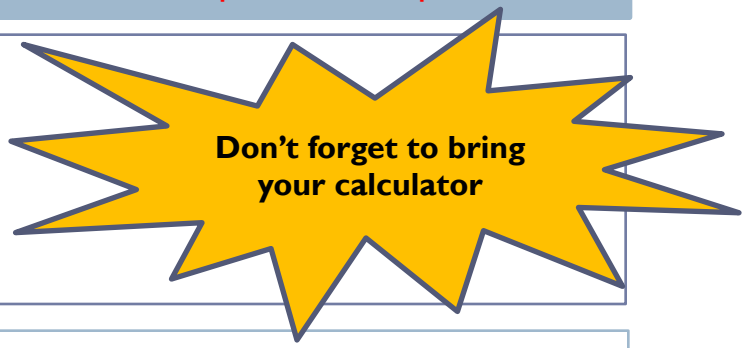
BLOOD PRACTICAL

- This slides contain both (Slides + Handout الملزمة)
- Procedures in Female slide and handout are the same .Therefore ,we put it as one procedure

Red : important
Green: only found in males' slides.
Purple: only found in females' slides.
Gray: notes.



Physiology practical Team 436 – Foundation block

(CBC & ESR)

{ يامالك الملك وكلناك أمرنا واستودعناك همومنا
{فبشرنا بما يفتح مداخل السعادة إلى قلوبنا

Aims of the Practical

1. Counting Red blood cells.
2. Counting White blood cells.
3. Determination of hemoglobin concentration.
4. Determination of packed cell volume (PCV) hematocrit.
5. Calculation of red blood cell indices.
6. Determination of ESR.

Objectives(CBC & ESR)

1- Recognize the method used to measure the different hematological values, and compare it with the normal values.

2- Do the calculation of indices, their normal values and their importance in diagnosis of different types of anemia.

3- To know how to measure the erythrocyte sedimentation rate .

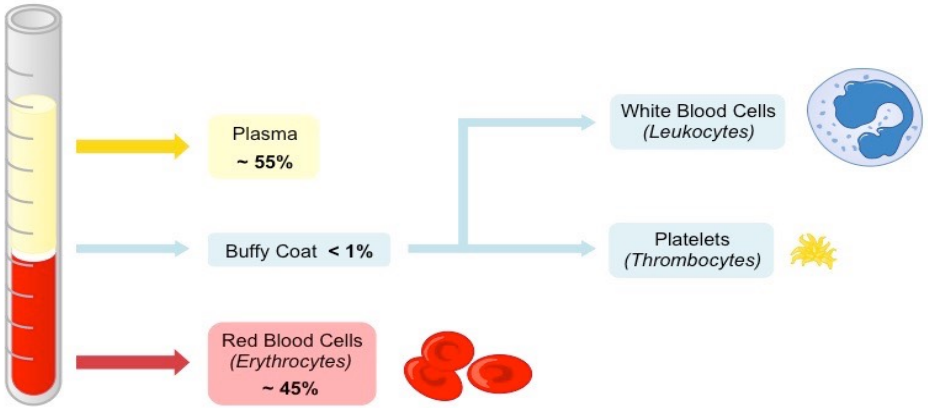
4-To recognize what is the clinical value of these measurements

Blood Composition

The average blood volume of adults is about 7% of body weight (5 - 5.5 L).

Cellular component **45%**	Plasma **55%**
• RBCs • WBCs • Platelets	• Water 98% • Ions • Plasma Protein

- Ions like : (Na , K , HCO3 , PO4 … etc.)
- Plasma Protein like : (Albumin , Globulin , Fibrinogen)



Plasma has same ionic composition as Interstitial fluid

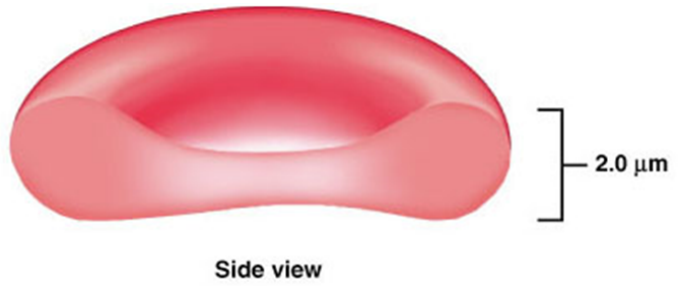
In male slides and female doctor said take it as a revision

Red blood cell (Erythrocytes)

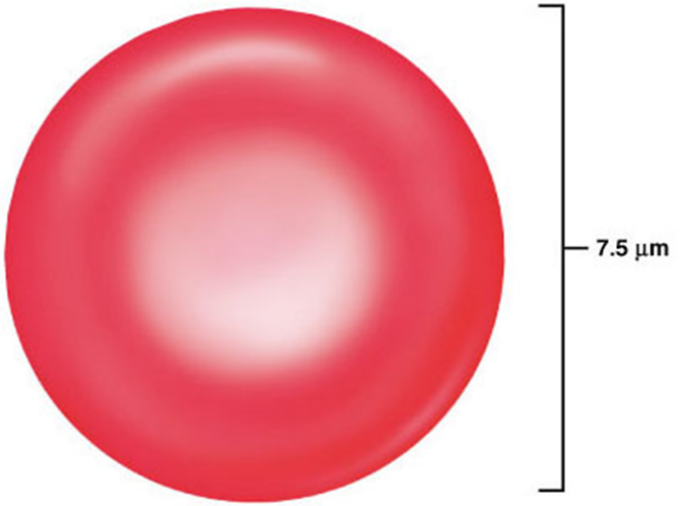
STRUCTURE :

- Flat biconcave disc
- Non-nucleated
- Dimensions:
  - Diameter: 7.5 µm
  - Thickness 2 µm
- Contain a red pigment (hemoglobin)
- RBC membrane is elastic so it is flexible

(can easily pass through the capillaries).



