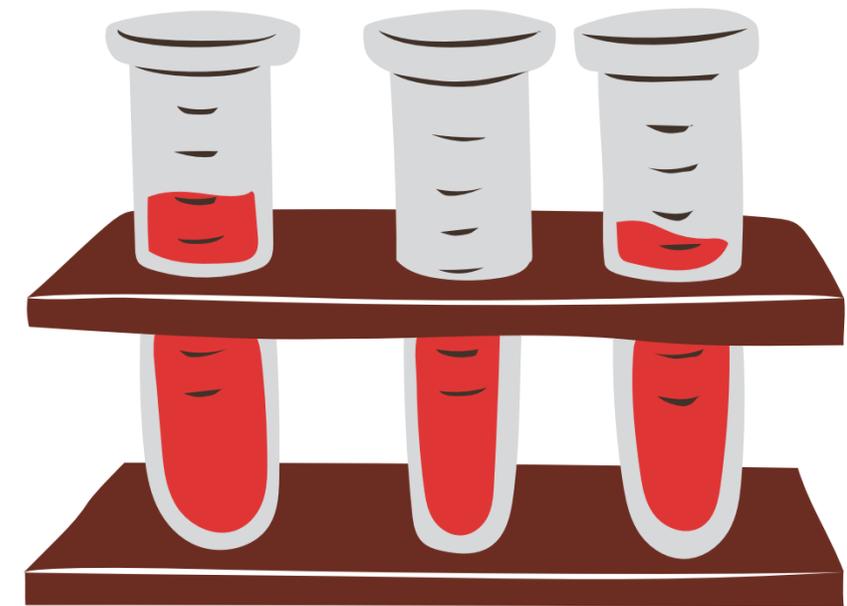
Lecture 4: Haematocrit (Packed Cell Volume – PCV)

Determination



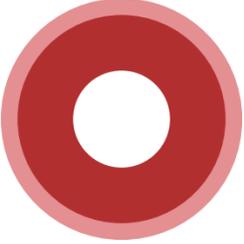
Question (Individual Activity):

- How can accurate **RBC count** and **hemoglobin measurements** help calculate absolute RBC values?





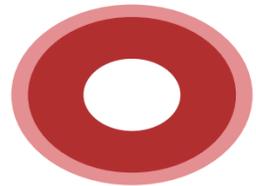
The haematocrit (PCV)



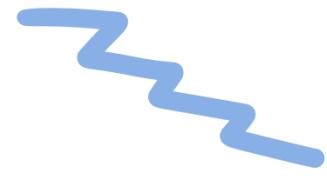
The haematocrit (PCV) is the percent of the packed red cells in a volume of whole blood. The hematocrit may also be referred to as Packed Cell Volume (PCV) or erythrocyte volume fraction (EVF). It reflects the combination of total number of RBCS , and the volume that they occupy in plasma . When accurate measurements of Red cell count and Hb concentration are available, the absolute values can be calculated



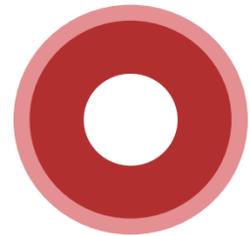
The haematocrit (PCV)



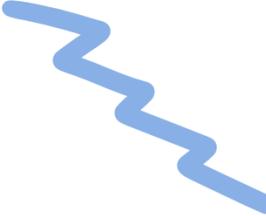
Note: Remember the Hematocrit is a reflection of the RBC concentration, not the RBC mass. It is a screening test for anemia or polycythemia. In comparison, hemoglobin estimation is less accurate, and RBC count far less accurate.



Principle:



A volume of anticoagulated blood is placed in a glass tube which is centrifuged so the blood will be separated into three layers: Red cells, Buffy coat (WBC and platelets) and plasma. Ideally there should be complete separation of the three layers. Haematocrit is the ratio of the height of red cells column to that of the whole blood in the tube.



The two methods of direct measurement of the PCV which are in current use are



- 1. Macro-method using Wintrobe tubes.**
- 2. 2. Micro-method using capillary tubes.**
- 3. 3. Electronic cell counting**

The more popular one is the micro-method, as it has the advantage of short time of centrifugation and better packing of the red cells.

