BLOOD PRACTICAL-1
CBC & ESR

- Very important
- Extra information
- Terms

عن أبي الدرداء رضي الله عنه قال: فَإِنِّي سَمِعْتُ رَسُولَ اللَّهِ صلى الله عليه وسلم يَقُولُ: **«مَنْ سَلَكَ طَرِيقًا يَطْلُبُ فِيهِ عِلْمًا سَلَكَ اللَّهُ بِهِ طَرِيقًا مِنْ طُرُقِ الْجَنَّةِ ... وَإِنَّ الْعُلَمَاءَ وَرَثَةُ الأَنْبِيَاءِ، وَإِنَّ الأَنْبِيَاءَ لَمْ يُوَرِّثُوا دِينَارًا وَلاَ دِرْهَمًا، وَرَّثُوا الْعِلْمَ فَمَنْ أَخَذَهُ أَخَذَ بِحَظٍّ وَافِرٍ »**

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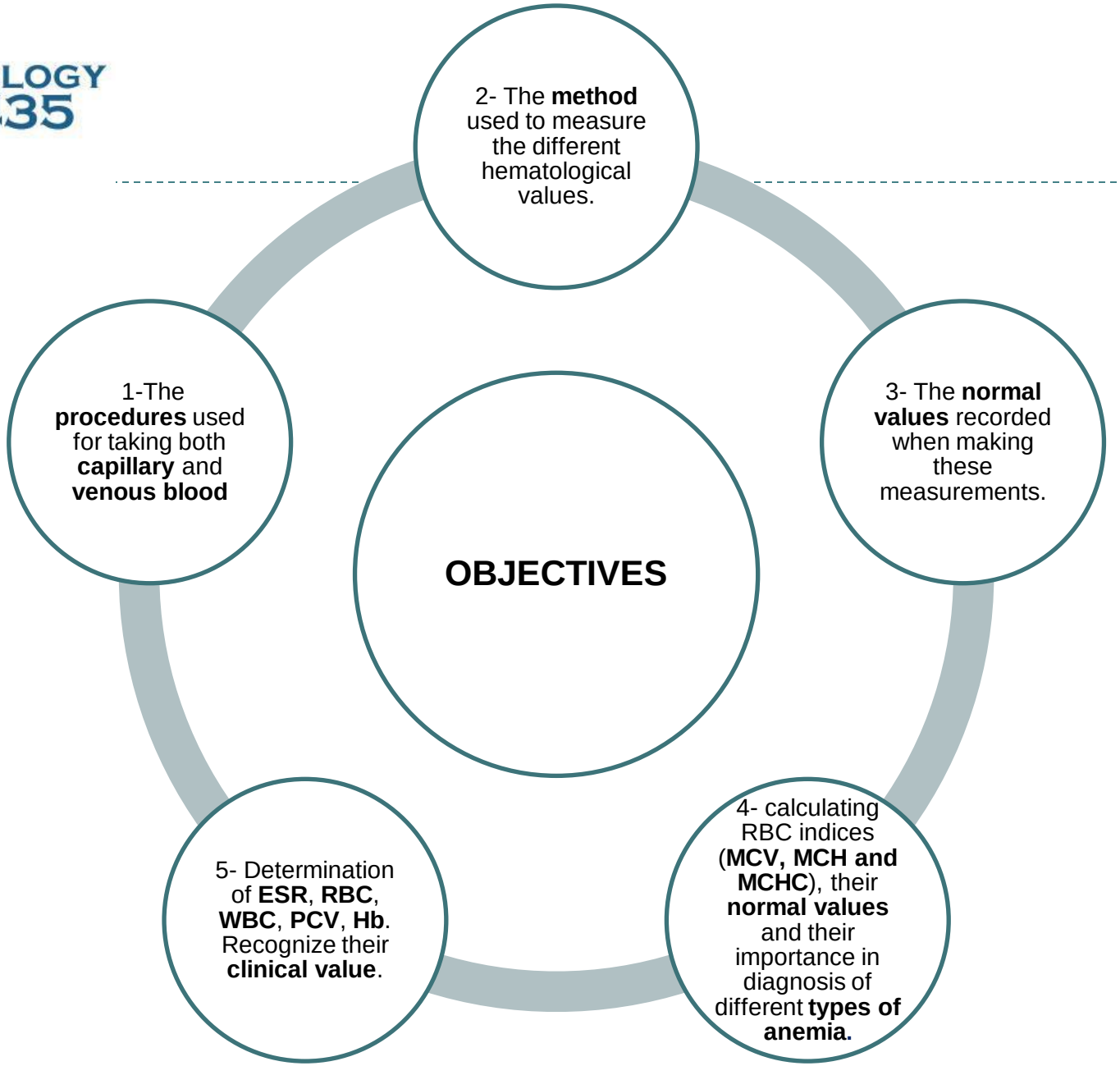

