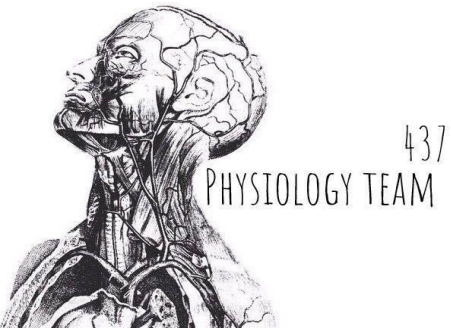
PHYSIOLOGY
PRACTICAL

BLOOD PRACTICAL

CBC & ESR

slides + handout

Red: Important
Green: Notes
Gray: Extra Information
Blue: Only boys slides
Purple: Only girls slides



MED437
KING SAUD UNIVERSITY

Objectives (CBC):

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.

Objectives (ESR):

1.To know how to measure the erythrocyte sedimentation rate.

2.To recognize what is the clinical value of these measurements.

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

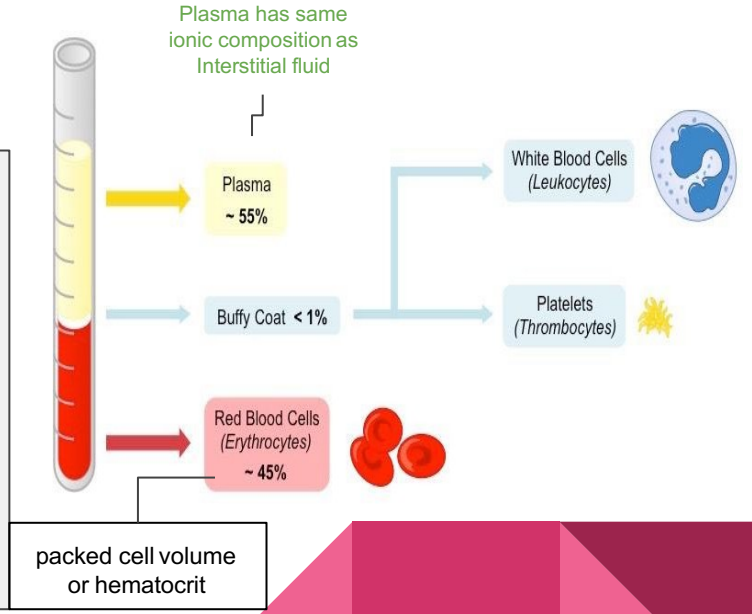
BLOOD

*the whole slide is from boys

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

Blood Composition

Cellular components: 45%Contains: -Red blood cells -White blood cells -Platelets	**Plasma:** 55%Consists of: -98% water -Ions: Na,K,HCO3,PO4 …. etc. -Plasma proteins (Albumin, Globulin, Fibrinogen). -It has the same ionic composition of the interstitial fluid.



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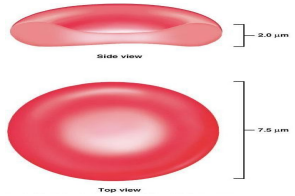
Red Blood Cells (RBC) (Erythrocytes) :

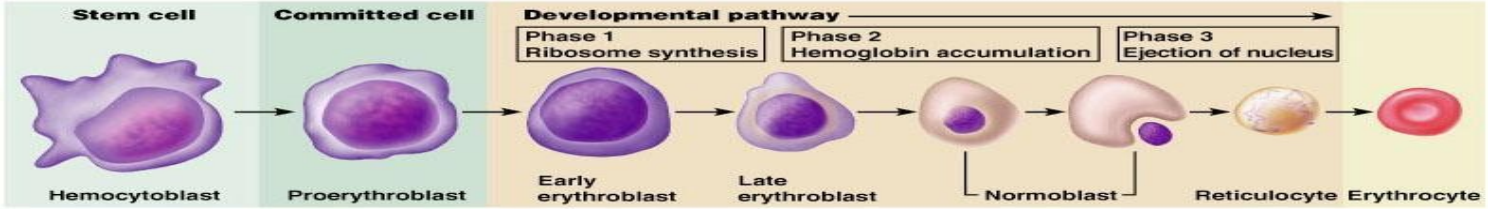


Structure	1- Flat biconcave disk 2- Non-nucleate 3- Dimensions: 7-8 µmx 2.5 µm x 1 µm 4- contain a red pigment haemoglobin 5- RBC membrane is elastic so it is flexible (can easily pass through the capillaries)
RBC count	In *males*: 4.8-6.5 million cells/mm3 In *females*: 3.8-5.8 million cells/mm3 In different textbooks there are different values but they are nearly similar
Life span	120 days
Functions	*1Carry haemoglobin* *2Transport of oxygen;* transport oxygen from lungs to tissues\ and carbon dioxide from tissues to lungs (because they contain haemoglobin) *3buffer* (pH regulation) *4Metabolism;* metabolically active cells uses glucose for energy

