جامعة تكريت

كلية طب الاسنان

مادة الفسلجة عملي

المرحلة الثانية

م.مادلين قاسم عباس & م.م. نغم حسن علي

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Lec. 1
2nd class

Blood Specimen Collection and Processing

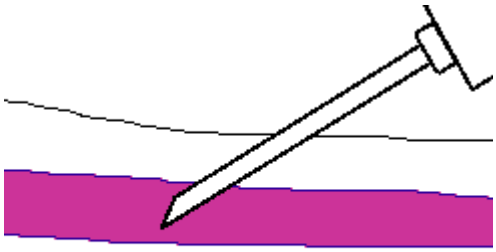
The first step in acquiring a quality lab test result for any patient is the specimen collection procedure. The venipuncture procedure is complex, requiring both knowledge and skill to perform. Several essential steps are required for every successful collection procedure:

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, beginning at the site and working outward. Allow the area to air dry. After the area is cleansed, it should not be touched or palpated again. If you find it necessary to reevaluate the site by palpation, the area needs to be re-cleansed before the venipuncture is performed.
10. Ask the patient to make a fist; avoid “pumping the fist.” Grasp the patient’s arm firmly using your thumb to draw the skin taut and

anchor the vein. Swiftly insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Avoid excess probing.

11.



12. When the last tube is filling, remove the tourniquet.
13. Remove the needle from the patient's arm using a swift backward motion.
14. Place gauze immediately on the puncture site. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
15. Dispose of contaminated materials/supplies in designated containers.

Note: The larger median cubital and cephalic veins are the usual choice, but the basilic vein on the dorsum of the arm or dorsal hand veins are also acceptable. Foot veins are a last resort because of the higher probability of complications.

Lec 2

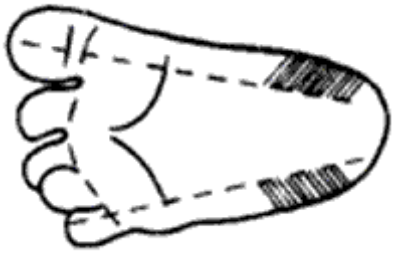
Finger stick Procedure:

1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers of the non-dominant hand. Do not use the tip of the finger or the center of the finger. Avoid the side of the finger where there is less soft tissue, where vessels and nerves are located, and where the bone is closer to the surface. The 2nd (index) finger tends to have thicker, callused skin. The fifth finger tends to have less soft tissue overlying the bone. Avoid puncturing a finger that is cold or cyanotic, swollen, scarred, or covered with a rash.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.
4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.

7. Collect drops of blood into the collection tube/device by gentle pressure on the finger. Avoid excessive pressure or “milking” that may squeeze tissue fluid into the drop of blood.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Heelstick Procedure (infants):

The recommended location for blood collection on a newborn baby or infant is the heel. The diagram below indicates the proper area to use for heel punctures for blood collection.

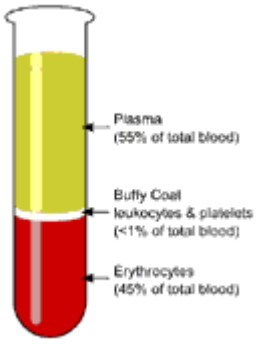


1. Prewarming the infant's heel (42° C for 3 to 5 minutes) is important to increase the flow of blood for collection.
2. Wash your hands, and put gloves on. Clean the site to be punctured with an alcohol sponge. Dry the cleaned area with a dry gauze pad.
3. Hold the baby's foot firmly to avoid sudden movement.
4. Using a sterile blood safety lancet, puncture the side of the heel in the appropriate regions shown above. Make the cut across the heel print lines so that a drop of blood can well up and not run down along the lines.
5. Wipe away the first drop of blood with a piece of clean, dry cotton gauze. Since newborns do not often bleed immediately, use gentle pressure to produce a rounded drop of blood. Do not use excessive pressure because the blood may become diluted with tissue fluid.
6. Fill the required microtainer(s) as needed.
7. When finished, elevate the heel, place a piece of clean, dry cotton on the puncture site, and hold it in place until the bleeding has stopped. Apply tape or Band-Aid to area if needed.
8. Be sure to dispose of the lancet in the appropriate sharps container. Dispose of contaminated materials in appropriate waste receptacles.
9. Remove your gloves and wash your hands.

Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes and labeling the sample.

Blood Sample Centrifugation – It is recommended that serum be physically separated from contact with cells as soon as possible, with a maximum time limit of 2 hours from the time of collection.

- Complete gel barrier formation (gel barrier tubes) is time, temperature and G-force dependent. The uniformity of the barrier is time dependent; an incomplete barrier could result from shortened centrifugation times.



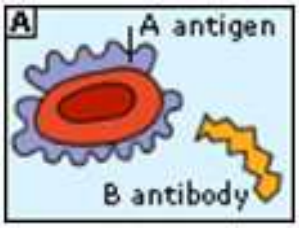
- In general, for a horizontal, swing-bucket centrifuge, the recommended spin time is 10 minutes. For a fixed-angle centrifuge, the recommended spin time is 15 minutes.
- NOTE: Gel flow may be impeded if chilled before or after centrifugation.
- Tubes should remain closed at all times during the centrifugation process.
- Place the closed tubes in the centrifuge as a “balanced load” noting the following:
    - Opposing tube holders must be identical and contain the same cushion or none at all.
    - Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
    - If an odd number of samples is to be spun, fill a tube with water to match the weight of the unpaired sample and place it across from this sample.

Lec 3

ABO blood grouping system:

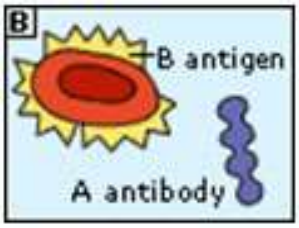
According to the ABO blood group system there are four different kinds of blood groups: **A, B, AB and O** (null).

Blood group A



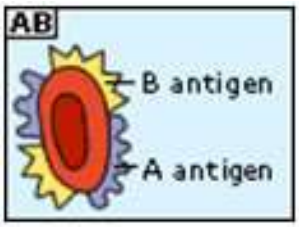
Blood group A: If you belong to the blood group A, you have A antigen on the surface of your red blood cells and B antibodies in your blood plasma

Blood group B

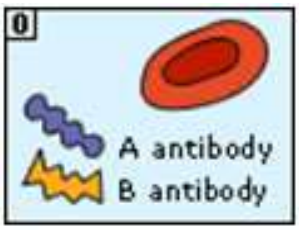


Blood group B: If you belong to the blood group B, you have B antigen on the surface of your red blood cells and A antibodies in your blood plasma

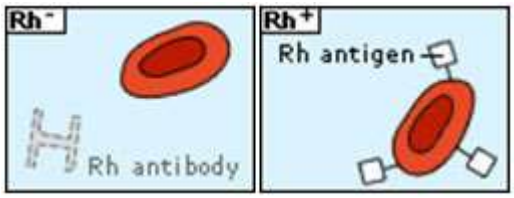
Blood group AB



Blood group O



Rh factor



Rh (Rhesus) factor is found on the RBC's surface in most people. Like A and B, this is also an antigen and those who have it are called Rh+. Those who lack the antigen on the surface of RBCs are called Rh-. A person with Rh- blood does not have Rh antibodies naturally in the blood plasma. But a person with Rh- blood can develop Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can trigger the production of Rh antibodies (as the immune system is triggered by the presence of an unknown antigen in the system). A person with Rh+ blood can receive blood from a person with Rh- blood without any problems.

