اسم المادة	باللغة العربية	وظائف الأعضاء	السنة الدراسية	عدد الساعات الأسبوعية			
	باللغة الانكليزية	Physiology					
لغة التدريس للمادة	اللغة الانكليزية		الأولى	نظرية	عملي	مجموع	عدد الوحدات
				2	4	6	4

أهداف المادة:

الهدف العام: يكون الطالب قادرا على معرفة الوظائف الرئيسية لخلايا واجهزة واعضاء جسم الأنسان الهدف الخاص:

يكون الطالب قادرا على:-

1. معرفة وظائف ومكونات اجهزة الجسم) الهضمي، العصبي، التنفسي، المفاوي-----،الخ

2.) التعرف على العوامل المؤثرة على فسلجة أجهزة وأعضاء الجسم

الفصل الدراسي الأول	
week	تفاصيل المفردات
First	The microscope: type, parts and how to use it
Second	Hematology, collection of blood, capillary blood, venous blood, plasma and serum
Third & Fourth	Hemoglobin estimation by Cyanamithaemoglobin method (photometer method), and by acid hematic Sali method
Fifth	Packed cell volume (PCV)
Sixth & Seventh	Red blood cells count: repeat in two weeks
Eight & Ninth	Total leukocyte: repeat in two weeks
Tenth & Eleventh	Blood smear, staining
Twelfth & Thirteenth	Differential leukocyte count (type of WBC): repeat
Fourteenth	Scientific movies showing blood structures
Fifteenth	ESR by westergreem method and by wintrob method

First	ABO blood type: slide method, true method
Second	Rh factor: slide method, tube method
Third	Cross match test
Fourth & Fifth	Blood coagulation test, platelets count-repeat
Sixth	Bleeding time (Ducks method; ivy's method)
Seventh & Eighth	Clotting time: capillary tube method, lide method, Lee and while method
Ninth	Scientific movies showing bleeding and blood transfusion
Tenth & Eleventh	Fragility test (RBC fragility test)-repeat
Twelfth & Thirteenth	Examination of the urine; urine collection physical examination
Fourteenth	The chemical examination of urine; urine creatinine
Fifteenth	The microscopic examination of urine-repeat

فسلجه عملي

Microscope

Microscope:

Is the instrument that produces enlarged images of small objects, allowing the observer an exceedingly close view of minute structures at a scale convenient for examination and analysis.

kinds of microscope:

1- Simple Microscope
2- Compound Microscope
3- Stereo Microscope (dissecting)
4- Confocal Microscope
5- Electron Microscope