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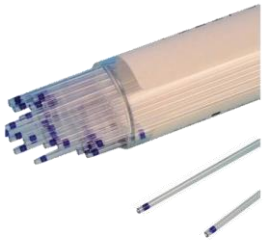
Equipments



- Coulter analyzer
- Heparinized capillary tubes
- Plasticine

- EDTA tubes
- Alcohol swabs
- Centrifuge machine

- lancets
- Tourniquet
- Micro hematocrit reader

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CBC : Materials and methods

Complete Blood Count (CBC) is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood.

EDTA anticoagulant blood

Is an alternative to the whole blood reference method for calibration

Calibrator kit

Coulter analyzer

Diluent reagents (REAGENT I)

Lytic reagent (REAGENT II)

Lysis RBCs for WBCs count and hemoglobin measurement.

Is an isotonic electrolyte solution, **used for Dilution and counting RBC by:**

1. Stabilizing cell membrane for accurate counting and size
2. Conducting aperture current
3. Rinsing instrument components between analysis
4. Preventing duplicate cell counts

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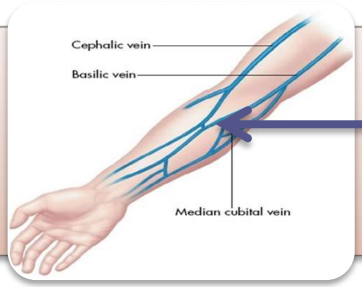
CBC Procedure

Measurement of blood cells counts by using coulter analyzer in order to analyze the blood for various hematological values:

* هذي الخطوات ما سويناها كانوا مجهزين لنا الدم في المعمل .



1. We need to draw 5 ml of blood from a superficial vein (Usually from median cubital vein in front of the elbow joint) using a disposable syringe (needle).
2. Don't forget to clean the area of skin to be pricked..

3. Apply the tourniquet to the elbow joint to impede(prevent) the flow of venous blood toward the heart for a while.

4. Immediately transfer the collected blood from the syringe(needle) to **EDTA** anti-coagulated tube to prevent blood from clotting.

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TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
CBC With Differential/Platelet					
WBC	5.7		x10E3/uL	4.0-10.5	01
RBC	5.27		x10E6/uL	4.10-5.60	01
Hemoglobin	15.4		g/dL	12.5-17.0	01
Hematocrit	44.1		%	36.0-50.0	01
MCV	84		fL	80-98	01
MCH	29.2		pg	27.0-34.0	01
MCHC	34.9		g/dL	32.0-36.0	01
RDW	13.7		%	11.7-15.0	01
Platelets	268		x10E3/uL	140-415	01
Neutrophils	47		%	40-74	01
Lymphs	46		%	14-46	01
Monocytes	6		%	4-13	01
Eos	1		%	0-7	01
Basos	0		%	0-3	01
Neutrophils (Absolute)	2.6		x10E3/uL	1.8-7.8	01
Lymphs (Absolute)	2.6		x10E3/uL	0.7-4.3	01
Monocytes(Absolute)	0.4		x10E3/uL	0.1-1.0	01
Eos (Absolute)	0.1		x10E3/uL	0.0-0.4	01
Baso (Absolute)	0.0		x10E3/uL	0.0-0.2	01
Immature Granulocytes	0		%	0-1	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01

This is the probe for the aspiration in step 5

5- Activate the coulter analyzer machine and a probe will move across and down into aspirate. The aspiration syringe draws 12 µl of whole blood into the probe.

6- The coulter analyzer makes the necessary dilution with the reagents automatically.

*** reagent I : used for Dilution and counting RBC.**

*** reagent II : Lysing RBC and counting WBC and Hb.**

7- it accurately counts and measures the sizes of cells by detecting and measuring changes in electrical resistance when cells in the conductive liquid pass through a small aperture

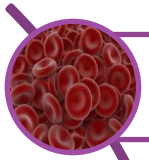
The number of pulses signals the number of particles.

The height of each pulse is proportional to the volume of that cell or particle.

