- ***All bloods could be contaminated***
- ***You should report all the results you gain in the lab***

First session

Packed cell volume (PCV) or hematocrit (hct)

Definition: volume percent of blood cells to the total volume of blood by centrifugation

Normal values:

Men: 40- 52%

Women: 37-47%

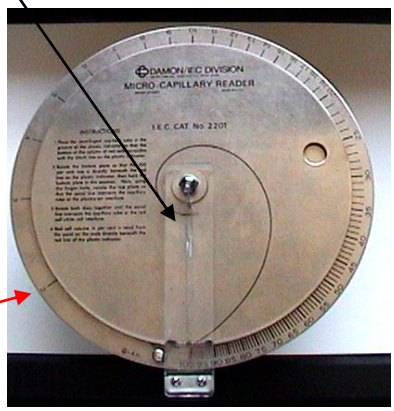
Newborn: 50- 62%

Materials;

heparinized micropipette marked by a red ring

Special wax

Microcentrifuge



Micro-hematocrit reader

Lancet, cotton & alcohol

Procedure: by micro-hematocrit

Fill at least 2/3 of the micropipette with blood from tip of your finger or of venous blood already prepared.

Use the wax to block the end of the micropipette

Put the micropipette in micro-centrifuge with the wax outside

Keep the micro-centrifuge on for 5 minutes

Read the hct by micro-hematocrit reader

Questions:

1. *Name some normal(physiological) variations of hct*
2. *Why hct of venous blood is more than arterial blood?*

❖ ***Put all disposable sharp instruments in the safety box***

Erythrocyte Sedimentation Rate (ESR)

Definition*:* rate of precipitation of red blood cells in non-coagulated blood per first, second or 24 hour (mm/hr)

Factors affecting ESR:

Shape and size of erythrocytes

Number of erythrocytes

Zeta potential

Normal values:

Men: up to 5 mm/1st hr and up to 15 mm/2thhr

Women: up to 10 mm/1st hr and up to 15 mm/2thhr

Materials:

Westergren pipette and holder

Sodium citrate 3.8%

Special rubber

Procedure: by westergren method

Add 1.6 ml blood to 0.4 ml Sodium citrate 3.8%

Drain the blood up to zero using the rubber (never use your mouth for draining the pipette)

Put the pipette in the holder properly

Read the amount of serum over the precipitated blood cells after one hour

Questions:

1. *What are the effects of plasma proteins on zeta potential and ESR?*
2. *How are the changes of ESR in newborn and pregnancy?*
3. *In which pathologic conditions does ESR rise?*
4. *In which pathologic conditions does ESR decrease?*

Blood grouping

To detect blood group in **ABO** and **Rh** systems

It is based on the agglutination of red blood cells under the reaction of **agglutinogen** present on the surface of the cells and **agglutinin** (The antibody which is against these antigens)

Blood group	Agglutinogen on RBC	Agglutinin in plasma	Prevalence %
A	A	Anti B	32
B	B	Anti A	23
AB	A & B	---	8
O	---	Anti A & anti B	38

Some racial differences of blood group and Rh systems:

Racial group	Blood groups				
	O	A	B	AB	Rhesus^{--}
Most Europeans	43	44	9	4	17
UK(south)	44	45	8	3	17
Scots	52	34	11	3	20
Basques	52	45	2	1	29
Lapps	20	59	16	5	2
Africans(Nigeria)	52	24	21	3	5
Indians	31	21	40	8	7
Vietnamese	42	22	30	6	0
Indonesians	40	27	26	7	0
Chinese	44	24	26	6	1
Japanese	32	36	23	9	0
Australian	70	20	9	1	0
Native Americans	77	16	6	1	0
Eskimos	36	55	5	4	0

*never confuse ***agglutination*** with ***precipitation***

Questions:

1. *"Blood group O is the general donor." Is it a correct sentence? Why?*
2. *What are the differences between agglutination and precipitation?*