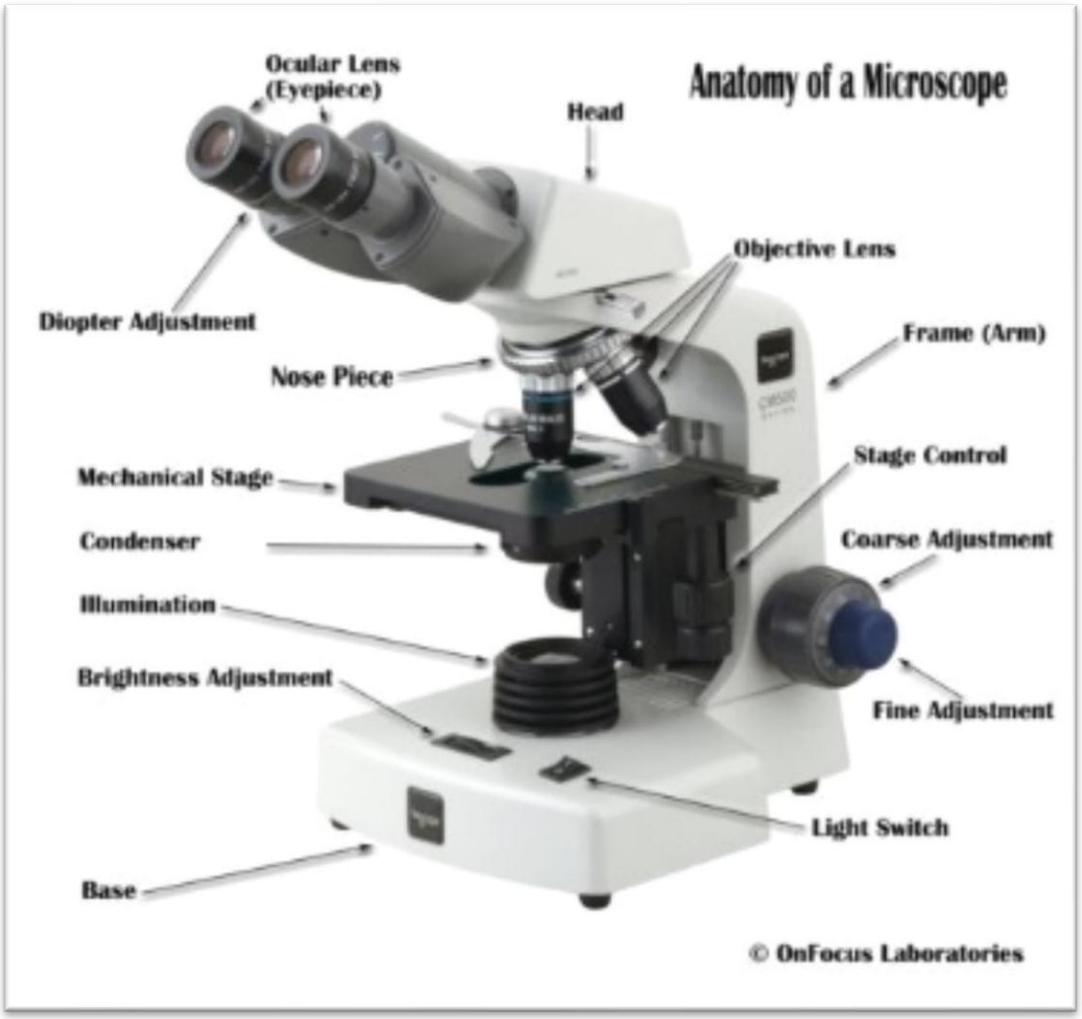
parts of compound (light) microscope:

1- **Eyepiece:** The lens the viewer looks through to see the specimen. The eyepiece usually contains a 10X or 15X power lens.
2- **Diopter Adjustment:** Useful as a means to change focus on one eyepiece so as to correct for any difference in vision between your two eyes.
3- **Body tube (Head):**
4- **Arm:** The arm connects the body tube to the base of the microscope.
5- **Coarse adjustment**: Brings the specimen into general focus.
6- **Fine adjustment:** Fine tunes the focus and increases the detail of the specimen.
7- **Objective lenses:** One of the most important parts of a compound microscope, as they are the lenses closest to the specimen.A standard microscope has three, four, or five objective lenses that range in power from 4X to 100X. When focusing the microscope, be careful that the objective lens doesn't touch the slide, as it could break the slide and destroy the specimen.
8- **Specimen or slide:** The specimen is the object being examined. Most specimens are mounted on slides, flat rectangles of thin glass.
9- **Stage:** The flat platform where the slide is placed.
10- **Stage clips:** Metal clips that hold the slide in place.
11- **Stage height adjustment (Stage Control):** These knobs move the stage left and right or up and down.
12- **Aperture:** The hole in the middle of the stage that allows light from the illuminator to reach the specimen.
13- **On/off switch:** This switch on the base of the microscope turns the illuminator off and on.
14- **Illumination:** The light source for a microscope. most microscopes now use a low-voltage bulb.

15- **Iris diaphragm:** Adjusts the amount of light that reaches the specimen.

16- **Condenser:** Gathers and focuses light from the illuminator onto the specimen being viewed.

17- **Base:** The base supports the microscope and it's where illuminator is located.



How Does a Microscope Work?

All of the parts of a microscope work together - The light from the illuminator passes through the aperture, through the slide, and through the objective lens, where the image of the specimen is magnified. The then magnified image continues up through the body tube of the microscope to the eyepiece, which further magnifies the image the viewer then sees.

(lab1) .....**practical physiology**

(BLOOD COLLECTION, TECHNICS OF BLOOD COLLECTION)

BLOOD COLLECTION :

Blood is the body fluid used most frequently for analytical purposes. Blood must be collected with care and adequate safety precautions to ensure test results are reliable, contamination of the sample is avoided and infection from blood transmissible pathogens is prevented.