Side view



Top view

In male slides and female doctor said take it as a revision

Red blood cell (Erythrocytes) male slides

RBCs Count

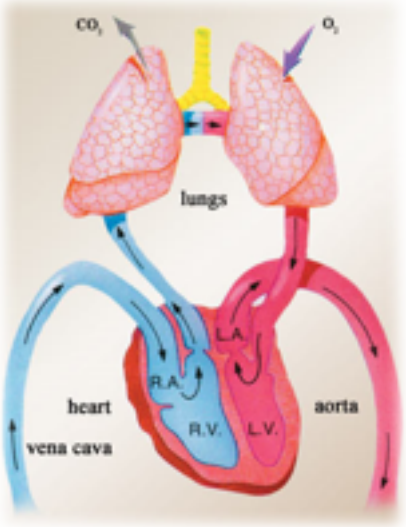
Male	Female
4.8-6.5 million cells/mm3 ·	3.8-5.8 million cells/mm3 ·

Life span is 120 Day

Functions

- Carry Hemoglobin
- Buffer (decrease pH; regulation)
- Transport oxygen from lungs to tissues and carbon dioxide from tissues to lungs ; (because they contain hemoglobin) .

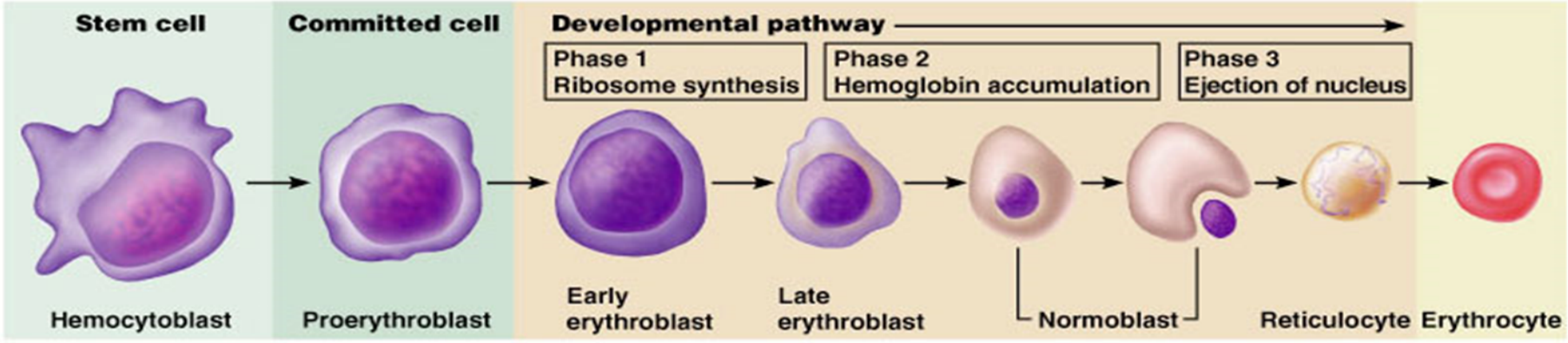
RBCs are metabolically active cells; uses glucose for energy



In Different textbooks there are different values but they are nearly similar

Erythropoiesis

The process of Production of Erythrocytes :



Hemocytoblasts: give rise to all formed elements .

Features of the maturation process of RBC
1. Reduction in size
2. Disappearance of the nucleus
3. Acquisition of hemoglobin

In male slides but female doctor said take it as a revision

Control of Erythropoiesis

Erythropoiesis is stimulated by erythropoietin hormone

Stimulated by:

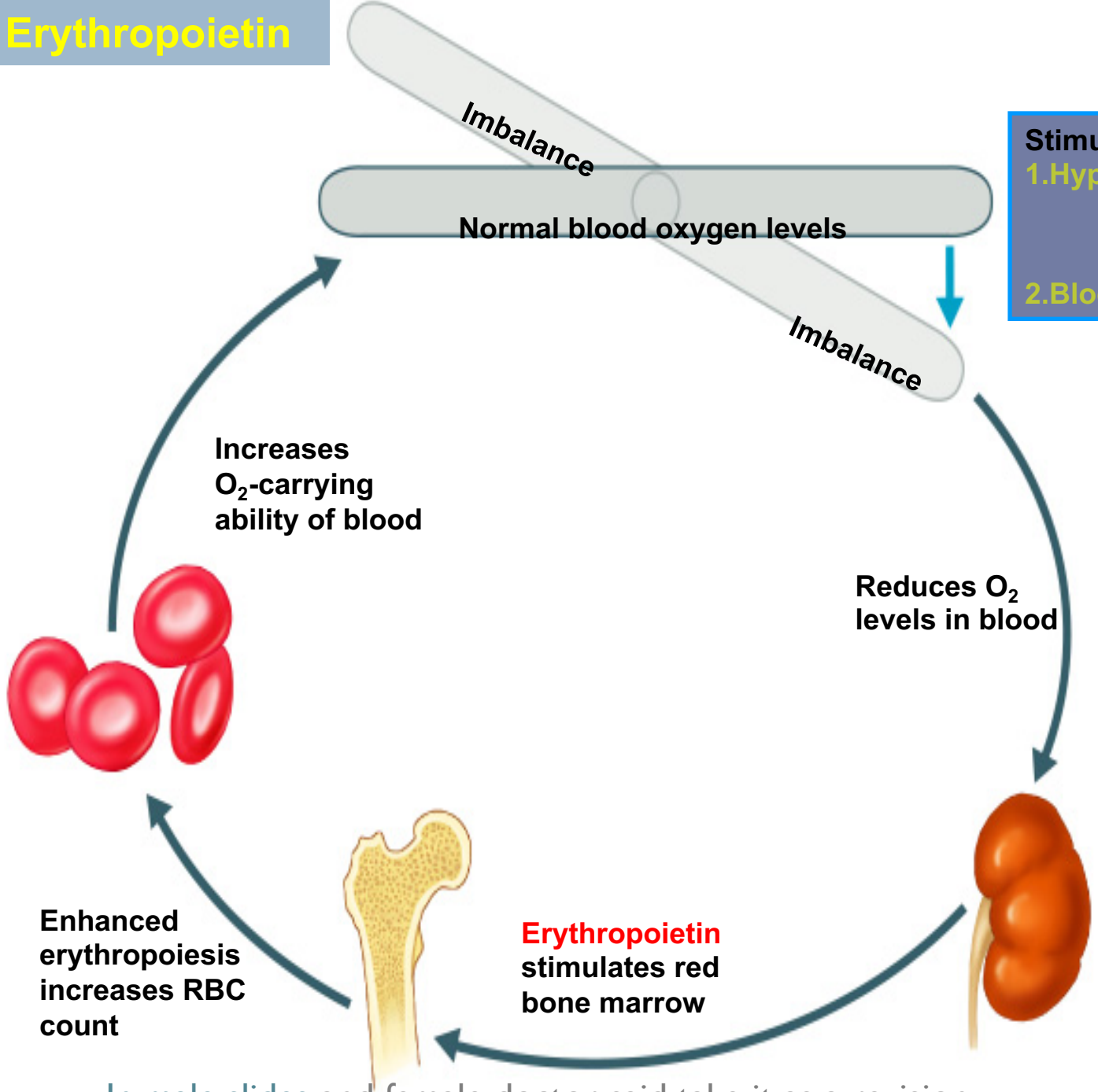
- Hypoxia (low oxygen)
- Anemia
- Hemorrhage
- High altitude
- Lung disease
- Heart failure

Nutritional requirements of RBC production :

1. Amino Acids
2. Iron
3. Vitamins (B12 , Folic Acid , c)
4. Trace Element (Copper , Cobalt , Zinc)

In male slides and female doctor said take it as a revision

Erythropoietin



Start

Stimulus:

1.Hypoxia :

- High altitude
- Heart or lung diseases
- Anemia

2.Blood loss

Structure: 24% CHO
67% protein

Half life: 6.7 hours.

In male slides and female doctor said take it as a revision

Hemoglobin

- Hemoglobin is the protein molecule in red blood cells. carries oxygen from the lungs to body's tissues and returns carbon dioxide from the tissues to the lungs .
- It is red pigment present in RBCs and gives red blood cells their color.
- 280 million molecules / cell .

Male	Female
13-18 g/dl .	11.5 -16.5 g/dl .

Anemia :

- **Decrease number of RBC and Hb .**
- **Symptoms :** Tired , Fatigue , shortness of breath .
- **Signs :** pallor , tachycardia .

Tachycardia: rapid heart rate.

In male slides and female doctor said take it as a revision

Complete Blood Count (CBC)

- It is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood.

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	0.7-4.5	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

Materials and methods

Detail
Next slide

Coulter analyzer

EDTA anticoagulant blood

To prevent blood from clotting

Calibrator kit

Is an alternative to the whole blood reference method for calibration

(to know if the machine work efficiently)

(مثال : يكون عندي عينة مكتوب عليها كميات المكونات اللي فيها (كمية الهيموجلوبين , كمية الكريات الدم الحمراء/البيضاء) فندخل العينة بالجهاز ونشوف هل المكونات اللي طالعة من الجهاز هي نفسها المكتوب على العينة

Diluent reagents

- Is an isotonic electrolyte solution that:
  - Dilute the whole blood sample .
  - Stabilize cell membrane for accurate counting and size (الخلايا تكون متراصة فنفرق بين الخلايا عشان يصير العد صحيح)
  - Conduct aperture current
  - Rinse instrument components between analysis
  - Prevent duplicate cell counts