2nd session

Hemoglobinometry

Definition*:* Amount of hemoglobin in 100ml of blood

Factors affecting:

Plasma volume

Volume of erythrocytes

Altitude and oxygen pressure

Iron content of the body

Normal values:

Men: 13-16 gr/dl

Women: 12-15 gr/dl

Materials:

1. sampler 10 lambda (10µl)
2. helligee apparatus

3. acid chlorhydric 0.1 normal

Procedure: by Sahli method; this is the simplest way to assay the hemoglobin in the field. It is based on the conversion of hemoglobin to hematin acid by Hcl and comparison of its color with the color of standard tubes.

1. Pick up 10µl of blood using the sampler (charge the sampler as the figure).
2. Repeat the first step.
3. Pour it into the test tube and add 5 drops of Hcl and mix them well.

4. Add some water to get the same color as that of the standard tubes.
5. More hemoglobin in the blood needs more water to have the same color.
6. Total time should not exceed 5 minutes from the first.
7. Read the concentration of hemoglobin on the test tube.

How start to pick up the solution

How to release the solution

Questions:

1. *What is the effect of living in high altitude on hemoglobin concentration?*
2. *Explain the physiological changes of hemoglobin in pregnancy and infancy.*

White blood cell count (Wbc)

Definition*:* number of all leukocytes in cubic millimeter

Factors affecting:

Different conditions of activity of the immune system

Normal values:

Men & women: 4000-11000/mm^3

Newborn: 18000-20000/mm^3

Materials:

Hemocytometer (Neubauer) slide

Sampler 100 lambda

Acetic acid 2%+ violet (diluting solution).

Procedure:

Add 100µl blood to 1.9ml diluting solution (1/20 dilution) and mix them well for 2 minutes.

Pour some of the mixed solution between the slide and lamella using a micropipette.

Count the nucleated cells (Wbc) in the specific squares in the corners.

Each square's volume equals: $1\times1\times0.1=0.1mm^3$.

Total volume of four squares equals: $4\times0.1= 0.4\ mm^3$.

Thus coefficient correction number is ***50*** because: $0.4\times1/20= 1/50$.

Questions:

1. *What are the physiological conditions that change the number of white blood cells?*
2. *Why do we use Acetic acid and violet for WBC count solution?*

3rd session

Red blood cell count (Rbc)

Definition*:* number of red blood cells in cubic millimeter.

Factors affecting:

Total body water

Altitude

Hematopoietic factors

Normal values:

Men: 4.5-5.5×10^6/mm^3

Women: 4-5 ×10^6/mm^3

Newborn: 5-6.5 ×10^6/mm^3

Materials:

Hemocytometer (Neubauer) slide

Sampler 10 lambda

Normal saline (diluting solution)

Procedure:

Add 10µl blood to 1.9ml diluting solution (1/200 dilution) and mix them well for 2 minutes.

Pour the mixed solution in the space between slide and lamella using a micropipette.

Count the cells (Rbc) in the specific squares in the center.

Each square's volume equals: 0.2×0.2×0.1=0.004mm^3.

Total volume of four squares equals: 5×0.004= 0.02mm^3.

Thus coefficient correction number is ***10000*** because: 0.02×1/200= 1/10000.

Questions:

1. *Name some physiological changes in Rbc count.*
2. *Describe the effects of hematopoietic vitamins.*

Blood indices

MCV (Mean Corpuscular Volume)

Tells you how volume each erythrocyte has

$$MCV = \frac{Hematocrit \times 10}{Number\ of\ RBCs\ in 1 mm^3}$$

Normal values:

Men and women: 74-95μ^3 (famtolitre)

MCH (Mean Corpuscular hemoglobin)

$$MCH = \frac{Hemoglobin\ (gr/dl)}{Number\ Of\ RBCs\ In\ 1 mm^3}$$

Normal values:

Men and women: 27-32Pgr (picogram)

MCHC (Mean Corpuscular hemoglobin concentration)

$$MCHC = \frac{Hemoglobin \times 100}{Hematocrit}$$

Normal values:

Men and women: 30-36%(gr/dl)

Blood pressure (BP)

Definition*:*

Indirect measurement of blood pressure by 2 methods:

1. Palpatory method
2. Auscultatory method

Materials:

Sphygmomanometer

Stethoscope

Factors affecting:

Blood volume

Blood viscosity and density

Peripheral resistance and vascular status

Position

Contraction power of the left ventricle

Elasticity of aorta and large arteries

Heart rate

Normal values:

Men: and women: 120/80 mmHg

Newborn: 80/50 mmHg

Procedure:

Palpatory method:

The patient (subject) should stay at the same position at least for 5 minutes.

His/her arm should be at the same level of the heart.

Palpate the radial pulse at the wrist by your three mid fingers.

Wrap the cuff around the arm in the correct position (figure).

Inflate the bag to 180mmHg.

Open the valve to release the air by the maximum speed of 2-3mmHg per second.

The pressure at which you feel the first pulse is called *systolic pressure*.

Auscultatory method:

The patient (subject) should stay at the same position at least for 5 minutes.

His/her arm should be at the same level of the heart.

Wrap the cuff around the arm in the correct position (figure).

Put the head of the stethoscope between the cuff and arm where the brachial artery lies.

Inflate the bag to 180mmHg.

Open the valve to release the air by maximum speed of 2-3mmHg per second.

The pressure at which you hear the first tapping sounds is called *systolic pressure.*

As inflation pressure continues to fall the sounds become louder.

As the inflation pressure approaches the diastolic level, the sounds gradually become muffled.

And when the sounds disappear it is called *diastolic pressure.*

Blood pressure in different positions***:***

Measure the blood pressure in different positions: supine, sitting and standing.

Measure the blood pressure in supine position then measure it in standing position at 0 , 2 , 4 and 6 minutes after standing.

Draw the diagram of alterations of the blood pressure (systole and diastole) during this period (supine and standing positions).

Questions:

1. *Explain about Korotkoff sounds*
2. *What is the most important cause of orthostatic hypotension? How could it be examined?*
3. Calculate the *pulse pressure*. How important is it?
4. Calculate the *mean arterial pressure*. How important is it?

Heart sounds

Four heart sounds and four heart valve sounds can be heard at the surface of the chest with the aid of a stethoscope.

Look at the figure to find the proper site of the each valve at the surface of the chest

Materials:

Stethoscope

Hyperemia

Definition:

Hyperemia means increasing the volume content of an organ; it might be *direct* or *indirect*

Materials:

Sphygmomanometer

Procedure:

Stage 1:

Wrap the cuff around the arm

Raise the hand for 3 minutes

Inflate the cuff to 180mmHg and try to stabilize the pressure

Put the hand on the desk and leave it for 5 minutes

Release the air suddenly and completely

Look at the changes in the hand and compare it with the other hand.

Stage 2:

Wrap the cuff around the arm

Inflate the cuff to 70mmHg and try to stabilize the pressure

Put the hand on the desk and leave it for 5 minutes

Release the air suddenly and completely

Look at the changes in the hand and compare it with the other hand.

Stage 3:

Wrap two cuffs around both arms

Raise one of the hands for 3 minutes

Inflate the cuff of the raised hand to 180mmHg and try to stabilize the pressure

Put the hand on the desk and leave it for 5 minutes

Inflate both cuffs to 70mmHg and try to fix the pressure

Look at the changes in hands and compare them with each other.

Release the air from both cuffs after 3 minutes maximally.