Principle behind blood tests: *Blood clumping or Agglutination observation.*

Compatibility between the blood groups of donor and recipient determines the success of a blood transfusion. The AB0 and Rh blood groups are looked at while conducting the test. In a diagnostic lab, Monoclonal antibodies are available for A, B and Rh antigen. Monoclonal antibody against Antigen A (also called Anti-A), comes in a small bottles with droppers; the monoclonal suspension being BLUE in colour. Anti-B comes in YELLOW colour. Anti-D (monoclonal antibody against Rh) is colourless. All the colour codes are universal standards. When the monoclonal antibodies are added one by one to wells that contain the test sample (blood from patient), if the RBCs in that particular sample carry the corresponding Antigen, clumps can be observed in the corresponding wells. A drop of blood is left without adding any of the antibodies; it is used as a control in the experiment. The monoclonal antibody bottles should be stored in a refrigerator. It is recommended to

tilt the bottle a couple of times before use in order to resuspend the antibodies that have settled at the bottom of the bottle.

Materials Required:

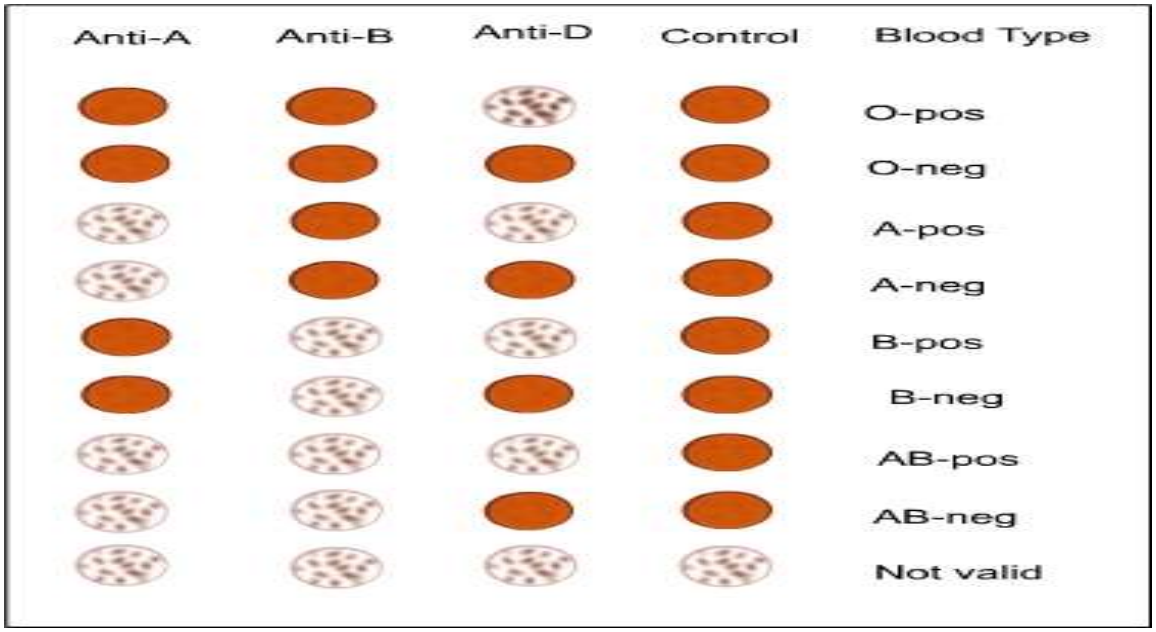
- Monoclonal Antibodies (Anti-A, B and D)
- Blood Lancet
- Alcohol swabs
- Tooth picks
- Sterile cotton balls
- Clean glass slide
- Ice tray
- Biohazard disposal container

Procedure:

1. Set the table with all the materials required. Remember to place the Monoclonal Antibody (Mab) kit in an Ice tray.
2. Open an Alcohol swab, and rub it at the area from where the blood will be sampled (finger tip). (Discard the swab)
3. Open the Lancet cover, put pressure at the tip of the finger from where blood will be sampled (maintain it). Prick the finger tip with the opened Lancet.(Discard the Lancet)
4. As blood starts oozing out, make 1 drop fall on the three depressions of the glass slide. (in clinical setup, there will be a fourth well used as a control).
5. Place a cotton ball at the site where it was pricked. Using the thumb, put pressure on the area to stop blood flow.
6. Take the Anti-A (blue) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 1st spot. Place the bottle back in ice.
7. Take the Anti-B (yellow) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 2nd spot. Place the bottle back in ice.
8. Take the Anti-D (colorless) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 3rd spot. Place the bottle back in ice.

9. Take a tooth pick and mix the content in each well. Discard the tooth pick after using in one well (take a new one for the next well).
10. After mixing, wait for a while to observe the result.

Result:



Lec4

Hematocrit or Packed cell volume (PCV)

Principle

Hematocrit is the ratio of the total volume of RBC's to that of whole blood expressed as percentage(%) (whole blood = total volume of cells + plasma). The second synonym for hematocrit is PCV (Packed Cell Volume). The procedure is easy to perform, whole blood is centrifuged in a narrow tube (capillary tube,) cellular elements will be separated from the plasma, after centrifugation blood will be separated into 3 layers : (1) Bottom layer contains packed RBC's, (2) Middle layer contains WBC's and Platelets (on top of RBC's), (3) Upper plasma layer.

The hematocrit value is determined by comparing the volume of RBC's to the total volume of the whole blood sample, it is usually reported as a %.

Apparatus and Materials

1 -Microhematocrit centrifuge.
2 -Modeling clay (seal material)
3 -Capillary tubes (7 cm long, 1mm diameter(
4 -Hematocrit measuring device reader or a conventional ruler.

Procedure:

1. Fill the capillary tube with blood by capillary attraction. Either from free flowing finger punctured
2. by a sterile lancet/ or from a well mixed anticoagulated whole venous blood (this requires only few microliters of blood. Seal with the modeling clay the empty end of the capillary tube.
3. Place and position the capillary tube in the radial grooves of the microhematocrit centrifuge with the sealed end away from the center pointed toward the outside.
4. Centrifuge for 5 minutes so that additional centrifugation does not pack the red blood cells more.
5. The height of the RBC column, and the total column should be measured with the aid of a ruler in cm and mm, simply with the aid of a special HCT reader device read the result.
6. Express the results in percentage (%).