Micro-Haematocrit Method:

Test sample:

Heparin or EDTA venous or capillary blood.

Equipments:

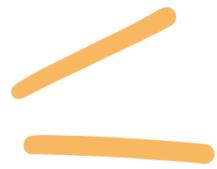
- **Micro-haematocrite centrifuge.**
- **Plastic sealer or Bunsen burner.**

Disposable materials:

- **Capillary tubes 75 mm long and internal diameter of 1 mm.**



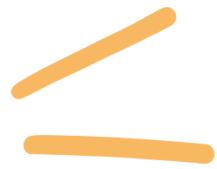
Method:



- 1. The blood sample should be used as fresh as possible, and well mixed.**
- 2. Using the capillary action, allow blood to enter the tube stopping at 10 15 mm from one end. Wipe the outside of the tube.**
- 3. Seal the dry end by pushing into the plasticine two or three times.**
- 4. If heat sealing is used rotate the dry end of the tube in a fine Bunsen Burner flame.**



Method:



- 5. Place the tube into one of the centrifuge plate slots, with the sealed end against the rubber gasket of the centrifuge plate.**
- 6. Keep a record of the patient number against centrifuge plate number.**
- 7. Centrifuge for five minutes.**
- 8. Read the PCV in the micro haematocrit reader.**
- 9. Unit: the haematocrit result is expressed in percentage**