Three general procedures for obtaining blood are:

(1) Skin puncture,
(2) Venipuncture,
(3) Arterial puncture

- Capillary blood (peripheral blood / micro blood samples) is frequently used when only small quantities of blood are required, e.g., for hemoglobin quantitation, for WBC and RBC counts and for blood smear preparation. It is also used when venipuncture is impractical, e.g. in infants, in cases of severe burns, in extreme obesity where locating the veins could be a problem and in patients whose arm veins are being used for intravenous medication.

-**Venous Blood Collection:**

A venous blood sample is used for most tests that require anticoagulation or larger quantities of blood, plasma or serum.

Apparatus and materials:

1-Syring, 2-tourniquet, 3-cotton, 4-alcohol, 5-test tube, 6-centerfuge, 7-pipette

Container for blood sample :

A container may or may not contain an anticoagulant depending on whether a sample of blood, plasma or serum is required.

Lab2…… practical physiology

(HB ESTIMATION BY CYANOMETHOMOGLOBIN METHOD)

Haemoglobin (Hb):

Haemoglobin is a substance which is the main constituent of the Red Blood Cells.

 It is a large complex molecule have molecular weight 68000.

 Haemoglobin is consists of:-

1-Basic protein globin (four-polypeptide chains closely linked together)

2- the iron-porphyrin complex containing complex haem is attached to each polypeptide chain and it is this part of the molecule.

Normal value of hemoglobin :

Adult male 13.5-17.5 g/ l

Adult female 11.5-15.5 g/ l

Methods for Hb estimation:

Various methods are available for estimation of hemoglobin in the laboratory.

I.Methods based on development of color. These are:

- Sahli's or acid hematin method
- Cyanmethemoglobin method
- Oxyhemoglobin method
- Alkaline hematin method

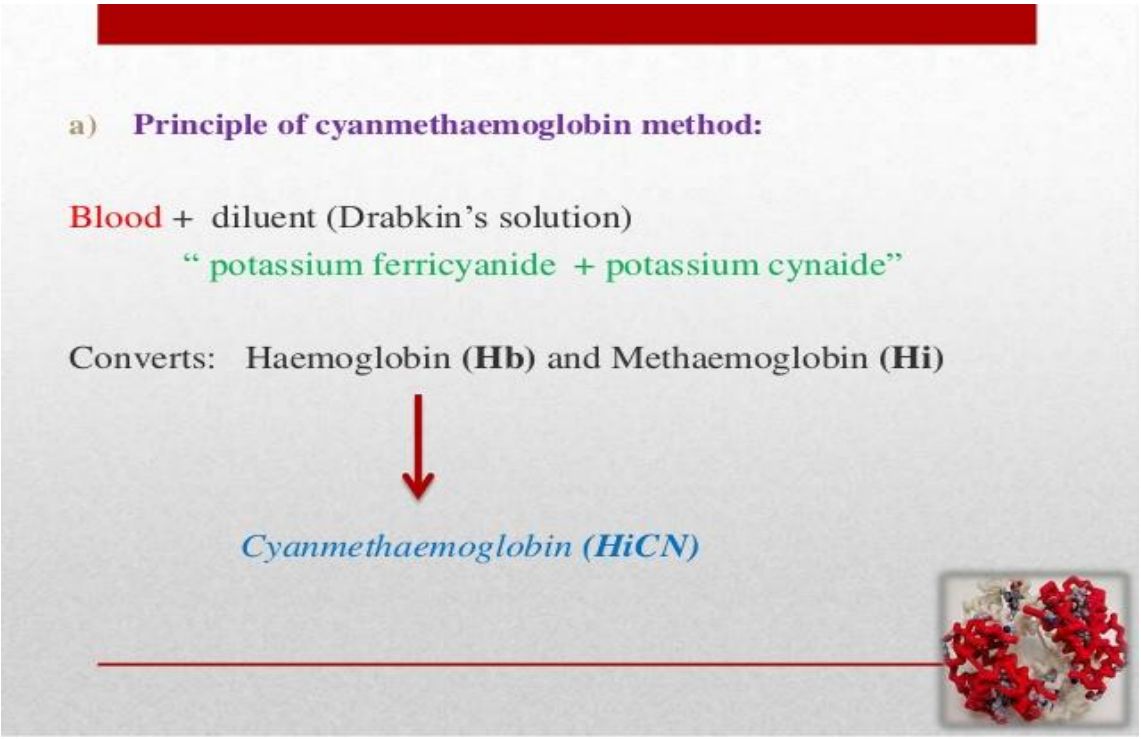
II. Measurement of oxygen combining capacity

III. Measurement of iron content

Hb estimation by Cyanmethaemoglobin method:

Cyanmethemoglobin method: This is the internationally recommended for determining hemoglobin.