Normal values : -
Males : 40 - 53%
Females : 37 - 47%
Newborns: 51 - 60%
Children : 34 - 49%

Higher values than the normal range is called **polycythemia**.

Lower values than the normal range is called **anemia**.

Clinical Significance:

HCT is used to detect anemia's, polycythemias, hemodilution, hemoconcentration, and also is used in the laboratory to calculate the MCV, and the MCHC manually.

Lec.5

Hemoglobin (Hb) estimation

Introduction and principle :

Haemoglobin is composed of haem (organic iron) and globin protein, by adding HCI, Hb is converted in to colored acid hematine (Sahli method) and the intensity of the color is measured by comparing it with the standard, this could be done visually or spectrophotometrically.

Concentration of Hb is measured either as a percentage or better in absolute figure (gm/dl)

Normal value

- Male : 13.5 – 17.5 gm/dl

- Female : 11.5 – 15.5 gm/dl

Materials and method

Sahli haemometer consists of

1- Color matching standard chamber.

2- Pipette marked to contain 20 microliter of blood.

3- Graduated glass tube.

4- Glass starrier

5- Distilled water (D.W.).

6- 0.1 N HCI solution .

Procedure

- Put in the graduated glass tube 2 ml of 0.1 N (HCI) .

- Draw blood by hemoglobin pipette to mark (20) µl.

- Dip the tip of the pipette in the graduated tube to blow the blood into the tube, mix content with stirrer.

- Place the tube in the hemoglobinmeter for (3) minutes for complete reaction.

- Add D.W. drop by drop until the color in the graduated tube is identical to the color of the standard.

- Read the result in gm/dl.

Medical consideration:

Increased Hb : polycythemia

Decreased Hb : anemia

Hb the major function of it is

1- Carrying oxygen to the tissues to the lungs.

2- Transport carbon dioxide from the tissues to the lungs.

3- Buffer against change in (H+).

Lec. 6

Anemia & Blood indices

Red Blood Cell Indices

Red blood indices are calculated parameters which determine red blood cell size, hemoglobin content of red cell, and hemoglobin concentration of red cells. These parameters are useful in classifying anemia's into microcytic, normocytic, or macrocytic; and hypochromic or normochromic or hyperchromic. These parameters are calculated from total red cell count, hematocrit and hemoglobin.

1. **MCV Mean Corpuscular Volume**, is the average volume of red cell. This parameter is useful in classifying anemia's into: **Microcytic**, **normocytic**, and **macrocytic.**

MCV is calculated from the hematocrit (HCT), and the Red Blood Cells Count (RBC count)

$$MCV = \frac{HCT}{RBC} \times 10$$

The results of MCV are expressed in femtoliters (fl).

MCV Normal Range: 80 – 96 fl

¨If results are less than 80 fl, the red cells are said to be **Microcytic**

¨If results are within 80-96 fl, the red cells are said to be **Normocytic**

¨If results are higher than 96 fl, the red cells are said to be **Macrocytic**

2. MCH (Mean Cell Hemoglobin) the average weight of hemoglobin per RBC. It is calculated from the hemoglobin concentration (Hb), and the total RBC count.

$$MCH = \frac{Hb\ g/dl}{RBC} \times 10$$

Results of MCH are expressed in picograms (pg).

MCH Normal Range: 27 – 32 pg

• Macrocytic red cells have higher MCH, because they are larger and contain more hemoglobin.

•Microcytic red cells have lower MCH, because they are smaller and contain less hemoglobin.

3. **MCHC Mean Cell Hemoglobin Concentration**: is the average hemoglobin concentration in 100 cc red blood cells. It indicates the average weight of hemoglobin as compared to the cell size. It correlates with the degree of hemoglobinization of the red cells on the peripheral blood film. MCHC is calculated from the hematocrit and hemoglobin.

$$MCHC = \frac{\text{Hb g/dl}}{\text{HCT}} \times 100$$

OR

$$MCHC = \frac{\text{MCH in picograms}}{\text{MCV in femtoliters}}$$

- Results of MCHC are expressed in percentage (%) or gm/dl.

Normal Range: 32 – 36 g/dl (%)

□ If results are within this range, it is said that red cells are **Normochromic.**

□ If results are less than normal, red cells are said to be **Hypochromic** .

□ If results are more than normal range , red cells are said to be **Hyperchromic .**

Anemia A reduction in red cell mass and O2-carrying capacity .It is expressed in terms of reduction in the concentration of Hb (or RBC or HCT%)

The symptoms of anemia which varies among individuals according to severity, rapidity of the developing anemia, age, and presence of other health problems includes:

- Feeling of tiredness.
- Breathlessness.
- Angina (chest pain(
- Headache.
- Dizziness
- Pale skin and gums
- Irregular heart beat
- Loss of appetite

Classification of anemia according to:

Cause

1. Blood loss (bleeding) as in trauma, menstruation.
2. Decreased RBC production
3. Increased RBC destruction

Morphology of RBCs (according to MCV)

A-Microcytic anemias:

- **Thalassaemia**.: inherited impaired hemoglobin production, in which there is partial or complete failure to synthesize a specific type of globin chain.

 Iron deficiency anemia: due to iron loss (e.g. bleeding) or inadequate intake (diet deficiency) or malabsorption.

 Sideroblastic anemia: due to defect in production of hemoglobin (defect in protoporphyrn of haem)

B-Normocytic anaemias:

 Acute post –haemorrhagic anaemia.

 Hemolytic anemia

a) **Hereditary spherocytosis** : deficiency of spectrin (red cell membrane protein) leading to abnormal RBC shape (spherical instead of biconcave)

b) **Glucose 6 phosphate dehydrogenase (G6PD) deficiency**.

c) **Sickle cell anemia**: alteration in amimo acid sequence of polypeptide chains of the globin in hemoglobin Results in defective hemoglobin synthesis, produces sickle shaped red blood cells.

 Aplastic anemia: basic structure of the marrow becomes abnormal. This occurs due to exposure to drugs (anti-cancer), radiation, chemicals.

 Anemia of chronic disease: (tumors, chronic infections such as malaria).

C- Macrocytic anemias:

 Megaloblastic anemia: due to deficiency of folate (folic acid).

 Pernicious anemia due to deficiency in vitamin B12: Inability to absorb vitamin B12 , B12 attaches itself to intrinsic factor in order to be absorbed .

Lec 7

Hemostasis

Bleeding time & Clotting time

Hemostasis means prevention of blood loss. Whenever a vessel is severed or ruptured, hemostasis is achieved by several mechanisms:

(1) Vascular constriction (vasoconstriction)

(2) Formation of a platelet plug.