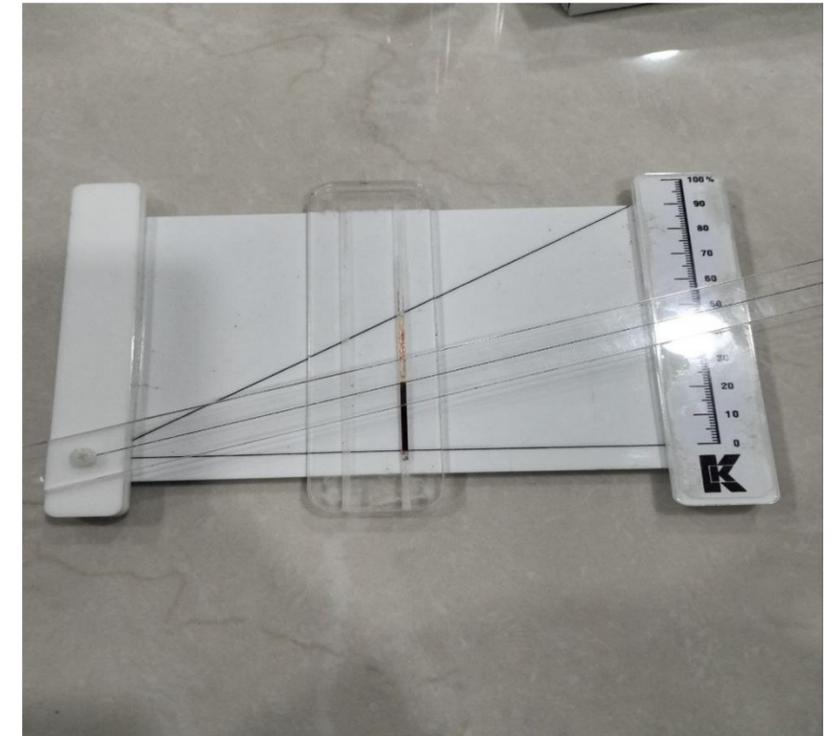
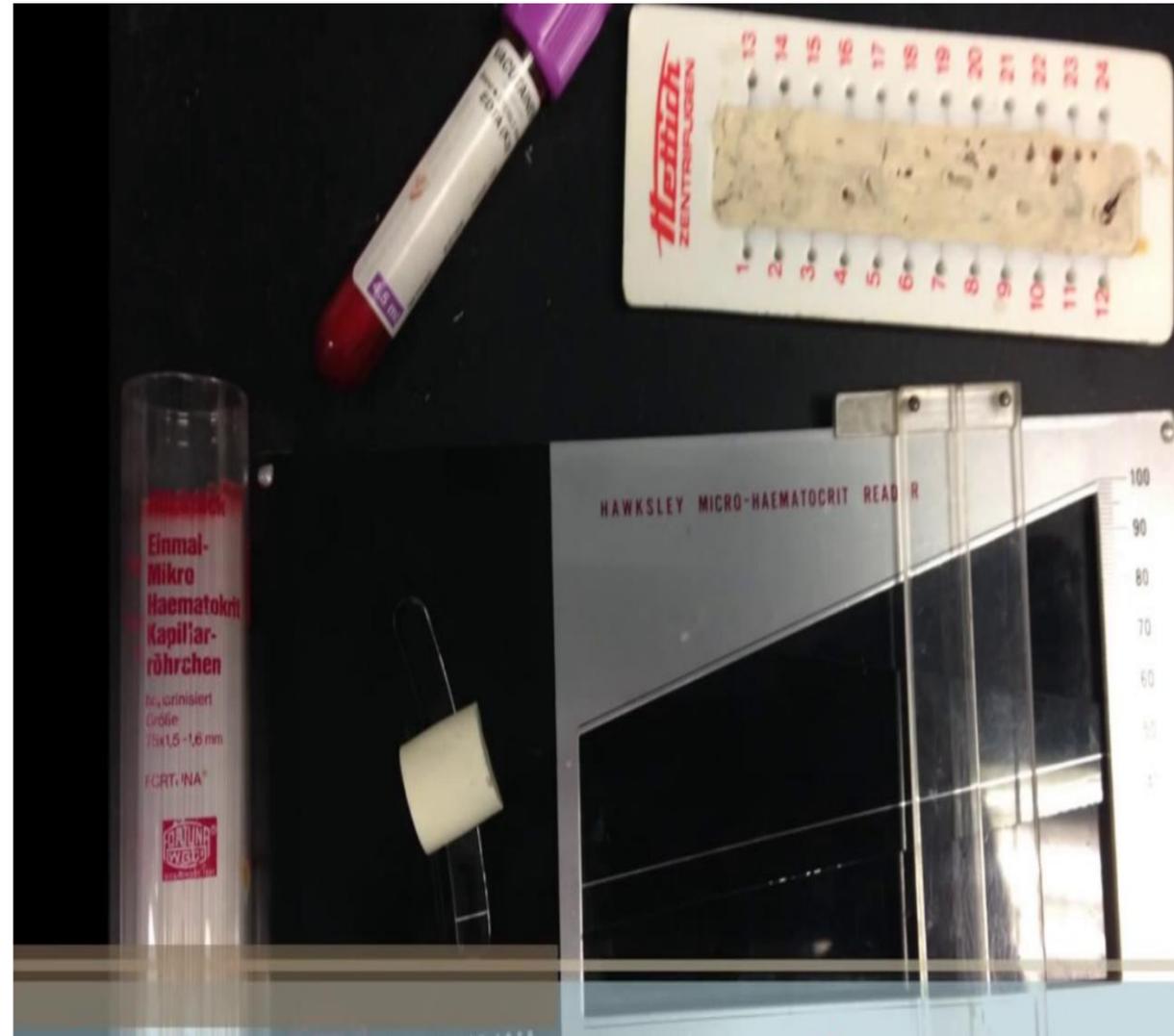
Question (Individual Activity):



- **Why should the tube be placed with the sealed end against the rubber gasket in the centrifuge?**
- **Why is centrifugation performed for only 5 minutes, and how could a longer or shorter duration affect the results?**



Tools



Note:

It is preferable to perform the test in duplicate.