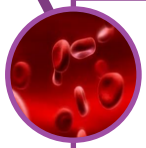
8- Finally all the hematological values are reported and printed.

YouTube

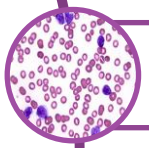
Clinical terms



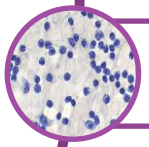
Polycythemia : Increased red blood cell count above normal. ↑



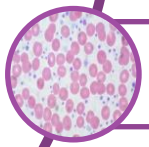
Anemia : Reduced ability of blood to carry Oxygen due to either decreased red blood cell count and/or hemoglobin concentration. ↓



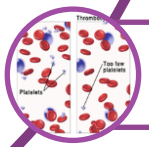
Leucocytosis :Increased white blood cell count above normal. ↑



Leucopenia : Decreased white blood cell count below normal. ↓



Thrombocytosis : Increased platelets count above normal. ↑



Thrombocytopenia : Decreased platelets count below normal. ↓

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Clinical applications

High numbers of RBCs may indicate:	RBCs normal values			Low numbers of RBCs may indicate:
	Male	Female	Average	
1. Low oxygen tension in the blood • Congenital heart disease • Cor pulmonale [1] • Pulmonary fibrosis 2. Polycythemia vera. 3. Dehydration (such as from severe diarrhea). 4. Renal (kidney) disease with high erythropoietin production.	4.5-6.5 x10^6/µl	3.8-5.8 x10^6/µl	4.7–6.5 x10^6/µl	1. Blood loss: • Anemia (various types). • Hemorrhage. 2. Bone marrow failure (for example, from radiation, toxin, fibrosis, tumor). 3. Erythropoietin deficiency (secondary to renal disease). 4. Hemolysis (RBC destruction).

1 cor pulmonale: abnormal enlargement of the right side of the heart as a result of disease of the lungs or the pulmonary blood vessels.

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Clinical applications-2

High numbers of WBCs (Leucocytosis) may indicate:	WBCs normal value	Low numbers of WBCs (leukopenia) may indicate:
• Infectious diseases. • Inflammatory disease (such as rheumatoid arthritis or allergy). • Leukemia. • Severe emotional or physical stress. • Tissue damage (burns).	$4 – 11 \times 10^3$ /µl	•Bone marrow failure (for example, due to infection, tumor or fibrosis). •Presence of cytotoxic substance. •Autoimmune/collagen-vascular diseases (such as lupus erythematosus). •Disease of the liver or spleen. •Radiation exposure.

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Clinical applications-3

High numbers of platelets (Thrombocytosis) may indicate:	**Platelets** normal value	Low numbers of platelets (Thrombocytopenia) may indicate:
- Chronic myeloid leukemia.	150-400x10^3 /µl	- A plastic anemia. - Chemotherapy.

	Male	Female	Average
HB Normal value (hemoglobin)	13-18 g/dl	11.5-16.5 g/dl	13 –18 g/dl