Questions:

1. *What is the reactive hyperemia?*
2. *In which stages you can see a reactive hyperemia?*
3. *Discuss about the reasons of hyperemia in the first stage.*

4th session

Differential count of white blood cells

	Cell type	*Diameter μm*	*Nucleus character*	*Cytoplasmic granules*	*Percent*
Granulocytes	Neutrophil	10-14	2-5 segmented nuclei connected by fine strands of chromatin	Very fine granules	50-70
	Eosinophil	10-15	Mostly 2 segmented and dumbbell-like shape	Large orange granules	1-4
	Basophil	10-15	2-3 segmented nuclei	Large black granules cover the nuclei	0-1
Agranulocytes	Monocyte	15-20	Large loose kidney-like nucleus	Pale blue	3-8
	Lymphocyte	7-9	Dark blue and fill most of the cell	Scanty blue cytoplasm	25-40

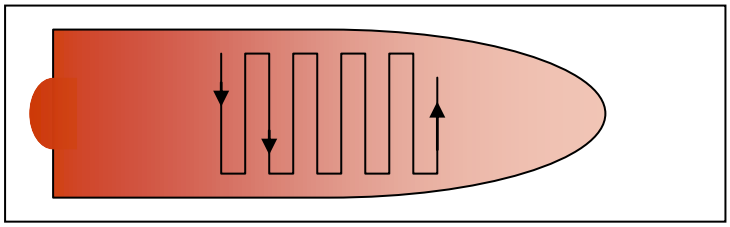
Procedure:

Use a drop of emersion oil on the slide

Select the subject lens on the power of 100

Find a proper field (somewhere with a layer of Rbcs)

Follow the following direction to count white blood cells with total number of 100



Questions:

1. *What is the neutrophilia?*
2. *what are the functions of Eosinophil and basophil?*

5th session

Homeostasis

Bleeding time:

Method of Duck:

Materials:

Lancet

Hydrophilic paper

Chronometer

Procedure:

First wash the lobule of the subject's ear with alcoholic cotton.

Use a lancet to make a standard incision in the lobule of the subject's ear and start the chronometer.

Use the hydrophilic paper to dry the pouring blood just below the incision

Continue the procedure to complete dryness

Count the numbers of the blood stains on the paper and subdivide it to 2

Clothing time:

Materials:

2 slides one as control and the other test

Lancet

A watch glass

A piece of wet cotton to prevent dryness of the blood

Chronometer

Procedure:

Pour a drop of blood on each two slides

Put a piece of wet cotton on the control slide and cover the slide with a watch glass

Start the chronometer

Use the tip of a lancet on the blood drop to see the first fibrin strand which is white

Stop the chronometer when you see the first strand of fibrin

To confirm your result repeat it with the control slide.

Effects of physical and chemical factors on coagulation time

Add 0.5 ml blood to the following tubes

1. Dry tube
2. Tube with a piece of cotton
3. Tube with a wooden bar
4. Tube impregnated with paraffin
5. Tube impregnated with paraffin in ice
6. Tube contained heparin
7. Tube contained thromboplastin

Compare the time of complete clothing in each tube and discuss about it

Prothrombin time (PT)

Definition:

The time needs to conversion of prothrombin to thrombin

Materials:

Potassium oxalate 0.1M

Centrifuge

Water bath 37^{o} C

Thromboplastin + calcium

Sampler 100µl

Wooden bar

Chronometer

Procedure:

Add 4.5 ml blood to 0.5 ml potassium oxalate 0.1M in a tube and mix them gently to decalcificate the plasma

Put the tube in centrifuge in 3000g for 5 minutes

Separate the decalcified plasma

Put a tube consists of 0.1ml (100µl) decalcified plasma as the test tube in water bath for 3 minutes

Put a tube consists of thromboplastin + calcium in water bath for 3 minutes

Add 200µl thromboplastin + calcium to the test tube and set the time

After 8 seconds look for the fibrin network using a wooden bar

Record time when you see the first strand of fibrin

Normal values:

11- 18 seconds

Questions:

1. *Which pathway of homeostasis could be assessed by Prothrombin time?*
2. *Blood contents of which one has no effect on PT: factor XIII, calcium, Prothrombin, factor X*

فیزیولوژی عملی، علی نقی‌نژاد. انتشارات دانشگاه علوم پزشکی تبریز