(3) Formation of a fibrin blood clot as a result of coagulation cascade.

Hemorrhage or blood loss can result from trauma, vascular defects (e.g., esophageal avarices, peptic ulcer), platelet abnormalities, or deficiencies of one or more of the plasma coagulation factors.

Thrombosis is a process in which a platelet aggregate and/or a fibrin clot forms in the lumen of an intact blood vessel or in a chamber of the heart.

Bleeding time is the time taken for a standardized skin wound to stop bleeding. It is a crude qualitative test of hemostasis. It indicates one or more combination of the following:

1. Reduced platelet number (thrombocytopenia).(normal:150,000-300,000)

2. Defected platelets function to interact with blood vessel walls to form blood clots after the wound.

3. Defected blood vessel wall to interact with the platelet.

The bleeding time test is usually used on patients who have a history of prolonged bleeding after cuts, or who have a family history of bleeding disorders. Also, the bleeding time test is sometimes performed as part of preoperative investigation to determine a patients bleeding response during and after surgery. However, in patients with no history of bleeding problems, or who are not taking anti-inflammatory drugs (e.g. aspirin), the bleeding time test is not usually necessary.

Duke method for bleeding time:

Introduction and principle: a standardized puncture (5 mm long × 1 mm deep) of the ear lobe or at the lateral aspect of the volar surface of the forearm is made and the time needed for the bleeding to stop is recorded.

The disadvantage of dukes forearm method is that the pressure on the blood veins in the stab area is not constant and the results are less reliable.

Material & instrument: lancet, stop watch, circular filter paper, alcohol, sphygmomanometer.

Procedure:

- **Ear lobe method**: the ear lobe is cleaned with alcohol and is allowed to dry (alcohol should be left on the skin long enough to kill bacteria).

- **Forearm method**: select a site free of veins, bruises, edematous areas, and scars and is approximately 5 cm below the antecubital crease. Place the sphygmomanometer around the patient's arm approximately 5 cm above elbow and maintain blood pressure at 40 mm Hg.

- A standardized puncture is made using a lancet or (scalpel in case of forearm).

- Time recording is started at the time of puncture.

- Using a circular filter paper the blotted every 30 sec. without allowing the filter paper to touch the wound.

- Sto the watch as the bleeding ceases and this is the bleeding time (bleedinpg stop not due to clothing but due to spasm of the capillaries and formation of platelet plug).

Normal range: (2-6 min. if use the ear lobe)

(2-9 min. if use the forearm)

Prolonged bleeding time may be due to:

1. Decreased platelets number (thrombocytopenia) (<100,000 platelet/uL).

2. Defected platelets function (thrombosthenia) : acquired (aspirin use) and inherited (Von-willebrand's disease)

3. Defected blood vessels (e.g. vitamin C deficiency).

Coagulation time or whole blood clotting time : the time needed for a measured amount of blood to clot under special condition.

Capillary tube method

Material and instrument

Capillary tube (non-heparinized), Lancet , Cotton , Alcohol, Stop watch

Procedure:

Clean the finger by the cotton and alcohol then prick the finger and note the time using a stop watch then fill a non-heparinized capillary tube (blue colored end) to at least 1/2 full.

After 30 sec. take the filled capillary tube between your thumb and forefingers and gently break it in half. Slowly pull the ends apart to view the insoluble fibrin threads. If you don't see any fibrin, wait a little longer and break the tube again every 30 sec. once the clot is formed we record the time.

Normal clotting time: 2- 6 min.

Prolonged clotting time may be due to:

1- Decreased clotting factor VIII (Hemophilia A) or clotting factor IX (Hemophilia B) Hemophilia occurs only in men.

2- Liver failure (clotting factors produced in liver)

3- Vitamin K deficiency (clotting factors production is vitamin K dependent)

Lec.8

RED BLOOD CELL (RBC's) COUNT

Blood cell counts can be performed using the hemocytometer. This instrument possesses a platform with microscopic grid scoring. Rails on either side hold up a cover slide so that a specified quantity of fluid is held. By properly diluting blood, counting all cells in specified squares, and multiplying by the proper conversion factor, the number of cells per cubic millimeter (µl) can be determined.

Material & Equipment:

Hemacytometer (counting chamber)

Coverslide

RBCS pipette with Mouthpiece

Lancet

Microscope

70% ethanol

Bottle ringer's solution (a clear diluent for RBCs).

Procedure:

1. Swab towards the side of the tip of a little-used finger with 70% alcohol.

2. by using lancet make a finger puncture.

3. Using the dilution pipette with RED mixer from hemacytometer kit, draw blood. up to the 0.5 mark. (Do not allow air to be drawn into the pipette or it will not draw the correct volume of blood. Do not allow blood to congeal in pipette! Immediately proceed to the next step.

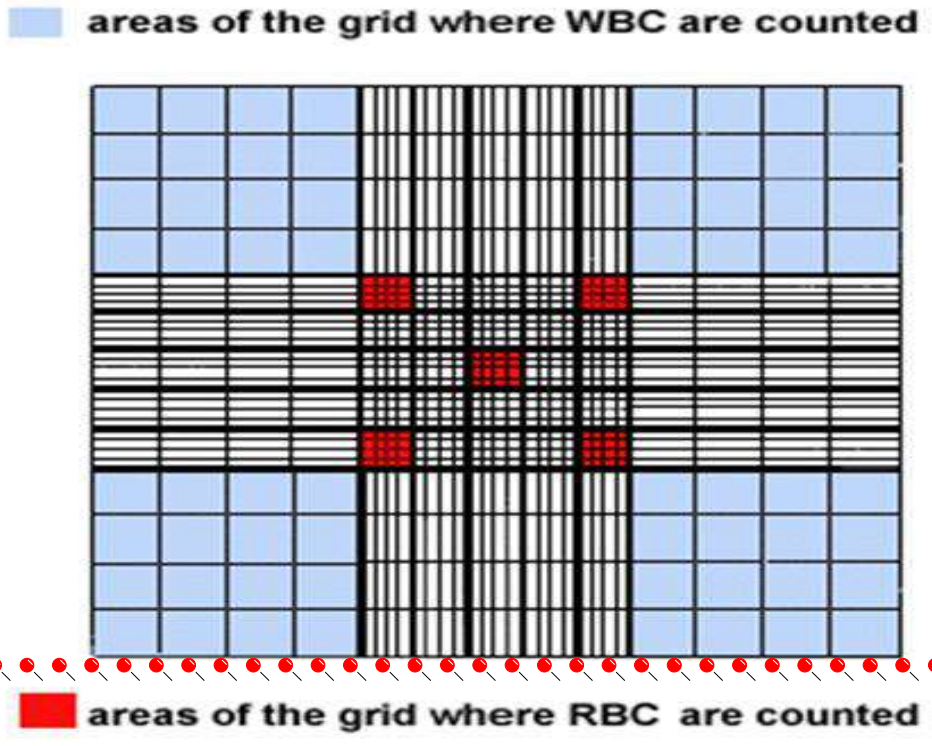
4 .Continuing to hold the pipette as horizontal as possible, draw Ringer's solution diluent up to the 101 mark. (Dilution of 1 to 200).