Lytic reagent

Lysis RBCs for hemoglobin measurements and WBCs count

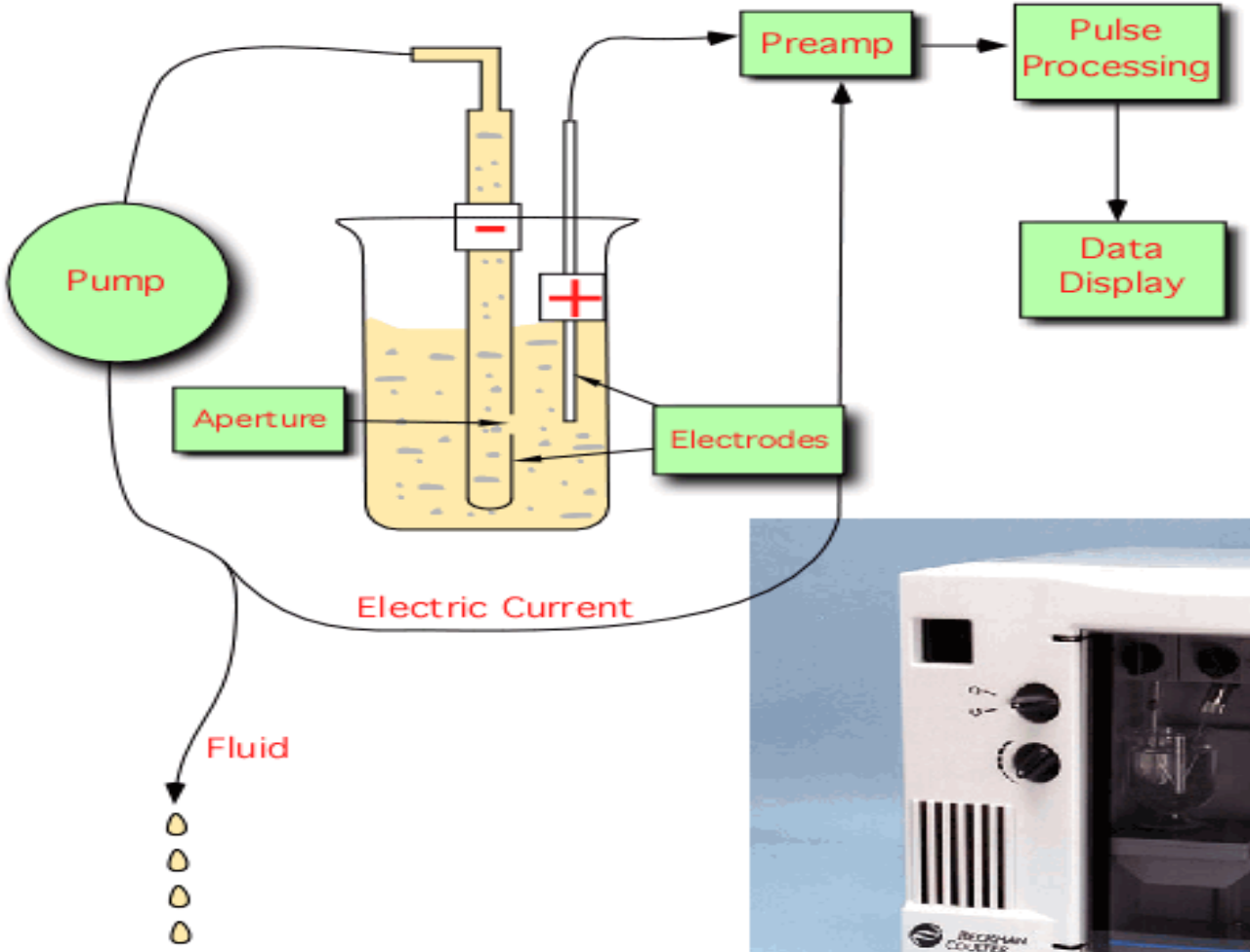
Coulter Counter Principle = Coulter analyzer

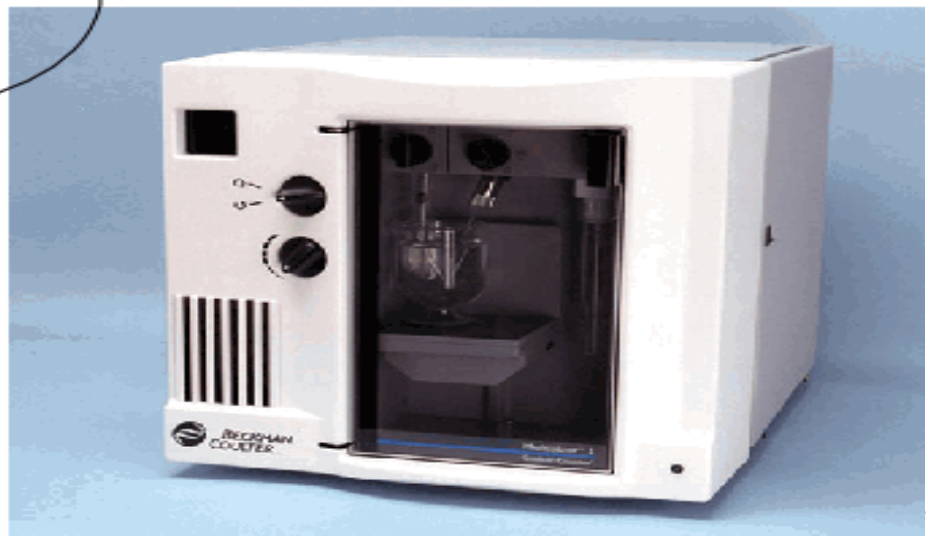
- It count and measure the size of the cells by detecting and measuring electrical resistance when a liquid pass through aperture.
- While passing the aperture, the cells impedes the current and causes a measurable pulse.

- Number of pulses --> number of particles.
- Height of pulses --> volume of particles.

