Hematology

Clinical examination of blood

1.making blood film.

2.anticoagulant agents, coagulating time and bleeding time.

3.total red blood cell count

4.estimation of Hb

5.packed cell volume or hematocrit value
6.erythrocyte sedmindation rate ESR and osmotic fragility test.
7.blood groups ABO system
8.total white blood cell count
9.Differential leukocytes count
10.total platelets count.

sites of blood collection:

Animals	Site of collection
1.mouse	Tail and heart
2.rat	Tail ,heart and angle of eye
3.rabbit	Ear vein and heart
4.human	Finger tip and brachial artery
5.dog	Femoral artery
6.cow and horse	Jugular vein
7.chiken	Wing vein

Making blood film methods:

1.slide film method.

2.cover slip method.

Preparation of blood film or smear:

1.should made as soon as possible after collected of specimen because leukocytes and other cells have tendency to degenerate rapidly.

2.should made from fresh blood contain no anticoagulant.

3.blood film prepared either on microscopic slide or cover slip.

4.routine laboratory work , the microscopic slide is preferable to cover slip because cover slip is difficult to clean and store.

5.slide must be dipped in alcohol and dried with cloth prior to use.

6.not touch the surface of slide because deposit of grease interferes by finger with making the smear.

7.small drop of blood is placed on one end of slide.

8. the spreader slide is placed in front of the drop of blood at 30 degree.

9. as soon as spreader touch the drop of blood spread by capillary action along the edge of slide and should be pushed forward smoothly and rather quickly at angle 30 degree (proper speed) because if spread too rapidly the leukocyte will be concentrated near the end of film.

10.the angle influence the thickness of blood smear , as the greater the angle the thick and short film, the smaller the angle the thin and long smear.

11.the slide should be wave in air to dry .

12.marking the slide with a pencil on the thick end of blood film.

Properties of a good blood film:

1.Thick in one end and thin in other end.

2.the edge of film should be at least 2mm from the slide end.

3.the film should be smooth even appearance free from holes which mean presence of grease and stream mean dirty.

Procedure of slide method:

1.prick the finger tip and wipe away the first drop of blood and take next drop over one end at the slide.

2.use other slide to spread.

3.dry the smear by waving then stained with leishman stain for one minute.

4.add equal volume of distilled water and blow genteelly on the slide for about 7-8 minute.
5.wash with the tap water.
6.dry it and examine under oil immersion.

Objective:

1.morphology of RBC :

a.the size and shape of RBC biconcave disk.

b.normal diameter: microcytosis has small diameter

macrocytosis large diameter

c.anisocytosis (variation in size)that occur by iron and vit B12 deficiency.

d.poikilocytosis (variation in shape) occur in cases of anemia.

2.blood parasite rapid diagnosis such as trypansoma and anaplasma.

Examination of slide:

Exam the slide begin with thin end and systematic meander this will insure all area of slide examined.

Procedure of cover slip method:

Superior method because leukocytes are better distributed.

1.holding the clean dry cover slip by its edges in one hand.

2.place small drop of blood on the center of cover slip.

3.the drop should be small enough so that the spreading will not reach the edge of cover slip.
4.place a second clean dry cover slip on the first one forming an eight pointed star.
5.grasp the top cover slip by its corner and using a smooth motion sliding them a part.
6.wave the cover slip to enhance drying.
7.stained and exam.

Blood groups

Introduction

The surface of RBC s contain numerous glycoprotein markers known as antigens (Ag).there are many different types of antigens, but the most common are the A,B and D antigen (D is also known Rh factor).

the presence or absence of these antigens determines the type of individual. In addition, about six months after birth, lymphocytes begin to produce certain (Ab), which are proteins that circulate in blood plasma. These Abs are isolated from the plasma and used as anti-sera in the laboratory to determine blood types. Mixing these anti-sera with whole blood stimulates a process called agglutination (clumping).

For example: mixing anti- A serum with type A blood will cause the anti-A antibodies in the serum to (stick to) the A antigen on erythrocytes. This Ag-Ab reaction will give the slide a beaded appearance. Mixing anti-B serum with type A blood will *not* show agglutination.

Blood types	Antigen present	Antibody present
A	A	B
B	B	A
AB	AB	-----
O	------	AB
Rh+	D	-----
Rh-	-----	IF exposure to Rh+ antibodies will produced

Procedure

1.on prepared microscopic slide with 3 circles drawn with pencil and labeled A,B and Rh or D, place a small drop of blood in each circle (make sure you have enough blood, but do not completely fill the circles.

2.add anti-A serum to the circle labeled A, and anti-B serum to the one labeled B, and anti-D serum (or Rh)to the circle labeled D. mix the contents of each circle with clean toothpick, the reaction will appear in 20-60 sec.

Check for agglutination by naked eye or by microscope and the test should be performed in warm room temperature to prevent the action of cold agglutinin which leads to confusion.

Rh system: there are 6 common types of Rh antigens these are C,D,E,c,d,e the most common one is D antigen, any body that has D agglutinogens is Rh positive and the antibodies to Rh antigen do not occur naturally but can occur after blood transfusion and during pregnancy. the percentage of people who Rh+ve are 75-85%.