5. Seal the tip with your finger and shake well to mix.

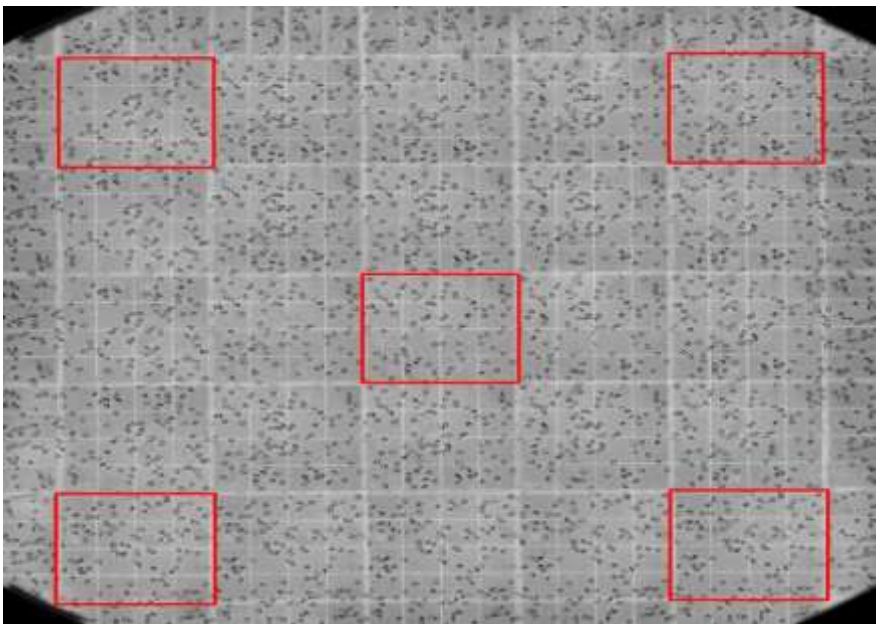
6 . Empty ~1/2 of pipette into waste container. add a small amount of the diluted blood to one chamber of the hemacytometer to just fill the chamber of the hemacytometer. (Do not over fill).

7. Let the preparation sit for a minute (for cellsto settle).

8. Center the grid at 100x, switch to 400x and count and record the RBCs



in each of five fields (each with 16 smallest squares) with a clicker



(fields: top R & L, bottom R & L, center).

Calculate the RBCs/cmm by adding the cells in the 5 groups and multiplying by **10,000**

$$\text{RBC's Count/mm}^3\text{ Blood} = \frac{\text{No. of RBC's counted/ 80 small square}}{\text{No. of small square (80)}} \times \frac{1}{\text{Volume of small square}} \times \frac{1}{\text{Dilution}}$$

- No. of RBC's counted 80 small square = ??? /
- No. of small square = 80
- Volume = Length X Width X Height = 1/20 X 1/20 X 1/10 = 1/4000
- Dilution = 1:200 = 1/200

i.e. Factor = 10000

RBC's Count/mm^3 Blood = ??? X 10000

9 .Wash out the pipette thoroughly with soap and water, rinse well, finish with distilled H2O rinse.

Normal Reference Range:

Males : 4.6 – 6.2 x 10^{12}/L
Females : 4.2 – 5.4 x 10^{12}/L
Children: 4.5 – 5.1 x 10^{12}/L

WHITE BLOOD CELL (WBC's) COUNT

The blood contains non nucleated red blood cells and nucleated white blood cells. The white cells are far less in number than red cells. It will be necessary to destroy the red cells and to stain the white cells for easy identification (as they are colorless) and counting.

Material & Equipment:

Hemacytometer (counting chamber)

Coverslide

WBCs pipette with Mouthpiece

Lancet

Microscope

70% ethanol

WBC Diluting Fluid (Turk's solution) is used. It is a 3% solution of acetic acid (for destruction of red cells) with trace of Gentian Violet (for staining the white cells).

Procedure

1. Make a finger prick and wipe out the first drop and allow a second drop to from.

2. Hold the pipette horizontally and draw blood in the pipette up to 0.5 mark. Wipe any blood on the exterior of the pipette tip and insert the pipette vertically and draw the diluting fluid up to 11 mark.

3. Cover the other end of the pipette by the right thumb so that the pipette is held in the right hand such as its both ends are closed. Shake the pipette thoroughly for about one minute. (Now the pipette contains blood diluted 20 times and haemolysed).

4. Place the counting chamber on the table with the cover-slip in place.

5. Discard two or three drops from the pipette Then, carefully controlling the escape of the fluid with a fingertip applied to the Upper end of the pipette, allow a small drop to from at the tip of the pipette. Apply this drop to the opening between the cover-slip

and the platform. (The counting chamber will be charged by the surface tension pulling the fluid inwards.

6. Examine the counting area under the microscope for general distribution of cells. If uneven distribution is seen, clean the counting chamber and re-charge the counting chamber.

7. Start with the lowest magnification (x 4). Identify the WBC counting areas and move one counting area to the center of the field. Turn to the next magnification (x 10) and start counting the cells systematically.

Calculations:

$$\text{WBC's Count/mm}^3\text{ Blood} = \frac{\text{No. of WBC's counted/ 64 big square}}{\text{No. of big square (64)}} \times \frac{1}{\text{Volume of big square}} \times \frac{1}{\text{Dilution}}$$

- No. of WBC's counted 64 small square = ??? /
- No. of big square = 64
- Volume = Length X Width X Height = 1/4 X 1/4 X 1/10 = 1/160
- Dilution = 1:20 = 1/20

i.e. Factor = 50

WBC's Count/mm^3 Blood = ??? X 50

Reference Range

- Adults : 4.5 – 11.0 x 10^9 /L
- Six years: 4.5 – 12.0 x 10^9 /L
- One year: 6.0 – 14.0 x 10^9 /L
- Newborn: 9.0 – 30.0 x 10^9 /L

- WBC count varies according to age but not to sex.

Lec.9

Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate is a non-specific indicator of inflammation. It is measured by the degree of settling of red blood cells in a specific time period, usually one hour.

There are several methods for determination of the ESR; the most common are the Wintrobe and the Westergren, named for the developers of the procedure. There is also an automated method for determining the sedimentation rate.

To perform the test, anticoagulated blood is placed in an upright tube, known as a Westergren tube, and the rate at which the red blood cells fall is measured and reported in mm/h.