Example : Short pules → small cell

Coulter Counter Principle





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Coulter Counter Principle

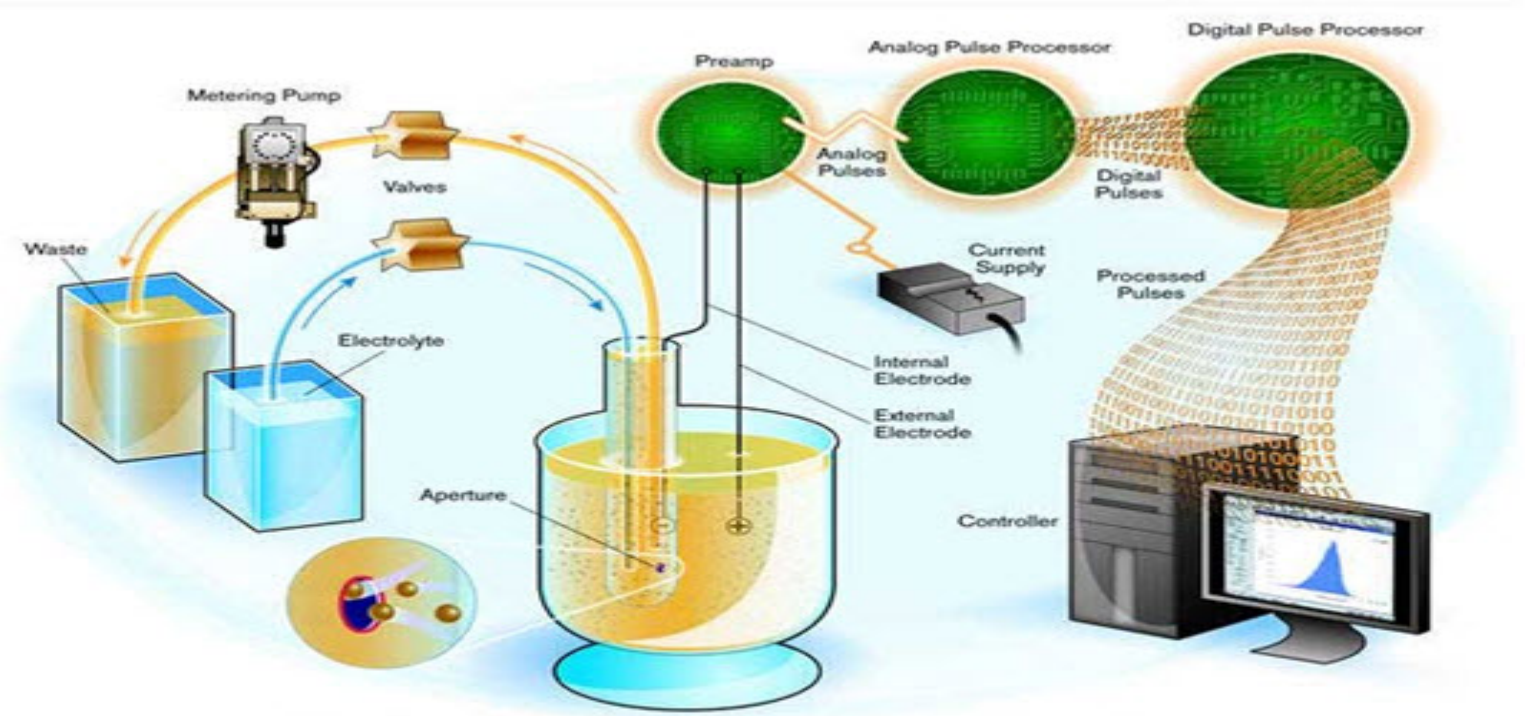


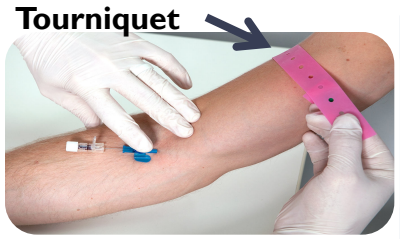
Figure 1. Schematic of a COULTER COUNTER

- In male slides and female doctor said take it as a revision

PROCEDURE 1:

MEASUREMENT OF BLOOD CELL COUNTS BY USING COULTER ANALYZER

Aim : draw blood from a superficial vein in order to analyse the blood for various haematological values using Coulter Analyser .



- **1.** Clean the area of the skin to be pricked .
- **2.** Apply the tourniquet above the elbow joint to impede the flow of venous blood towards the heart for a while.

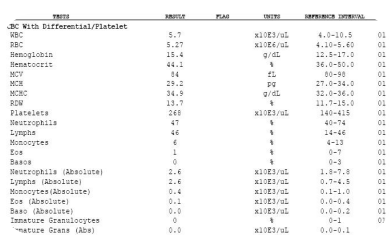
- **3.** Use a disposable syringe to draw 5ml of blood from the vein(Usually from median cubital vein in front of the elbow joint) then Immediately transfer it to EDTA anti-coagulated tube to prevent blood from clotting.

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

- The probe for the aspiration in step 4

Complete Procedure1

- **5**. The Coulter Analyzer makes the necessary dilutions with the reagents
- * reagent I : Diluted and used to count RBC.
- * reagent II : Lysing RBC and used for counting WBC and Hemoglobin.