*The whole slide is from boys

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 nuclues
3 Acquisition (addition) of haemoglobin

-Nutritional requirements of RBC production:

1-Amino acids
2-Iron
3 Vitamins (Vit: B12, folic acid, Vit C)
4 Trace elements (Copper, cobalt, zinc)

control of erythropoiesis

-Erythropoiesis is stimulated by erythropoietin hormone stimulated by:

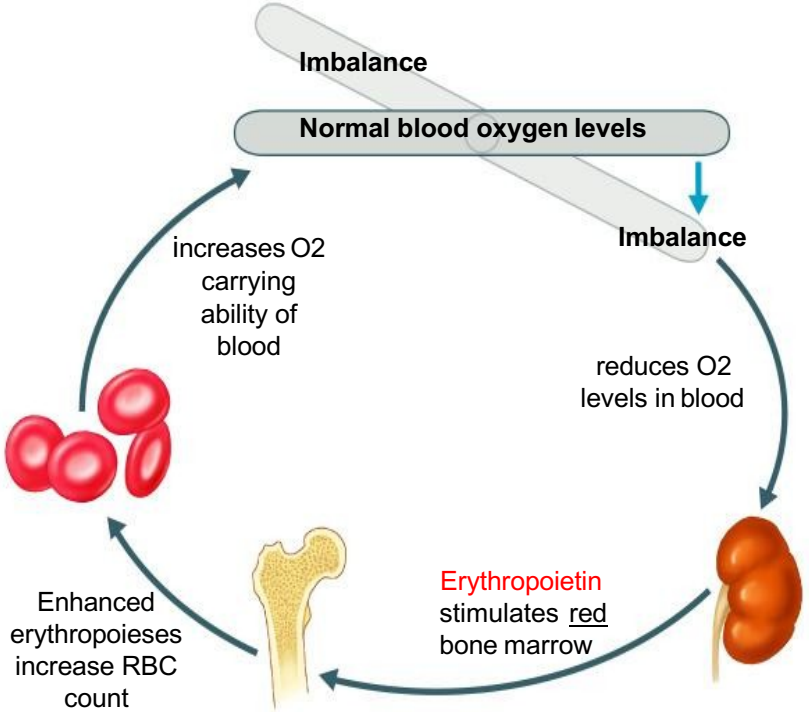
*erythropoietin hormone its function to synthesize RBCs

1-hypoxia (low oxygen)
2- anemia
3-Hemorrhage
4-High altitude
5-Lung diseases
6-Heart failure

start

Erythropoietin:

Stimulus: **hypoxia: high altitude** **heart or lung diseases** **Anemia** **blood loss**	**Kidney (and liver to small extent) release erythropoietin** **structure: 24% CHO** **67% protein** **half life: 6.7 hours**



Haemoglobin

*The whole slide is from boys

- **It is the protein molecule in red blood cells**
- **It carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues to the lungs**
- **It is a red pigment present in RBCs which gives them their color**
- **280 million molecule/cell**
- **Normal values:**
  male: 13-18 g/dl
  female: 11.5-16.5 g/dl
- **So what's anemia?**
  1 decrease in Hb
  2decrease in number of RBC
  3symptoms: tired, fatigue, shortness of breath
  4- signs: pallor, tachycardia

Obj1:

method used to measure the different hematological values, and compare it with the normal values

~

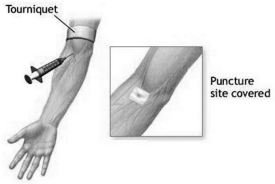
complete blood count (CBC): It is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood.

MEASUREMENT OF BLOOD CELL COUNTS BY USING COULTER ANALYZER or COULTER COUNTER

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

parts of the counter:

1. Capillary
2. Monitor (shows result)
3. Print out



Coulter Counter Principle, Materials, and Methods:

Principle

- 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. (every cell makes 1 pulse(curve) , large cells have large pulses, and small cells have small pulses)
- Number of pulses --> number of particles.
- Height of pulses --> volume of particles.