The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (**zeta potential**). When an inflammatory process is present, the high proportion of plasma proteins such as fibrinogen, C-reactive protein, immunoglobulins. causes red blood cells to stick to each other. The red cells form stacks called '**rouleaux**' which settle faster.

The ESR is increased by any cause or focus of inflammation. The ESR is increased in sickle cell anemia, arthritis, rheumatic fever, myocardial infarct, infections, some malignancies, menstruation, renal disease, leukemia , lymphoma tuberculosis and aging , normal pregnancy.

The ESR decreased in polycythemia, and congestive heart failure, spherocytosis .

Material & instruments:

- Westergen pipette (glass pipette: 30cm length &2.55 cm width), it is graduated (0-200mm) and open at both ends.
- Westergren pipette rack.

- 3.8% sodium citrate as anticoagulant in a ratio of 1:4 with blood (0.4 ml sodium citrate + 1.6ml blood) or EDTA.
- Syringe, cotton, alcohol, for blood draw by vein puncture.

Or:

- Disposable ESR set: plastic graduated pipette + small tube contain anticoagulant (sodium citrate).

Procedure:

1. Withdraw 2-3 cc of blood from patient vein using syringe.

2. Put 0.4 cc of (3.8% sodium citrate) in plain tube.

3. Immediately add 1.6cc of blood from syringe to plain tube and mix well.

4. Fill the westergren pipette to 0 mark , make sure that there are no air bubbles at all in blood.

5. Place the pipette vertically on the rack for 1hr.

6. At the end of 60 min. (1hr.) read the result.

Or: in case we used the Disposable ESR set: plastic graduated pipette + small tube contain anticoagulant (sodium citrate):

1. Withdraw 2-3cc of blood from patient vein using syringe

2. Immediately add blood from syringe to neck of small tube that contain anticoagulant and mix well.

3. Insert the plastic pipette through the plug of small tube and force the blood to go up by inserting pressure on pipette with screwing the pipette or small tube at the same time.

4. Place the pipette vertically on the rack for 1hr.

5. At the end of 60 min. (1hr.) read the result

Normal ranges:

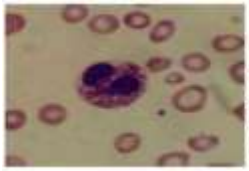
Adult men: 0-10 mm/hr. or 0-15 mm/hr.

- Adult women: 0-15 mm/hr. or 0-20mm/hr.

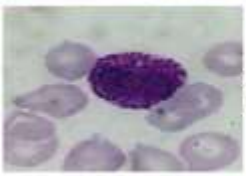
Lec.10

Differential WBCs count

White blood cells are evaluated by a differential count, which reports percentages of the types of WBCs present. These are neutrophils which fight infection (also known as segments (mature) and bands (immature), lymphocytes which produce antibodies and other immune system activities, monocytes which also fight infection , eosinophils and basophils which are involved with allergies. The red cells are also evaluated for size, shape, color and the presence of any abnormalities Manual differentials are performed by taking a drop of blood, spreading it on a slide, staining it, and evaluating 100 cells individually for quality and changes in morphology. For patients with elevated white cell counts,

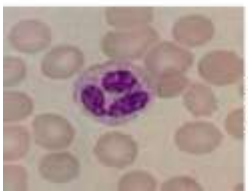


Cells whose granules stain bright red orange are called **eosinophils** and are part of the allergic response. Those granules contain histamine among other proteins. They should constitute between
0 - 4% of a normal differential for an adult.



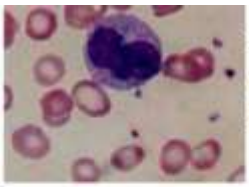
Cells whose granules stain dark blue are called **basophils** and are also involved in allergic reactions.

while those that favor the acid stain, eosin, stain bright red-orange. Some structures seem indifferent to the stain and are called neutral.

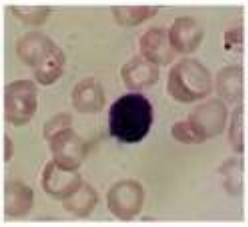


Neutrophils cells are usually between 50 - 70% of all of the cells seen in a normal differential performed on an adult.

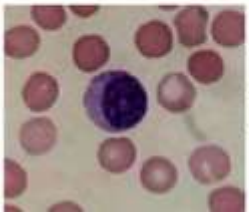
differentials of 200 cells or greater might be done. Automated differentials are performed by either testing for specific compounds within the cells or comparing their size, shape, and content.



Monocytes are a type of cross over cell. They are primarily responsible for removing dead or damaged cells and constitute less than **10%** of the adult differential.



Lymphocytes are cells that do not contain large numbers of any granules. They are responsible for producing antibodies against foreign material such as antigens found in viruses and some bacteria. They also are active against malignancy. In the adult, they range between **20 and 45%** of the cells seen in the differential.



When a **lymphocyte** is actively defending against an antigen, there will be changes seen in the cell and the cell is called a "reactive" lymphocyte.

Normal Values for Differential WBC count in Adults:

Cell Type/ Population	*%Normal Differential*	*Normal Absolute Count x 10^9*
Neutrophils- Segmented	50-70%	2.0-7.0 x 10^9/L
Neutrophil- Band	0-10%	0.0-1.0 x 10^9/L
Lymphocytes	15-45%	0.6-4.0 x 10^9/L
Monocytes	0-10%	0.0-1.0 x 10^9/L
Eosinophils	0-6%	0.2-0.7 x 10^9/L
Basophils	0-1%	0.0-0.2 x 10^9/L

Type Of Cell	Increase	Decrease
Red Blood Cells (RBC)	Erythrocytosis or Polycythemia	Anemia or Erythroblastopenia
White Blood Cells (WBC):	Leukocytosis	Leukopenia
-- Lymphocytes	-- Lymphocytosis	-- Lymphocytopenia
-- Granulocytes:	-- Granulocytosis	-- Granulocytopenia or Agranulocytosis
-- --Neutrophils	-- --Neutrophilia	-- --Neutropenia
-- --Eosinophils	-- --Eosinophilia	-- --Eosinopenia
-- --Basophils	-- --Basophilia	-- --Basopenia
Platelets	Thrombocytosis	Thrombocytopenia
All Cell Lines	-	Pancytopenia

Lec11

Obtaining Vital Signs and Measurements

Vital signs and body measurements are used to evaluate health problems. Provide information about patient's overall condition. Taken at each visit and compared to baseline. Accuracy is essential. Vital signs include:

- Temperature
- Pulse
- Respiration
- Blood pressure
- Body measurements

1. Height
2. Weight
3. Head circumference

Temperature

Febrile: body temperature above patient's normal range

Normal -36.6-37.2 C

Hypothermia- below normal

Pyrexia- higher than normal

Hyperpyrexia – extremely high temperature

Afebrile: normal body temperature

Body temperature varies with time of day. The recording temp.varies according to:

- Site of measurement
- Menstrual cycle can elevate temp .
- Physical activity
- Strong emotion

- Eating
- Heavy clothing
- Medications
- High humidity
- High room temp

Route	Normal Range °F / °C	Sites
Oral	98.6 °F / 37.0 °C	Mouth
Tympanic	99.6 °F / 37.6 °C	Ear
Rectal	99.6 °F / 37.6 °C	Rectum
Axillary	97.6 °F / 36.6 °C	Axilla (armpit)

Measurements of temperature

Degrees Fahrenheit (°F(

Degrees Celsius (centigrade; °C(

Temperature Measured using either electronic or disposable. Electronic digital more Accurate, fast, easy to read and Comfortable for the patient. While the Disposable, Single use and Less accurate

Method of measurements:

Oral temperatures

- Wait at least 15 minutes after eating, drinking, or smoking.
- Hold the thermometer from end away from the bulb with your thumb and index finger.
- Lower the mercury level by shaking.
- Place thermometer under tongue in either pocket.
- Wash thermometer by antiseptic solution with cotton wool.

Tympanic temperatures

- Proper technique specially with children
- Fast, easy to use, and preferred in pediatric offices

Rectal temperatures

- Standard precaution – gloves
- Patient is positioned on side or stomach
- Lubricate tip of thermometer
- Slowly and gently insert tip into anus
- ½inch for infants

- 1 inch for adults
- Hold thermometer in place while temperature is taken

Axillary temperatures

- Place patient in seated or lying position
- Place tip of thermometer in middle of axilla
- Probe must touch skin on all sides

Temporal temperatures

- Temporal scanner
- Noninvasive, quick
- Stroke scanner across forehead, crossing over the temporal artery

For children

Take temperature last if child cries or becomes agitated. Agitation will cause pulse, respiration, and blood pressure to elevate. Oral not appropriate for children under 5 years old.

Pulse : number of times the heart beats in 1 minute.

There are more than 9 sites to measure pulse in our bodies. We will discuss the most important sites:

- **Radial artery:**

Place your index and middle finger over the right or left redial artery at wrist of arm lateral to the flexor carpi radialis tendon (side of thumb). Count pulse for 15 sec. and multiply by four.

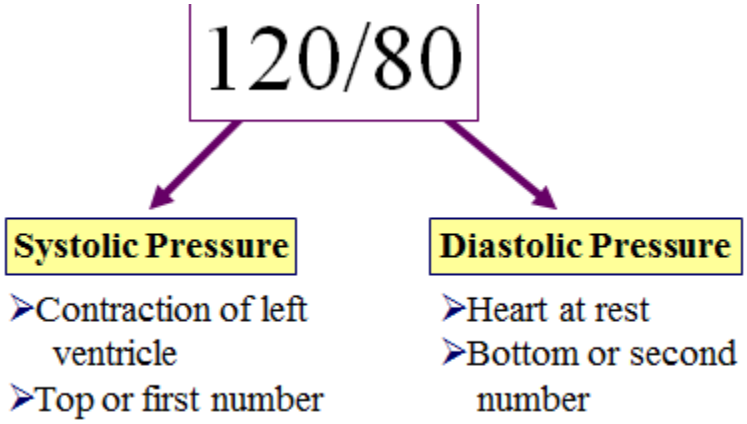
- **Brachial artery**: place your index and middle finger to feel the anticubital fossa medial to tendon of biceps muscle (feel side of arm between biceps and triceps muscles)
- **Carotid artery:** palpate the carotid pulse with the patient lying on or sitting. Never compress both left and right carotid arteries at the same time. Place your finger between the larynx and anterior border of the sternocleidomastoid muscle.

Locate pulse by pressing lightly with index and middle finger pads at the pulse site .Count the number of beats felt in 1 minute or Count pulse for 15 sec. and multiply by 4.

The normal adult resting pulse is between 60-100 bpm. Typical rate 73 bpm.
Electronic devices: measure blood pressure, pulse rate, oxygen saturation.

Respiratory rate indication of how well the body provides oxygen to the tissues. it is number of breaths in one minute.
1inhalation + 1 exhalation = 1 respiration (1 breath)
The ratio between pulse and respiration is 4 : 1
 Check by watching, listening, or feeling movement
 Count for one full minute: Rate, Rhythm (regular) and Effort (quality): normal, shallow, or deep
NOTE: If patients are aware that you are counting respirations, they may unintentionally alter their breathing.
Irregularities indication of possible disease:
Hyperventilation : excessive rate and depth
Dyspnea :difficult or painful breathing
Tachypnea : rapid breathing
Hyperpnea: abnormally rapid or deep breathing

Blood pressure: The force at which blood is pumped against the walls of the arteries (mmHg(
Two pressure measurements
Systolic pressure: measure of pressure when left ventricle contracts
Diastolic pressure: Measure of pressure when heart relaxes**.** Minimum pressure exerted against the artery walls at all times.



Hypertension (High blood pressure): Major contributor to heart attacks and strokes.
Hypotension (Low blood pressure): Normal for some people Severely low blood pressure readings occur with :

- Shock
- Heart failure
- Severe burns
- Excessive bleeding

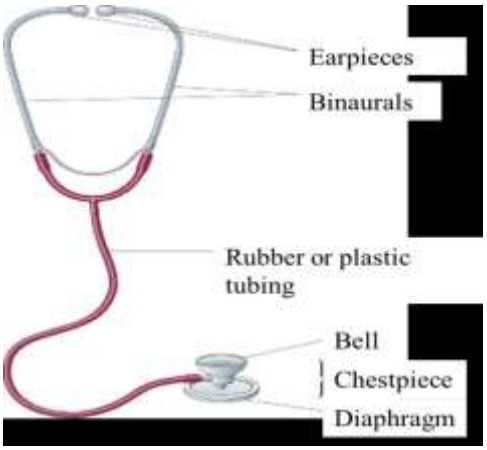
Types of sphygmomanometers

- **Aneroid:** Circular gauge for registering pressure, Each line 2 mmHg Very accurate, Must be checked, serviced, and calibrated every 3 to 6 months.
- **Electronic**: Provides a digital readout of the blood pressure, No stethoscope is needed, Easy to use, Maintain equipment according to manufacturer's instructions
- **Mercury:** A column of mercury rises with an increased pressure as the cuff is inflated, No longer available for purchase, If in use must be checked serviced and calibrated every 6 to 12 months.