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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	07
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	

- **6 .** It accurately counts and measures the sizes of cells by detecting and measuring changes in electrical resistance when a particle (such as cell) in the conductive liquid passes through a small aperture.
- As each cell goes through the aperture, it impedes the current and causes a measurable pulse .
- Number of pulses signals →Number of particles.
- height of each pulse →the volume of that cell

- **7.** Finally all the hematological values are reported and printed

YouTube

For videos click the YouTube icon or https://www.youtube.com/watch?v=1iY4Ufqh1XY

Red Blood Indices (team work 435)

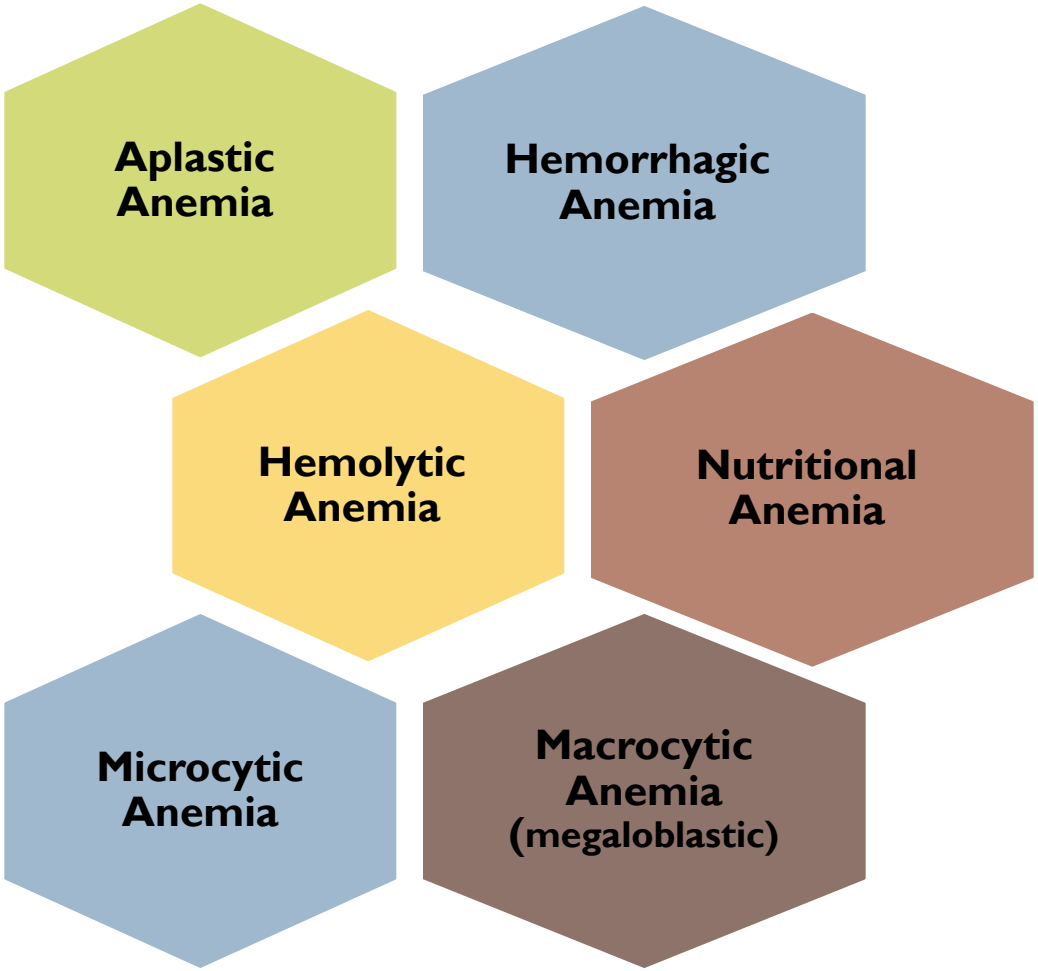
In males slides:
MCV is from 78-98 fl
MCHC is from 32-36

	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)	$MCV = \frac{PCV \times 10}{RBC\ count}$ PCV = packed cell volume	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 blood cells cell measured in picograms (pg).	$MCH = \frac{Hb \times 10}{RBC\ count}$ Hb = hemoglobin concentration	RBCs are Hyperchromic	27-32 pg	RBCs Are Hypochromic
Mean cell Hb concentration (MCHC)	Concentration of Hb (hemoglobin) per 100 ml of RBC measured in grams/deciliters (g/dl).	$MCHC = \frac{Hb \times 100}{PCV}$ PCV = packed cell volume Hb=hemoglobin concentration	-	30-36 g/dl	Iron deficiency Anemia

Normal values

	Male	Female	Average
RBC	4.5-6.5 $x10^6/\mu l$	3.8-5.8 $x10^6/\mu l$	4.7–6.5 $x10^6/\mu l$
WBC	4 – 11 $x10^3$ $/\mu l$	4 – 11 $x10^3$ $/\mu l$	4 – 11 $x10^3$ $/\mu l$
HB	13-18 g/dl	11.5-16.5 g/dl	13 –18 g/dl
Platelet	150-400$x10^3$ $/\mu l$	150-400$x10^3$ $/\mu l$	150-400$x10^3$ $/\mu l$
PCV % (percentage from the whole blood)	(40 – 54)%	(35 – 47)%	(35-54)%

Type of Anemia



- In male slides and female doctor said take it as a revision

Types of Anemia

(نحسب بالمعادلات بالسلايد السابق(رقم 18) ثم نقارن بالقيم الطبيعية سلايد رقم 19 ثم نقارن النتائج بهذا الجدول لمعرفة نوع الأنيميا)

	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

Important Terminology

POLYCYTHEMIA:

- Increased red blood cell count above normal.