Normal ranges:

The normal values of PCV vary according to the age and sex of the individuals. The normal ranges are

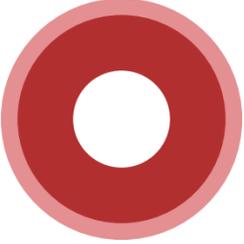
- Adult males = 40% - 52%.**
- Adult females = 37% - 47%.**
- Pregnant = 30%–46%**
- Neonates = 40%–68%**
- 3 months = 29%–54%**
- 1–2 years = 35%–44%**



Results extraction:



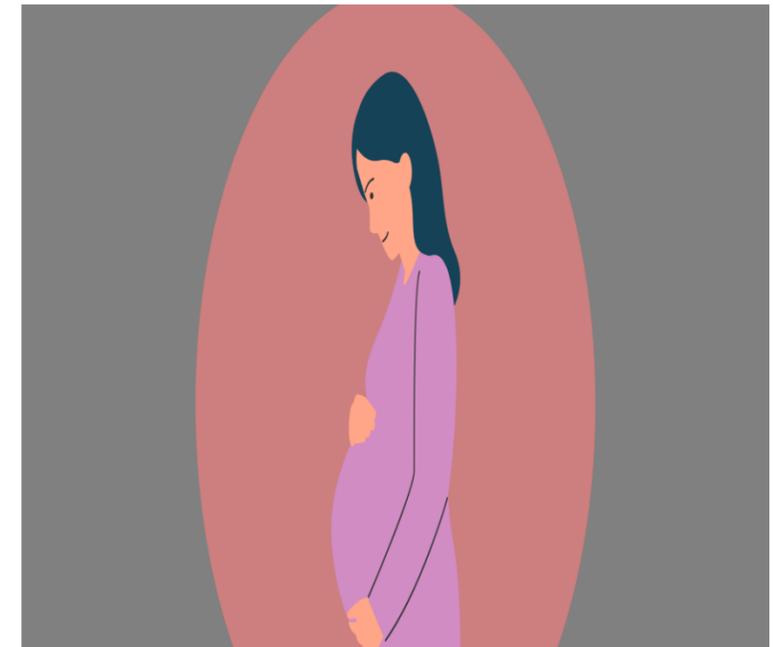
$$\text{Hct\%} = \{ \text{Height of RBCs (mm)} / \text{Height of RBCs and plasma (mm)} \} \times 100$$



For example, if the height of packed red cells is 45 mm, then $= 45 / 100 \times 100 = 45$ percent. It also means that out of 100 volumes (or parts) of blood 45 volumes (or parts) are red cells and 55 volumes (or parts) are plasma. Thus, out of 1 liter of blood, 450 ml are red cells and 550 ml are plasma

Question (Individual Activity):

- **Why is the hematocrit range wider in neonates compared to adults?**
- **What are the reasons that hematocrit levels are lower in pregnant women compared to non-pregnant women?**



Importance of the PCV :

**It is so important for the following reasons □ Simple , Accurate Reliable □
Screening for large clinic population**

PCV-Low

- 1. in pregnancy Cause is a hemodilution the RBCs are "diluted**
- 2. Low RBC production from the bone marrow (Toxins, cancer, lowEPO)**
- 3. IDA, aplastic anemia. Hemolytic anemia etc**

PCV-High :

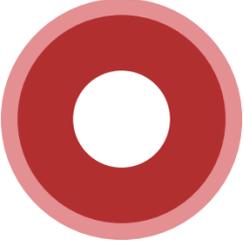
A high hematocrit value may truly reflect an increase in the fraction of RBCs

- 1. polycythemia vera**
- 2. secondary polycythemia (smoking, kidney cancer, high attitude living**
- 3-reactive polycythemia (vomiting and diarrhea, Burn)**



Diagnostic uses of the buffy coat



- 1. The buffy coat consist less than 1% of the total volume of the blood**
 - 2. The buffy coat is used to**
 - Extract DNA from the blood of mammals (since mammalian red blood cells are anucleate and do not contain DNA).**
 - Quantitative buffy coat (QBC) is a laboratory test to detect infection with malaria or other blood parasites.**
- 

How is the PCV in the following individuals ?

1. Fluid preservation - □ the Hct may be decreased explain that? □
the RBC mass normal
2. Patient with aplastic anemia ? Decrease □ low RBC mass
3. Patient with relative polycythemia? □ increase --. Low RBC mass
4. Patient with PRV? Increase □ increase RBC mass

THANK YOU!

I hope you learn something
new today!