Amplifies body sounds consist of:

- Earpieces
- Binaurals and tubing
- Chestpiece includes two parts:

Bell: low-pitched sounds
Diaphragm: high-pitched sounds



Measuring blood pressure

- Place cuff on the upper arm above the brachial pulse site
- Inflate cuff about 30 mmHg above palpatory result or approximately 180 mmHg to 200 mmHg

 Release the air in cuff and listen for the first heartbeat (systolic pressure) and the last heartbeat (diastolic pressure)

 Record results with systolic as the top number and diastolic as the bottom number (i.e., 120/76)

م.م. نغم حسن علي & م. مادلين قاسم عباس

Tikrit University – Collage of Dentistry

Lec. 12

Electrocardiogram ECG

Electrocardiograph is the interpretation of the electrical activity of the heart over a period of time, as detected by electrodes attached to the outer surface of the skin and recorded by a device external to the body.

The recording produced by this procedure is termed as Electrocardiogram

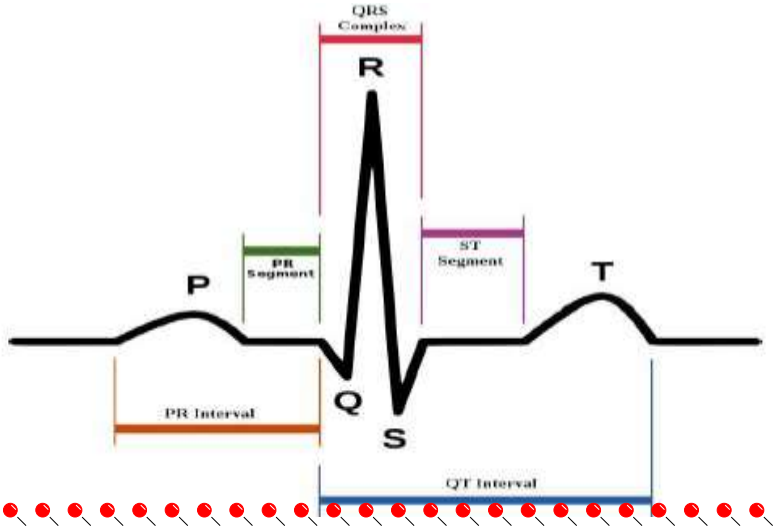
The normal electrocardiogram (see in figure 1) is composed of the following:

P wave caused by the electrical potential generated from depolarization of the atria before their contraction

QRS complex caused by the electrical potential generated from the ventricles before their contraction.

T wave caused by the potential generated from repolarization of the ventricles.

The P-Q or P-R interval on the electrocardiogram has a normal value of 0.16 second and is the duration of time between the first deflection of the P wave and the beginning of the QRS wave; this represents the time between the beginning of atrial contraction and the beginning of ventricular contraction. The Q-T interval has a normal value of 0.35 second, which is the duration of time from the beginning of the Q wave to the end of the T wave. This approximates the time of ventricular contraction.



ECG leads

There are two types of leads: **1. Limb leads 2. precordial (chest) leads.**

The reason we use these two leads is that the heart is a 3 dimensional structure, thus a single lead will not be able to describe its electrical activity properly. Hence the standard ECG consists of 12 leads. Each leads views the heart at a unique angle enhancing its sensitivity to a particular region of the heart.

Limb Leads

These leads view the heart in the vertical plane i.e. records the electrical forces (depolarization and repolarization) moving up, down, left and right.

To produce the 6 limb leads with 4 actual leads. Each of the electrodes is variably designed as positive and negative. This is done automatically by the machine.

Various limb leads again further grouped into Standard limb leads and Augmented limb leads. The standard limb leads are,

Lead 1: Left arm is made +ve and Right arm -ve.

Lead 2: Legs +ve and Right arm –ve.

Lead 3 : Legs +ve and Left arm -ve.

The augmented limb leads are,

AVR: Right arm is +ve and other limbs are –ve.

AVL: Left arm +ve and other limbs are –ve.

AVF : Legs are +ve and other limbs are –ve.

Note: these augmented are so named as they amplify the tracings to get an adequate recording.

Precordial leads

The precordial leads view the electrical forces moving anteriorly and posteriorly. These are:

V1: Placed in the 4th intercostal space right to the sternum.

V2: Placed in the 4th intercostal space left to the sternum.

V3: Placed between leads V2 and V4.

V4: Placed in the 5th intercostal space in the mid clavicular line.

V5: Placed between the leads V4 and V6.

V6: Placed in the 5th intercostal space in the mid axillary line.

The subject lies comfortably in a bed. The subject is instructed to lie still and to make no muscular movements. The limb electrodes are wetted by saline for better contact with skin and secured in place by a rubber strap

at the fore arms and legs above muscle belly. If the skin is hairy the place may have to be shaved to ensure god contact. When all leads are connected to the electrocardiograph according to the color code, the necessary connections can be obtained by positioning the appropriate knob.

ECG abnormal rhythm

Sinus Tachycardia

Sinus tachycardia occurs in adults when impulses originate at the SA node at a rate greater than 100 per minute. It may or may not be clinically significant, and is not in itself indicative of cardiac disease. (For example, sinus tachycardia may be associated with fever or exercise).

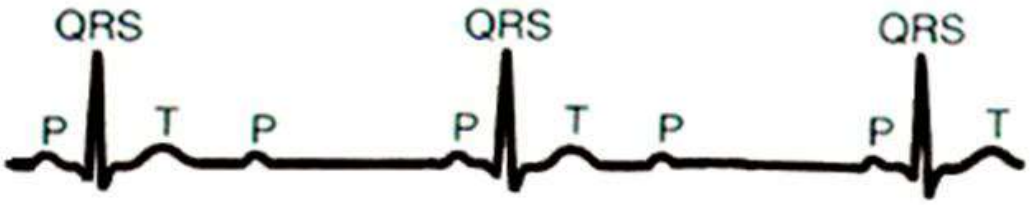
Sinus Bradycardia

Sinus bradycardia occurs when impulses originate at the SA node at a rate of less than 60 per minute. Sinus bradycardia is not necessarily indicative of cardiac disease, and is often seen in athletes and during sleep.

Atrioventricular (AV) block

Partial AV block occurs when AV node damage prevents some atrial impulses from being transmitted to the ventricles. In the particular case illustrated, every second P wave is not followed by QRS and T waves,

producing 2:1 AV block. It is said that there are "dropped beats" of the ventricles



Complete AV block: When the condition that is causing poor conduction in the AV node becomes severe, there is a complete block of the impulses from the atria to the ventricles. In that case, a subsidiary pacemaker can arise in the ventricles, which then paces the ventricular muscle. There is then no synchronization between atrial and ventricular electrical activity. The ventricles have "escaped" from atrial control, and are beating at their own natural rate, which is typically much less than the sinus rate.

Premature Contractions

A premature contraction occurs when the heart contracts prior to the time when normal contraction is expected. In the case of a premature contraction, the PR interval is often shorter for the premature beat than for the normal sinus beat.