Principle:



Equipment required :

Hb pipette , Spectrophotometer and Reagents required (Drabkin's solution).

Procedure:

1. Take 5ml of Drabkin's solution in a test tube.

2. Mix the blood sample by gentle inversion and draw 0.02ml of blood into the Hb pipette. Wipe the outer surface of the pipette to remove excess blood.

3. Place the pipette into the tube containing Drabkin's solution and slowly expel the blood into the solution. Mix well and let it stand undisturbed for 5min.

4. Measure the absorbance of this solution at 540nm in a spectrophotometer after adjusting the OD at 0 by using Drabkin's solution as blank.

Measure the absorbance of the solution by using a calorimeter at a wavelength = 540nm. Then compare it with the standard solution of HiCN.

Spectrophotometer

(Packed Cell Volume test (PCV))

Also called Erythrocyte Volume Fraction This is a benefit test for diagnosis of certain blood disorders, there are 2 methods used to doing this test:

1-Macrohaematocrit method (Wintrob's method)

In this method needs Wintrobs tube , capillary pipette, venous blood and centrifuge.

Procedure

1. Fill the tube with blood to mark 100 using pipettes

2. Spin the blood in centrifuge at 3000 RPM for ½ hour

3. Read the result (the cell packed)

2- Microhaematocritmethod

It is more used, because of many reasons, some of them, needing less blood, faster, we can use capillary blood and so on.

Material required:

1-Blood sample.
2-Microhaematocrite centrifuge.
3-Microheaematocrite reader.
4-Sealing material.
5-Cotton, alcohol.
6-Lancet.
7-Capillary tube.

Procedure:

▪Allow the blood to enter the capillary tube.

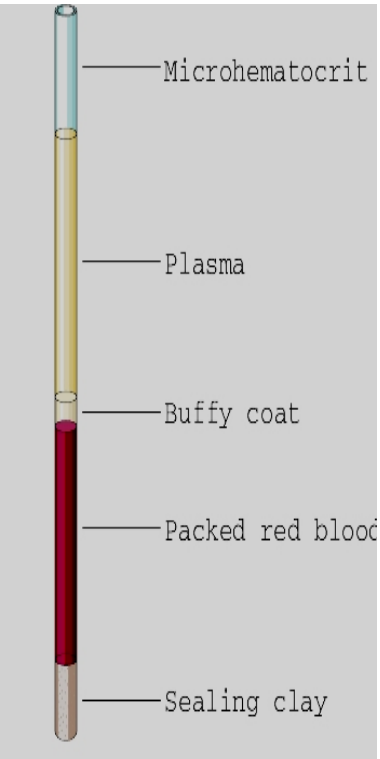
▪Seal the capillary tube by the sealing material

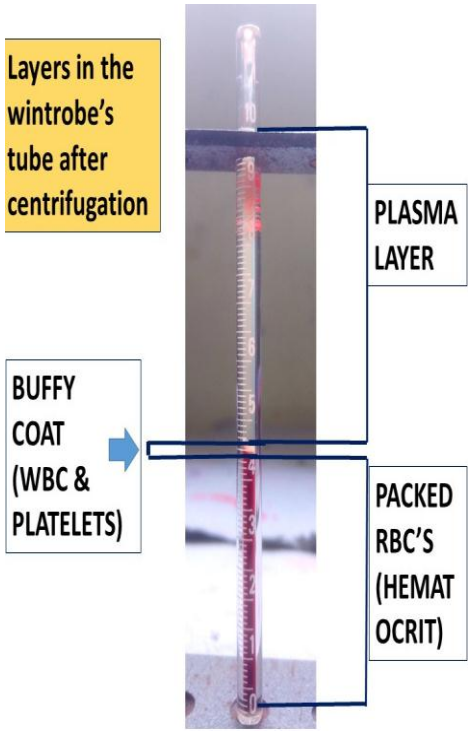
. ▪Spin the capillary tube by the microcenterfuge for 5min. at 10,000 R.P.M.

▪Using the haematocrite reader to read the value.

The normal value is as follows:

Man	40 – 54 %
woman	37 – 47 %
Children	30 – 38 %
Infant	42 – 52 %





Notes:

●Reduce plasma occurs in high altitude, burns and dehydration.
●Increase plasma occurs in pregnancy, server anemia, liver, spleen and kidney disease.