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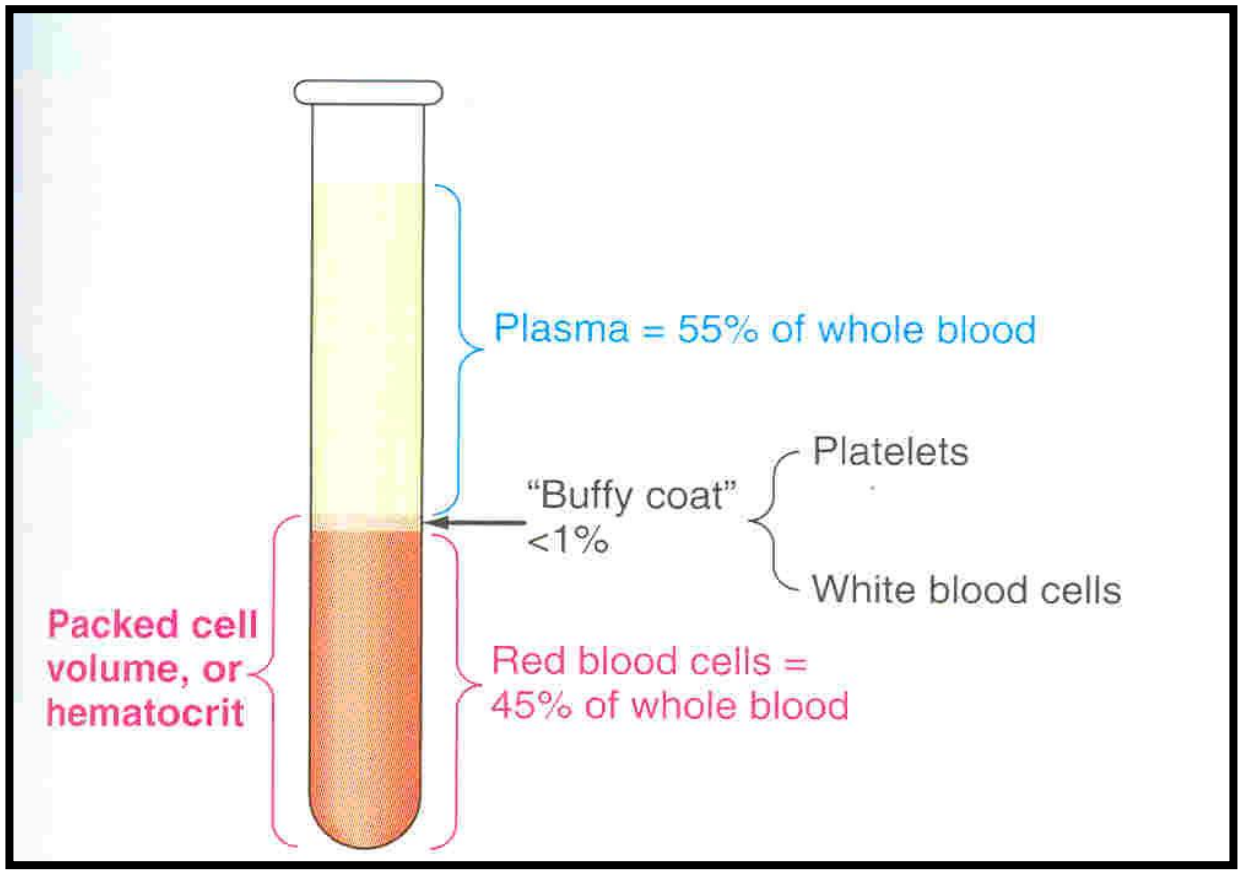
Packed Cell Volume (PCV) (Hematocrit)

The ratio of packed blood cells volume to plasma.



* **Plasma:** has anticoagulants.
* **Serum:** Without anticoagulants

PCV Procedure-1

Measurement of hematocrit or packed cell volume (PCV) : we need to draw blood from capillaries in order to measure hematocrit (PCV) using micro-hematocrit reader.

1. Clean the area of the skin of a finger-tip or an ear lobe with a sterilized alcohol swab.

2. Prick the skin using the pen lancet.

3. Discard the first drop of blood , because it is mixed with tissue fluid.
4. Allow the second drop of blood to be formed and allow it to become large enough to fill 75% of heparinized capillary tube by the capillary action when it is brought closer to the blood . apply only gentle pressure beneath the pricked skin to help the flow of blood, because if more pronounced pressure is exerted, blood is likely to be diluted with interstitial fluid.

5. Seal one end of the capillary tube with plasticine(الصلصال).
6. Repeat above steps 1-5 to **collect several capillary blood samples**.

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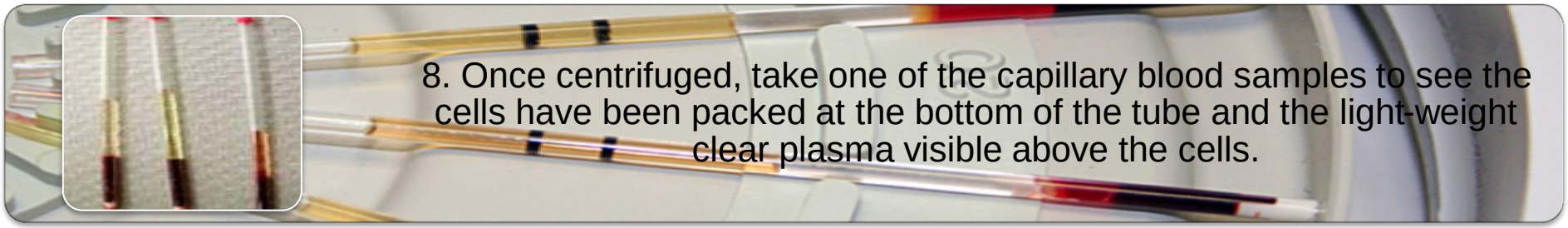
PCV Procedure-2

7. Put all the capillary blood samples in a **centrifuge machine** for 5 minutes at the speed of 3000-4000 RPM to separate plasma from cells.