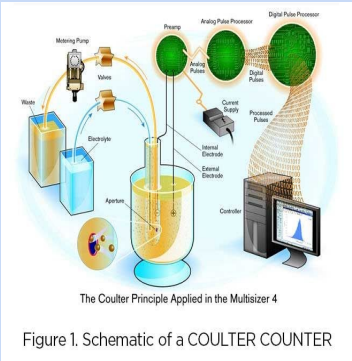
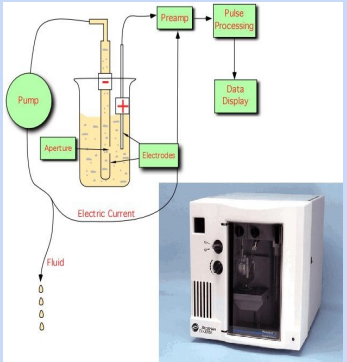


Figure 1. Schematic of a COULTER COUNTER



by the pulse we can know the type of cell that came through

Materials and Methods

- Coulter analyzer
- Diluent reagents
- Lytic reagent
- Calibrator kit
- EDTA anticoagulant blood

*calibrator kit is used to know the efficiency of the counter by having a sample that I know about its measurement

Diluent –Reagent

Is an isotonic electrolyte solution that

*three hoses are connected to the coulter counter

1Dilute the whole blood sample -

2Stabilize cell membrane for accurate counting and size -

3Conduct aperture current -

4Rinse instrument components between analysis -

5Prevent duplicate cell counts -

Lytic reagent Lysis RBCs for WBCs count and hemoglobin measurements

Calibrator Kit Is an alternative to to the whole blood reference method for calibration

RBC, WBC cell count & HB

Clinical terms

- 5 ml of venous blood will be drawn in EDTA anticoagulant tube.
- Diluted by the reagent I and used to count RBC.
- Lysing RBC using reagent II and used for counting WBC and Hb.

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 $x10^3$ /µl	4 – 11 $x10^3$ /µl	4 – 11 $x10^3$ /µl
HB	13-18 g/dl	11.5-16.5 g/dl	13 –18 g/dl
Platelet	150-400$x10^3$ /µl	150-400$x10^3$ /µl	150-400$x10^3$ /µl

clinical \ important terms:

↓ RBC = anemia

reduced the ability of red blood cells to carry oxygen due to either decreased red blood cell count or\and hemoglobin concentration

↑ RBC = polycythemia

increased red blood cell count above normal.

↓ WBC = leucopenia

decreased white blood cell count below the normal.

↑ WBC = leucocytosis

increased white blood cell count above the normal.

↓Platelets=thrombocytopenia

decreased platelets count below the normal.

↑ Platelets = thrombocytosis

increased platelets count above the normal.

PROCEDURE 1:

MEASUREMENT OF BLOOD CELL COUNTS BY USING COULTER ANALYZER

*found in the handout

Aim : we need to 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. Usually the blood is drawn from median Cubital Vein in front of the elbow joint to collect venous sample.

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 the blood from the vein.

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

5- 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.

probe for the aspiration

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.

6automatically and 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 a > the volume of that cell.

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CBC With Differential/Platelet					
WBC	5.7		x10E3/uL	4.0-10.5	01
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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	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01

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

Clinical Applications

Low numbers of RBCs	High numbers of RBCs	High numbers of WBCs (Leukocytosis)	Low numbers of WBCs (leukopenia)	platelet count
-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). causes hemolytic anemia	-Low oxygen tension in the blood – Congenital heart disease – Cor pulmonale – Pulmonary fibrosis -Polycythemia vera. -Dehydration (such as from severe diarrhea).loss of plasma so the count of rbc is more -Renal (kidney) disease with high erythropoietin production.	-Infectious diseases. -Inflammatory disease (such as rheumatoid arthritis or allergy). -Leukemia. -Severe emotional or physical stress. -Tissue damage (burns).	-Bone marrow failure (for example, due to infection, tumor or fibrosis).aplastic anemia -Presence of cytotoxic substance. -Autoimmune/collagen -vascular diseases (such as lupus erythematosus). -Disease of the liver or spleen. -Radiation exposure.	**Thrombocytosis :** - Chronic myeloid leukaemia. **Thrombocytopenia:** - A plastic anemia. - Chemotherapy.

*The whole slide is from girls

PCV=volume of rbc to the plasma (relative ratio)

*PCV is heparinized and CBC has editor

anything that causes RBC to be more causes PCV to become higher

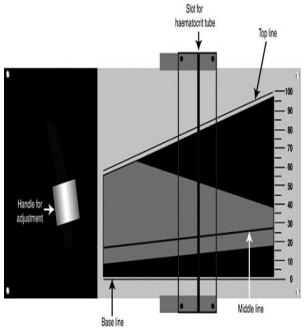
Packed Cell Volume (PCV) Hematocrit

*The whole slide is from girls

Normal value PCV

	Male	Female	Average
PCV %	40-54	35-47	35-54

Hematocrit Reader:



Procedure 2 measurement of hematocrit

- Capillary blood obtained from pricking finger tip after cleaning it with alcohol
- Fill a heparinized capillary tube, then seal one end by plasticine.
- Centrifuge for 5 minutes to packed the cells at one end of the tube leaving a clear plasma on top.
- Use the hematocrit reader to find the packed cell volume.