ANAEMIA:

- Reduced ability of blood to carry Oxygen due to either decreased red blood cell count and/or haemoglobin 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.

Clinical applications:

Low numbers of RBCs may indicate:

- Blood loss:
  - Anemia (various types).
  - Hemorrhage.
- Bone marrow failure (**for example**: from radiation, toxin, fibrosis, tumor).
- Erythropoietin deficiency (secondary to renal disease).
- Hemolysis (RBC destruction).

High numbers of RBCs may indicate:

- Low oxygen tension in the blood:(hypoxia)
  - Congenital heart disease
  - Cor pulmonale
  - Pulmonary fibrosis
- Polycythemia vera.(increase number of RBC without any cause)
- Dehydration (**such as :** from severe diarrhea).
- Renal (kidney) disease with high erythropoietin production.

Low numbers of WBCs (leukopenia) may indicate:

- 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.

High numbers of WBCs (Leucocytosis) may indicate:

- Infectious diseases.
- Inflammatory disease (**such as** : rheumatoid arthritis or allergy).
- Leukemia.
- Severe emotional or physical stress.
- Tissue damage (burns).

Low numbers of Platelet (Thrombocytopenia) may indicate:	**High numbers of Platelet (Thrombocytosis) may indicate:**
A plastic anemia. Chemotherapy.	Chronic myeloid leukemia.

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 Anaemia	loss of blood
Aplastic Anaemia	Bone marrow suppression by drugs or radiations etc.
Nutritional Anaemias	Deficiency of Iron, folic acid, Vitamin B12
Hemolytic Anaemia	Increased destruction of RBCs such as sickle cell disease

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

	SUBJECT "A"	SUBJECT "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	25%	25%

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

SUBJECT "A"	SUBJECT "B"
MCV = 25 x 10 /3.6 = 69.4 fl	MCV = 25 x 10 /2.5 = 100 fl
MCH = 7.2 x 10 / 3.6 = 20 pg	MCH = 8 x 10 / 2.5 = 32 pg
MCHC = 7.2 x 100 / 25 = 28.8 g/dl	MCHC = 8 x 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 anaemia (Iron deficiency anaemia)
Subject "B" ➔ Macrocytic normochromic anaemia (Megaloblastic anaemia or Pernicious anaemia)

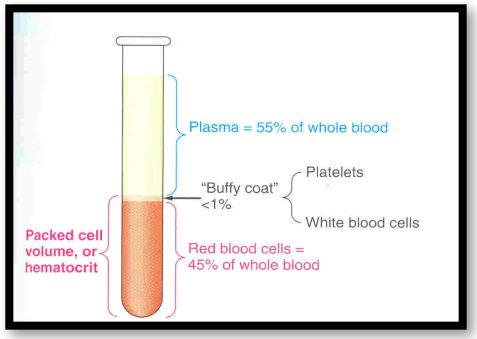
Don't forget to bring your calculator

Packed Cell Volume (PCV) or Hematocrit

- The ratio of packed blood cells volume to plasma

(packed cell volume : RBC's)

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



Low hematocrit may indicate:

- Anemia (various types).
- Blood loss (hemorrhage).
- Bone marrow failure (for example, due to radiation, toxin, fibrosis, tumor).
- Hemolysis(RBC's destruction)related to transfusion reaction.
- Leukemia.

High hematocrit may indicate:

- Dehydration
  - Burns
  - Diarrhea
- Polycythemia Vera.
- Low oxygen tension
  - smoking
  - congenital heart disease
  - living at high altitudes

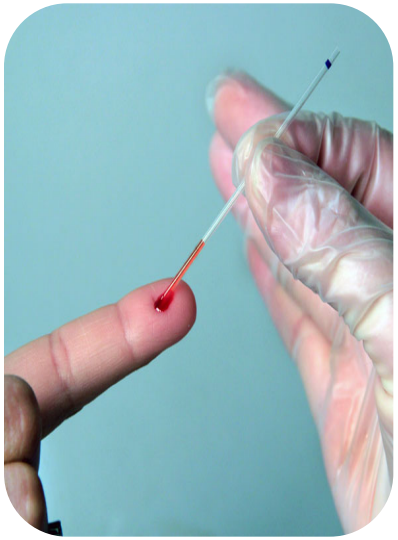
PROCEDURE 2:

MEASUREMENT OF HEMATOCRIT/PACKED CELL VOLUME (PCV):

Aim : draw blood from capillaries in order to measure Hematocrit (PCV) using micro-hematocrit reader.

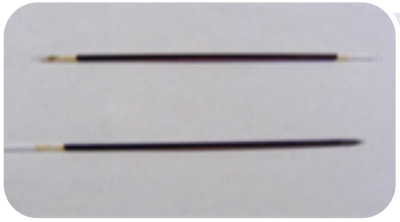
- **1. Clean the area of the skin of a finger-tip or an ear lobe with a sterilized alcohol swab.**

- **2.** Prick the skin using the pen lancet.