8. Once centrifuged, take one of the capillary blood samples to see the cells have been packed at the bottom of the tube and the light-weight clear plasma visible above the cells.

9. The PCV or hematocrit can then be determined as a percentage of the total volume using **hematocrit reader.**

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Clinical applications

High hematocrit may indicate:	PCV % normal value			Low hematocrit may indicate:
	Male	Female	Average	
• Dehydration - Burns - Diarrhea • Polycythemia Vera. • Low oxygen tension (smoking, congenital heart disease, living at high altitudes).	40-54	35-47	35-54	• Anemia (various types). • Blood loss (hemorrhage). • Bone marrow failure (for example, due to radiation, toxin, fibrosis, tumor). • Hemolysis (RBC destruction) related to transfusion reaction. • Leukemia.

Red Blood Indices

	Definition	The calculation of Red Blood Indices	higher than average	Average (normal value)	Lower than average
Mean cell volume (MCV)	The average volume of red blood cell measured in femtoliters (fl)	MCV = $\frac{PCV \times 10}{RBC\ count}$	RBC are large in size and they are called macrocytes.	77-98 µm3 (fl)	RBC are small in size and they are called microcytes
Mean cell hemoglobin (MCH)	The average weight of Hb in red cells cell measured in picograms (pg).	MCH = $\frac{Hb \times 10}{RBC\ count}$	hyperchromic	27-32 pg	hypochromic
Mean cell Hb concentration (MCHC)	Concentration of Hb per 100 ml of RBC measured in grams/deciliters (g/dl).	MCHC = $\frac{Hb \times 100}{PCV}$		30-36 g/dl	Iron deficiency Anemia

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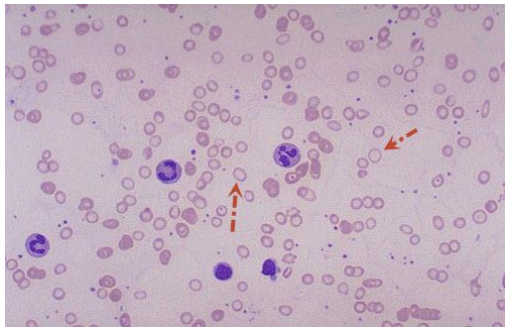
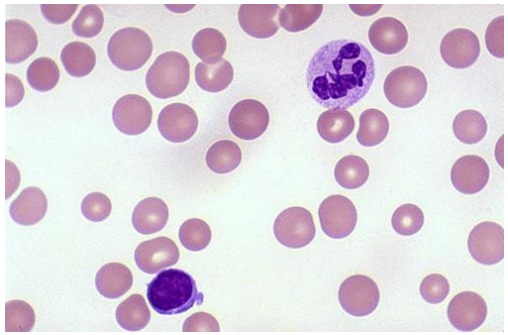
Types of Anemia

	Case A	Case B
RBC	Low	Low
HB	Low	Low
PCV	Low	Low
MCV	Low	high
MCH	Low	N/ high
MCHC	Low	N/low
Type of anemia	Microcytic Hypochromic	Macrocytic megaloblastic
Cause	Iron deficiency	Vit B12 or Folic deficiency



Microcytic Hypochromic anemia



Macrocytic megaloblastic anemia

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Clinical application

RBC Sedimentation

- **RBC Sedimentation is controlled by the balance between plasma protein fibrinogen, and the negative charge of the erythrocytes.**
- **In inflammatory, the high fibrinogen level causes RBCs to stick to each other to form stacks (rouleaux), which settle faster.**

Erythrocyte Sedimentation Rate (ESR)

- **ESR is the rate at which RBCs sediment in a period of 1 hour.**
- **<u>Prognostic not diagnostic</u>, because it is a nonspecific marker of inflammation and is affected by other factors , therefore, ESR results must be used along with other clinical findings.**
- **Monitor disease activity and response to therapy.**

C-reactive protein

- **C-reactive protein is an acute phase protein produced by the liver during an inflammatory reaction.**
- **Since C-reactive protein levels in the blood rise more quickly after the inflammatory or infective process begins, ESR is often replaced with C-reactive protein measurement.**

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ESR
Materials and Procedure

Disposable sterile syringes (needles).