●Yellow colour plasma occurs in jaundice.

●Turbid plasma occurs in haemolytic anemia.

●Light plasma occurs in iron deficiency anemia.

(Lab4) .....**practical physiology**

Erythrocyte Sedimentation Rate (ESR)

The ESR is an easy, nonspecific test that has been used for many years to help diagnose conditions associated with acute and chronic inflammation, including infections, cancers, and autoimmune diseases.

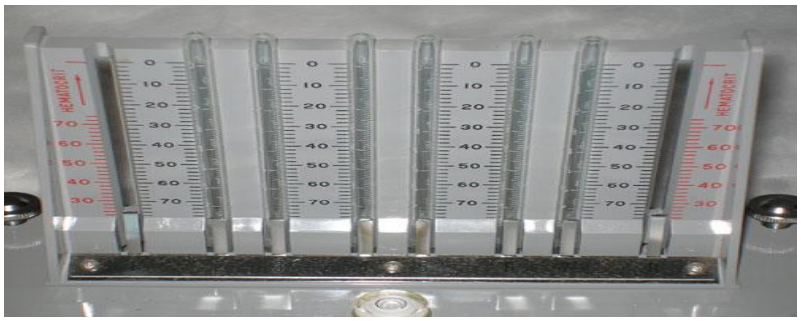
Principle:

Blood collected into an anticoagulant tube is placed in a long graduated tube held in a vertical position. The erythrocytes-under the influence of gravity - settle to the bottom, leaving a layer of plasma above. The height of the column of plasma after 1 hour is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr)., indicates the sedimentation rate of the erythrocytes (erythrocyte sedimentation rate (ESR).

Method of E.S.R tests:

1-Westergreen method.

2-Wintrops method.



(The Wintrobe sedimentation rack)

Materials and reagents

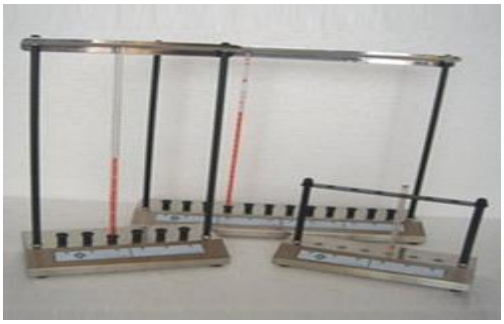
_ Westergren ESR tube: internal diameter 2.5mm; graduated from 0 to 200mm
_ Westergren stand
_ Test-tubes

_ Graduated syringe, 5ml

_ Graduated pipette, 5ml _

Timer _ Anticoagulant: trisodium citrate, 3.2% solution

Westergren ESR tube →



Method:

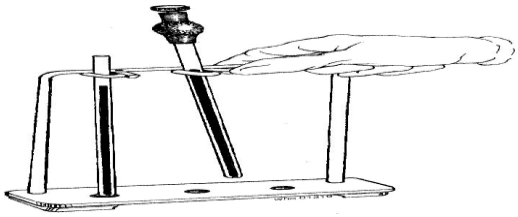
1. Pipette 0.4 ml of trisodium citrate solution into a test-tube or bottle.

2. Collect a venous blood specimen 2 ml .

(adding a blood sample to the the trisodium citrate solution)

3. Remove the needle from the syringe and add 1.6 ml of blood to the test-tube containing anticoagulant (marked to contain a total of 2.0 ml).

4. Draw the citrated blood into the Westergren tube (using a rubber safety bulb) up to the 0-mm mark.



5. Place the tube in the Westergren stand, Check that there are no air bubbles in the tube,Check that the stand is level.

6. Leave on a bench away from vibration (e.g. not on the same bench as a centrifuge.

7. Wait 1 hour, then note the height of the column of plasma in mm graduations starting from the 0-mm mark at the top of the tube .

Results : The result is expressed in millimeters per hour (mm/h).

Normal value: Man: 2------15 mm/hr Women: 2------20 mm/hr

Lab5) .....**practical (physiology**

(BLOOD COLLECTION, TECHNICS OF BLOOD COLLECTION)

BLOOD COLLECTION :

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-**Venous Blood Collection:**

A venous blood sample is used for most tests that require anticoagulation or larger quantities of blood, plasma or serum.