Clinical Application

High hematocrit may indicate:

Dehydration (Burns, Diarrhea) / Polycythemia Vera/ Low oxygen tension (smoking, congenital heart disease, living at high altitudes)

Low hematocrit may indicate:

Anemia (various types)/Blood loss (hemorrhage)/ bone marrow failure (for example due to radiation, toxins, fibrosis,tumor) / Hemolysis (RBC destruction) related to transfusion reaction/ Leukemia

PROCEDURE 2 :

MEASUREMENT OF HEMATOCRIT OR PACKED CELL VOLUME (PCV)

*found in the handout

AIM : We need to draw blood from capillaries in order to measure Hematocrit (PCV) using microhematocrit reader.

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

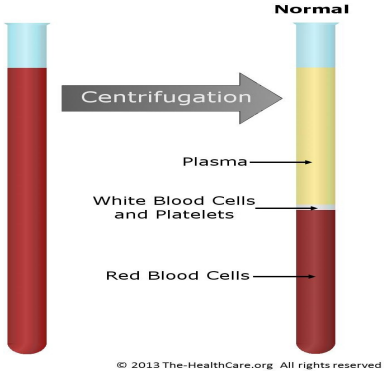
2- Prick the skin using the pen lancet.

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 pronounce pressure is exerted, blood is likely to be diluted with interstitial fluid.

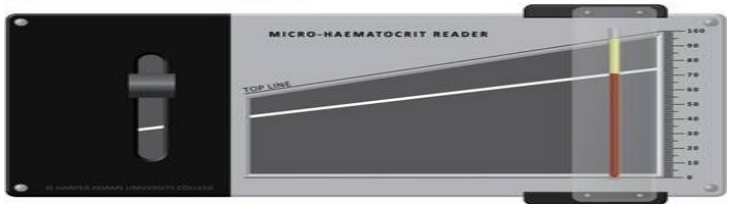
5.Seal one end of the capillary tube with plasticine(should have several capillaries) .

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.



Obj2 .The calculation of Red Blood Indices

(can differ between patient by seeing the calculation)

1.Mean cell volume (MCV):

–The average volume of red blood cell measured by femtoliters(fl).

–MCV = Packed cell volume "PCV" x (10\RBC count) = 85 ± 8 mm^3 (fl)

-MCV of a normal person range from 78-98 fl.

- if MCV is low , it means that RBC are small in size and they are called microcytes.

- but if MCV is high it means that RBC are lange in size and they are called macrocytes.

macrocytic(megaloblastic anemia)caused by b12 or folic acid deficiency

2.Mean cell hemoglobin (MCH):

–The average weight of Hb in red cells measured by picogram (pg)

–MCH = Hb concentration x (10\RBC count) = 29.5 ± 2.5 pg

-MCH of a normal person range from 27-32pg.

-High value of MCH tell us that RBC are hyperchromic.

-Low value of MCH will be seen if RBC are hypochromic.

*Chromic means color

3.Mean cell Hb concentration (MCHC):

–Concentration of Hb per 100 ml of RBC measured in grams \ deciliters (g\dl)

–MCHC = Hb x (100\packed cell volume) = 33 ± 3 g/dl PCV

-MCHC of a normal person ranges from 32-36 g\dl.

-value of MCHC below normal suggests iron deficiency anemia.

Normal values	average
MCV	78-98 µm3
MCH	27-32 pg
MCHC	30-35 g/dl

-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.

CLASSIFICATION \ TYPES OF ANEMIA :

* this part only found in boys slides

1- Hemorrhagic Anemia > caused by loss of blood

Aplastic anemia: less production of RBCs by the bone marrow

2Aplastic Anemia > bone marrow suppression by drugs or radiations etc.

3Nutritional Anemias > deficiency of iron, folic acid, vit B12.

4Hemolytic Anemia > Increased destruction of RBCs such as sickle cell disease.

*found only in girls slides

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 Acid Deficiency

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

1

	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.