Medical application:

Hemolytic disease of newborn which occurs in case the mother is Rh-ve and the fetus is Rh +ve and due to the attack on fetal RBC by maternal antibodies which pass across the placenta, seldom the first pregnancy is affected unless the mother is previously sensitized.

Note :

The person who is of blood group AB is called universal recipient.

The person of blood group O is called universal donor.

Packed cell volume (PCV)

or

Hematocrit (HCT) value

Introduction and principle :

Anticoagulated whole blood is centrifuged for maximum red blood cell packing. The space occupied by the red blood cells is measured and expressed as a percentage of whole blood volume.

So PCV is the volume of RBCs per unit volume of whole blood.

Methods:

1.microhematocrit

2.electronic cell counting

Microhematocrit method

Material and instruments:

1.microhematocrit tube (capillary tube) 75mm in length and 1mm in diameter which contain heparin and show a red ring at the end of the tube.

2.microhematocrit centrifuge device.

3.plastic seal to seal one end of microhematocrit capillary tube.
4.microhematocrit reader.

Procedure:

1.blood is drawn into the tube by capillary phenomena. By holding the tube in a horizontal manner and allow 2/3 to3/4 to be filled with blood.
Air bubbles denote poor technique but do not affect the results of the test.
2.seal the dry end of the tube by plastic seal.

3.the sealed tube is then placed in radial grooves of microhematocrit centrifuge for 5 minute.

4.when looking at a centrifuge hematocrit tube, you can see three distinct layers.

a.top layer of clear slightly milky plasma.

b. a thin Buffy coat layer consisting from WBC and thrombocyte.

c. a dark packed RBC layer.

5.obtain the result using the microhematocrit tube reading device, adjust the movable line to touch the top of the RBC in the tube.

Normal range in adult male 40-54%

Normal range in adult female 36-47%

Medical application:

PCV is affected by the shape and the number of RBC and the plasma volume. High PCV indicate either increase in number of circulating RBC or decrease in plasma volume(seen in cholera due to loss of water in stool).

A low PCV indicate either decrease in RBC or increase in plasma volume.

Anticoagulants, coagulating time and bleeding time

Anticoagulants: are agents used in order to obtain blood sample free from clot, which used in blood transfusion and blood analysis.

1.heparin: anticoagulant present in the body naturally, Synthesized by the basophilic white blood cell. It function inhibit the clotting factor (stuart X).

2.EDTA (ethylene diamine tetra acetic acid) : this agent chalet calcium to prevent formation of clot.

Coagulating time: also called clotting time it is the time needed for measure amount of blood to clot.

Blood coagulation is a complex process involving over 30 substances called pro coagulants.

Thrombocytes (platelets) are responsible for releasing many of these substances, include mineral ,vitamine that are crucial to the clotting process.

Hemostasis (stoppage of bleeding) chain of events that lead to formation of insoluble fibrin strand which form a meshwork that trap blood cells and seal the damaged vessel.

Method:

Capillary tube method

Material and instrument:

1.capillary tube (non hepranized)

2.lancet

Procedure:

1.prick the finger and load a capillary tube (non hepranized) to at least ½ full.

2.after about 1-5 minute take capillary tube between thumb and forefinger and gently break in half. Slowly pull the ends apart to view the insoluble fibrin strand. Usually we do a break every 30 second. Once the clot is formed we record the time.

Normal clotting time is 6 min.

The clotting time is a measure of the time needed for the clotting mechanism to occur.

Medical application:

Prolong of clotting time may be due to :

1.decrease in factor VIII (hemophilia).

2.decrease in factor IX (Christmas disease)

3.liver failure

4.vitamine K deficiency

Bleeding time: is a measure of time needed for the spasm of capillaries to occur and platelet plug formation.

Normal range for bleeding time 2-6 minutes.

Bleeding time measure by Duk method.

Duk method: Using a circular filter paper the blood is blotted every 15-30 sec, without allowing filter paper to touch the wound.
The moment that the bleeding ceased this will represent the bleeding time.

Medical application:

The prolong of bleeding time is due to:

1.decrease number of platelets (thrombocytopenia)

2.defect in function of platelets (as in case of aspirin used).

3.defect in vessels themselves.

Bleeding time

Bleeding time:

is a measure of time needed for the spasm of capillaries to occur and platelet plug formation.

Introduction and principle:

A puncture of finger tip is made and the time needed for the bleeding to stop is recorded. Cessation of bleeding indicates the formation of a haemostatic plug which depended on an adequate number of platelets and the ability of platelets to adhere to the sub endothelium and to form aggregates.

Method

We can measure bleeding time by Duke method

Material and instrument

1.lancet

2.circular filter paper.

Procedure:

1.puncture the finger tip by lancet.

2.the recording of time is started at the time of puncture.

3.using the circular filter paper the blood is blotted every 30 second without allowing the filter paper to touch the wound.

4.the moment that the bleeding ceases and this will represent the bleeding time.

Normal range 2-6 minutes.

Note: the stop of bleeding is not due to clotting but due to the spasm of the capillaries and formation of platelet plug.

Medical application:

The prolongation of bleeding time is due to:

1.decrease number of platelets(thrombocytopenia).

2.defect in function of platelets(as in case of asprin use).

3.defect in vessels themselves.