Apparatus and materials:

1-Syring, 2-tourniquet, 3-cotton, 4-alcohol, 5-test tube, 6-centerfuge, 7-pipette

Container for blood sample :

A container may or may not contain an anticoagulant depending on whether a sample of blood, plasma or serum is required.

Bleeding time (B.T)

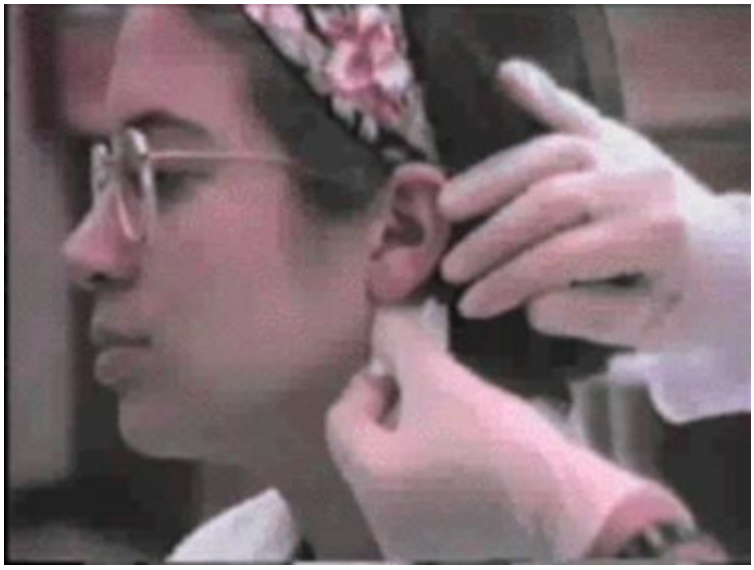
Learning objectives:

1. Will be able to measure (B.T).2. Learn about bleeding Time (B.T). Is the time course for bleeding to stop is required The normal bleeding time is between 2-5 minutes.

The test is performed:

- For the diagnosis of certain haemorrhagic disorders
- Before surgical operation
- Before liver or spleen puncture.

Methods used:



1-Duke's method.

2-Ivy's method.

Material Required:

1-Filter paper.

2-Stop-watch.

3-Sterile Lancet,alcohol andcotton.

Duke's Method Procedure:

1-clean and sterilize the lobe of your ear with 70% alcohol. Allow the ear to dry.

2-Make a wound about 4 mm with a dry sterile lancet, remove the oozing blood from the wound **b**y a clean piece of filter paper, using a different area of the paper,each time(every 30 seconds).

3-Continue removing the oozing blood from the wound until the bleeding stop.

4 -Count the spots of blood on the filter paper and multiply those by two; it will give you the bleeding time in minutes.

Ivy's method Material used:
1-Sphygmomanometer.
2-Filter paper, lancet, cotton and alcohol.
3-Two stop-watches**.**
Procedure:
1. Select a site on the patient's arm on the lateral aspect volar surface that is free of veins, bruises, edematous areas, and scars and is approximately 5 cm below the antecubital crease.
2. Clean the site with the alcohol prep.

3. Place the sphygmomanometer around the patient's arm approximately two inches above the elbow and maintain 40 mm Hg.

4- Make two punctures 3 mm apart.

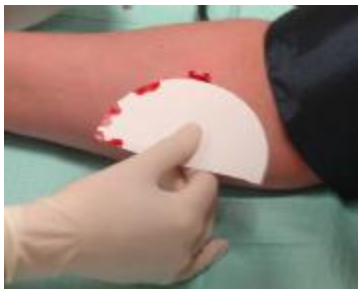
5-Start stop-watch for each puncture as soon as blood appears.

6-Touch the blood every 15 second and avoid touching the skin by filter paper.

7-Stop the watch for each puncture immediately if bleeding stop.