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

2

A\ The solution:

"SUBJECT "A	"SUBJECT "B
MCV = 25 x 10 /3.6 = **69.4 fl** **MCH** = 7.2 x 10 / 3.6 = **20 pg** **MCHC** = 7.2 x 100 / 25 = **28.8 g/dl**	**MCV** = 25 x 10 /2.5 = **100 fl** **MCH** = 8 x 10 / 2.5 = **32 pg** **MCHC** = 8 x 100 / 25 = **32 g/dl**

3

B\The solution :

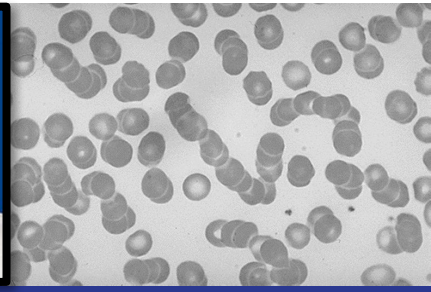
-Subject "A" > Microcytic hypochromic anemia (Iron deficiency anemia).

-Subject "B" > Macrocytic normochromic anemia (Megaloblastic anemia or Pernicious anemia). *Happen when there is a deficiency of b12

Erythrocyte Sedimentation Rate (ESR)

Obj1.To know how to measure the erythrocyte sedimentation rate"ESR".

- Is the rate at which red blood cells sediment in a period of 1 hour.
- measures how far RBCs fall through a column of anticoagulated blood in 1H it is really a length (35mm in the 1st hour) not rate.
- non-specific measure of inflammation.
- 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, thus increasing ESR.
- **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.
- Materials & Methods\Equipment:
  - Westergren's sedimentation apparatus.
  - Anticoagulant (EDTA TUBE).
  - Disposable sterile syringes and needles.



PROCEDURE 3 :

1. Using a sterile syringe, draw 1.6 ml of blood from a suitable vein
2. Transfer the blood to a test tube containing EDTA to prevent clotting.
3. Fill the Westergren's tube with blood up to the zero mark.
4. Place the tube upright in the stand and leave like this for one hour.
5. Note down the depth of the column of clear plasma at the top of red blood cells in the tube after one hour and again at the end of the 2nd hour. These will be your E.S.R. readings.

ESR Results:

- Normal ESR in male = 3.5 mm/ 1st hour and 7-15mm/2nd hour.
- Normally the value of E.S.R. ranges below 20 mm in the first hour.
- In females values are slightly higher (due to less number of RBC).
- conditions are associated with lower than normal ESR: Polycythaemia, Microcytosis and Sickle-cell anaemia.
- Moderately elevated ESR occurs\ conditions are associated with an increased ESR : Macrocytosis, Connective tissue disorders,inflammatory disorders eg. infections, inflammation, anemia, malignancies, pregnancy, and old age.
- A very high ESR associated with multiple myeloma, polymyalgia Rheumatica, temporal arteritis.

*ESR is not a diagnostic test but it used for prognosis

"normally the value or ESR ranges from 0mm to 7mm and it is slightly higher in females than males due to less number or RBC"

Rouleaux formation:

"Question and problem"

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.?

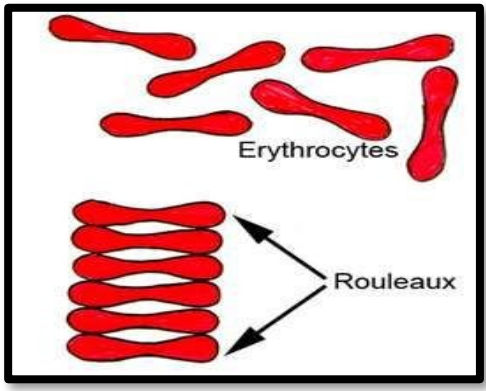
Rouleaux formation becomes rapid when plasma protein concentration is high and because of this E.S.R. also becomes increased.

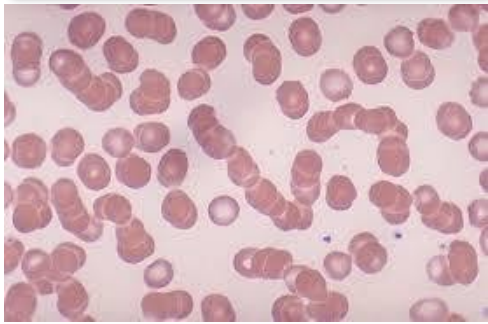
3-What is the clinical significance of E.S.R.?

·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





Clinical application of ESR

Obj2.To recognize what is the clinical value of these measurements(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 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.

*found only in girls slides

GOOD LUCK !

Team Members:
Rahaf Althnayan
Mashael Alkahtani
Dana Alrasheed

Team Leaders:
Reem ALQarni
Tareq ALOmaim

References :
1- Girls' & Boys' slides
2- Teamwork 436
3- The Handout