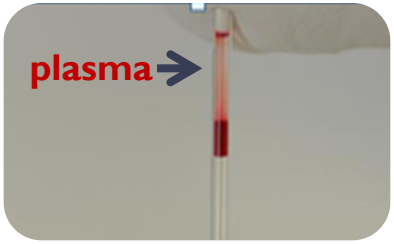
- **3. Discard the first drop of blood, because it is mixed with tissue fluid**
- **4. Allow the second drop of blood to be formed until it become large enough to fill 75% of the heparinized capillary tube (heparin inside the tube) by the capillary action when it is brought closer to the blood.**
- **5. 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.**

Complete Procedure2



- **5.Seal one end of the capillary tube with plasticine(صلصال).** (you should have several capillaries)

- **6. 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.**



- **7. 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.**

- **8.The packed cell volume or Hematocrit can then be determined as a percentage of the total volume using Hematocrit reader.**

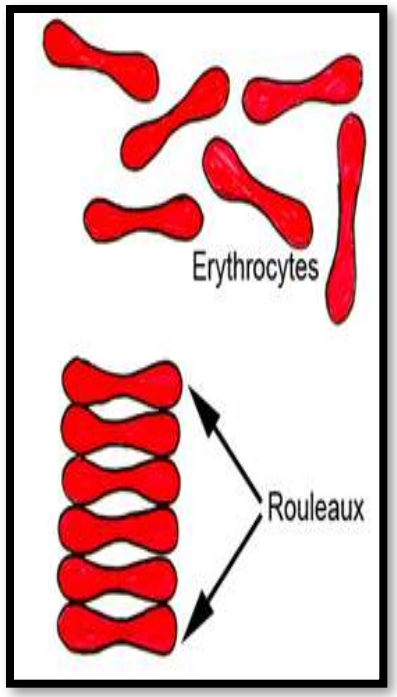
Erythrocyte Sedimentation Rate (ESR)

(سرعة الترسيب)

- Is the rate at which **red blood cells** sediment in a period of 1 hour.
- non-specific measure of **inflammation.**

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.



PROCEDURE 3:

ERYTHROCYTE SEDIMENTATION RATE (E.S.R.) EQUIPMENT

DISPOSABLE STERILE SYRINGES AND NEEDLES

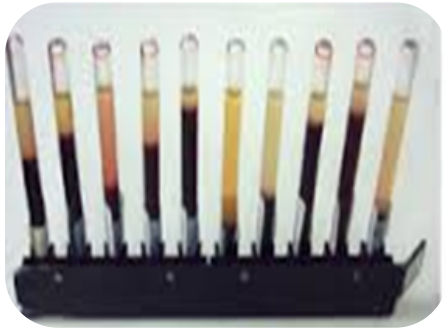
- **1-** Using a sterile syringe, draw 1.6 ml of blood from a suitable vein

ANTICOAGULANT EDTA TUBE

- **2-** Transfer the blood to a test tube containing EDTA to prevent clotting.

WESTERGREN'S SEDIMENTATION APPARATUS

- **3-** Fill the Westergren's tube with blood up to the zero mark.



- **4-** Place the tube upright in the stand and leave it undisturbed like this for one hour.
- **5-** Note down the height of the column of clear plasma at the top of red blood cells in the tube after one hour then we take notes after the 2nd our . This will be E.S.R. reading

Normally the value of E.S.R. ranges from (0-7)mm and it is slightly higher in females than males due to : less number of red blood cells.

3 Materials

ESR Results

In males slides normal range is from 0-7 mm

- Normal ESR range :

Male → 3-7mm\1st hour , 7-15 mm\2nd hour

Female → slightly higher than 7 mm due to less RBC

Moderately elevated ESR occurs :	**A very high ESR associated with :**
Infections	multiple myeloma
Inflammation	polymyalgia Rheumatic
Anemia	temporal arteritis
Malignancies	
Pregnancy	
old age.	

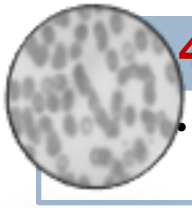
Clinical application of ESR

- Nonspecific test.
- Prognostic not diagnostic.
- Monitor disease activity and response to therapy.
- **ESR is :** a nonspecific marker of inflammation and is affected by other factors .
- ESR results must be used along with other clinical findings.

C-reactive protein & ESR

- C-reactive protein is an acute phase protien 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.

Question & problem



4- 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.

5- Why does rapid rouleaux formation increase the E.S.R.?

- Rouleaux formation becomes rapid when plasma protein concentration is high and because of this E.S.R. also becomes increased.

6- 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.

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

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

Thank you!

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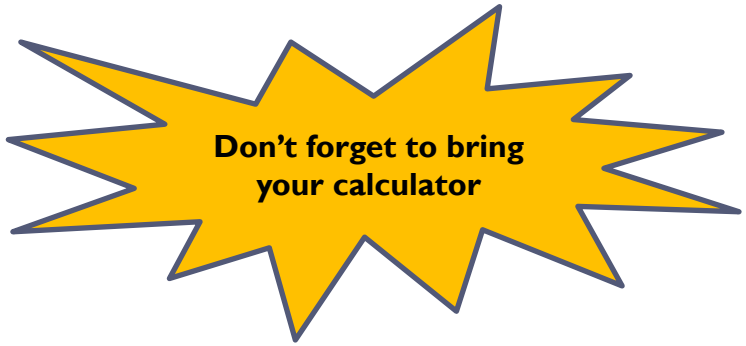

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