8-Take the average time for each puncture

Note: If the bleeding time exceeds 15 minutes:
1.stop the procedure
2.apply pressure to stop the bleeding
3.report as greater than 15 min.
Expected results :-Normal Values: 2- 9 minutes

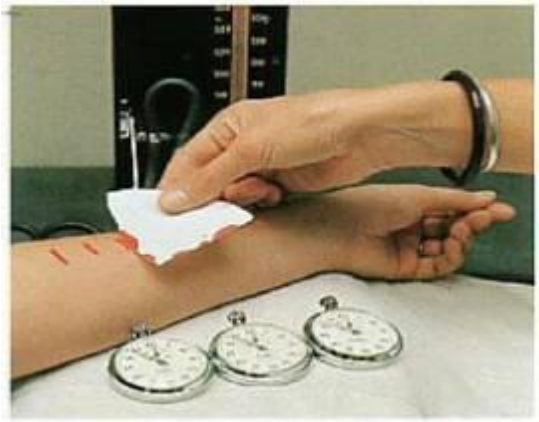


Procedure Notes: Sources of Error: Errors producing false positive results

- Blood pressure cuff maintained too high (>40mm Hg.)
- Incision too deep, caused by excessive pressure on the incision device.
- Disturbing the clot with the filter paper.

The causes of long bleeding time. ¤ Sever leukemia. ¤ Decrease number of platelets. ¤ Sever allergy. ¤ Low fibrinogen (<100 mg/dl) or platelet count (100,00 /mm3).
¤ Drug ingestions affecting platelet function (e.g. aspirin) **Errors producing false negative results**

- Blood pressure cuff maintained too low (<40 mm Hg).
- Incision too shallow.



Differential white blood cells count

Learning objectives:

1- Classify the WBC.

2- Know the types of fixed leukocytes**:**

Differential white blood cells count

Is performed to determine the relative number of each type of white blood cells is done on stained blood films on ordinary microscope slides.

Normal value

Neutrophil –the percent is 65%, Eosinophil – 1-3% Basophil 0.5-1% Monocyte 7% Lymphocyte 30%

Manual differential W.B.C.S count:

Staining the blood smear:- The air dried smear should be stained within one hour of preparation with one type of stains. If the staining cannot be done within that time, then the smears should be fixed.

Leishman's stain: contain → eosin.

- Acetone (free absolute methyl alcohol), acting as a solvent for these stain, it is also fixative.
- Methylene blue.

Materials & instruments:

1. Whole blood
2. Glass slides
3. Microscope
4. Leishman's stain
5. Alcohol 70%
6. Lancet

- **Staining method:**

1. Dry theblood films by air for at least 10 minutes using a fan
2. Place your prepared film on the staining rack for 2-3 minutes
3. Carefully dilute Leishman's stain with double the volume of buffered water or distilled water.
3. Leave for 10 minutes (allow the diluted stain to act).
4. Wash the stain in distilled water, shake excess water away.
5. Wipe the back of the slide & dry using fan.

- The dry stained blood film is examined without cover slip under the oil immersion objective.
- Every white cells seen should be recorded in a table under the following heading:

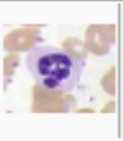
Neutrophil, Eosinophil, Basophil, Monocyte, Lymphocyte A total of 100 cells should be counted. Find the number, percentage of each type, present in each cubic millimeter of blood.

• A commonly performed laboratory test is the "*differential*"

– A high WBC count with an increase in granulocytes indicates a bacterial infection

– A high WBC count with predominantly immature WBCs present indicates leukemia

– A low WBC count with an increase in the lymphocytes indicates a viral infection.

Type	Microscopic Appearance	Diagram	Approx. % in adults[6] See also: Blood values	Diameter (µm)	Main targets	Nucleus	Granules	Lifetime
Neutrophil			54–62%[10]	10–12	• bacteria • fungi	multilobed	fine, faintly pink (H&E Stain)	6 hours–few days (days in spleen and other tissue)
Eosinophil			1–6%	10–12	• larger parasites • modulate allergic/inflammatory responses	bi-lobed	full of pink-orange (H&E Stain)	8–12 days (circulate for 4–5 hours)
Basophil			<1%	12–15	• release histamine for inflammatory responses	bi-lobed or tri-lobed	large blue	a few hours to a few days
Lymphocyte			25–33%	7–8	• B cells: releases antibodies and assists activation of T cells • T cells: Natural killer cells: virus-infected and tumor cells.	deeply staining, eccentric	NK-cells and Cytotoxic (CD8+) T-cells[1]	weeks to years
Monocyte			2–10%	14–17	Monocytes migrate from the bloodstream to other tissues and differentiate into tissue resident macrophages or dendritic cells.	kidney shaped		hours to days
Macrophage				21 (human)	Phagocytosis (engulfment and digestion) of cellular debris and pathogens, and stimulation of lymphocytes and other immune cells that respond to the pathogen.		none	activated: days immature: months to years
Dendritic cells					Main function is as an antigen-presenting cell (APC) that activates T lymphocytes.			similar to macrophages

Red Blood Cell Count

Learning objectives:

1-Know red cell counting method. 2- Identify haemocytometer.