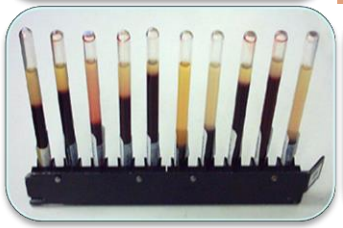
1. Using a sterile syringe draw 1.6ml of blood from a suitable vein.

Anticoagulant (EDTA).

2. Transfer it to a test tube containing EDTA

Westergren's sedimentation apparatus.

3. draw up blood into a Westergren tube exactly to the zero mark



4. Place the tube upright in the stand and leave undisturbed. ESR reading is the height of the column (depth) of clear plasma at the top of RBC in the tube, it is noted at the end of an hour and again at the end of 2nd hours.

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ESR results

Normal ESR	
Males	3-5 mm/ 1st hour 7-15mm/ 2nd hour
females	values are slightly higher than 7 mm due to less RBC

Moderately elevated ESR	very high ESR
• Infections. • Inflammation • anemia • Malignancies • pregnancy • old age	associated with: • multiple myeloma • polymyalagia Rheumatic • temporal arteritis

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Questions and Problems

1-What is the clinical importance of knowing the red blood cell indices?

They help to determine the type of anemia a patient is suffering from.

2- Discuss briefly the etiological classification of Anemia?

TYPE OF ANEMIA	CAUSE
Hemorrhagic Anemia	loss of blood
Aplastic Anemia	Bone marrow suppression by drugs or radiations etc.
Nutritional Anemia	Deficiency of Iron, folic acid, Vitamin B12
Hemolytic Anemia	Increased destruction of RBCs such as sickle cell disease

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Questions and Problems

3- An examination of the blood of 2 adult males (A and B) provided the following data.

	A	B
RBC count	3.6 X 10^6 / mm^3	2.5 X 10^6 / mm^3
HB concentration	7.2 g/dl	8 g/dl
Packed cell volume(PCV)	25%	25%

(a) Calculate MCV, MCH and MCHC for each of these subjects.

SUBJECT "A"	SUBJECT "B"
MCV = $\frac{25 \times 10}{3.6}$ = 69.4 fl	MCV = $\frac{25 \times 10}{2.5}$ = 100 fl
MCH = $\frac{7.2 \times 10}{3.6}$ = 20 pg	MCH = $\frac{8 \times 10}{2.5}$ = 32 pg
MCHC = $\frac{7.2 \times 100}{25}$ = 28.8 g/dl	MCHC = $\frac{8 \times 100}{25}$ = 32 g/dl

(b)What are the abnormalities encountered in these men. What are the possible causes of these abnormalities?

Subject "A" >> Microcytic hypochromic anemia (Iron deficiency anemia)

Subject "B" >> Macrocytic normochromic anemia (Megaloblastic anemia or Pernicious anemia)

The difference between Megaloblastic anemia and Pernicious anemia is:

1. **Megaloblastic anemia is due to deficiency of Vit12 or folic acid or both.**
2. **Pernicious anemia is caused by malabsorbation of Vit 12 due to lacking of intrinsic factor in the stomach**

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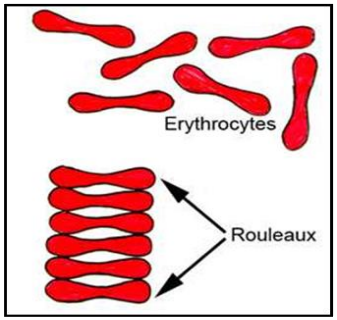
Questions and problems

1- what is meant by rouleaux formation?

When red blood cells are stacked together in long chains because of their biconcave disc like surfaces sticking to each other. It is called Rouleaux formation.



2- why does rapid rouleaux formation increase the E.S.R. ?

Rouleaux formation become rapid when plasma protine concentration is high and because of this E.S.R also become increased.

3- What is the clinical significance of E.S.R.?

- This is a non-specific indicator of presence of a disease.
- This is a useful prognostic tool.

4- What conditions are associated with an increased E.S.R.?

- Infections
- Connective tissue disorders
- Inflammatory disorders
- Malignancies
- Anemia
- Pregnancy

Contact us : Physiology435@gmail.com

Physiology practical team

عبدالعزيز رضوان

أمل العمران

YouTube Coulter analyzer Video

YouTube ESR Video

أفنان المالكي

لولوه الصغير

ريم البهلال

أسرار باطرفي

YouTube PCV Video

عمر الشهري

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Quiz

Easy

Expert

شكر خاص لخولة العماري

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