RBCs count : Is the number of red blood cells per cubic millimeter (volume) of whole blood?

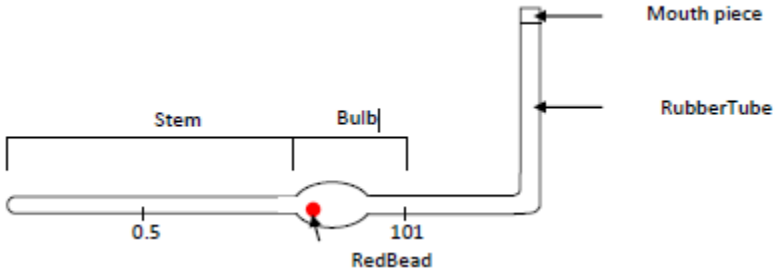
Principles:

The method consists in the accurate dilution of a measured quantity of blood with an isotonic solution which also prevents coagulation.

Manual Red Blood Cell Count Method & apparatus

1. Blood sample.

2. R.B.C. pipette. Consist of a capillary tube marked with figures 0.5, and 101 (stem), and bulb contains a small red bead to mixing the blood, and the rubber tube.

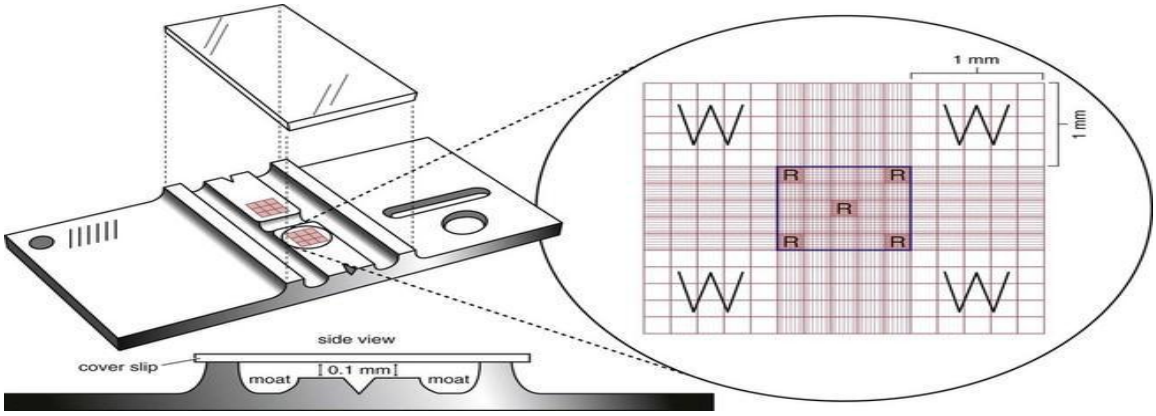


3-Diluting fluid (Formal Citrate): Sodium citrate 3gm Formaldehyde 4ml Safranine (few drops) D.W. 100ml

4-Alcohol 70%, lancet, cotton.

5-Neubour chamber+ coverslide (Haemocytometer) .

6-Microscope.



Procedure: 1-Wipe your partners finger with cotton soaked with alcohol and allows it to dry. With a sterile disposable lancet do small prick on the finger tip.

2-blood from a finger punctured is gently sucked into the pipette up to the mark 0.5. The tip of the pipette is then immediately dipped into the diluting fluid, this fluid sucked up to the mark 101.

3-Mixthe contents by holding the pipette horizontally and rotating it between the fingers.

4-Avoid trapping small bubbles of air while sucking up the blood or the diluting fluid, if you have air bubble, the dilution will be wrong & you will have to wash out the pipette and start again.

5-between 1 and 101, there is a volume of 100 which contains the diluted blood. The dilution of this blood is 0.5 in 100.

6-about one third of the contents of the bulb is blown out and discarded ,this washes away the diluting fluid in the stem. Then hold the pipette at an angle of 45_o.The tip is made to touch the space between the cover slid and the counting chamber, the fluid run under the cover slid, bubbles should be avoided.

7. Place the counting chamber under the microscope and allowed to settle for 2-3minutes.

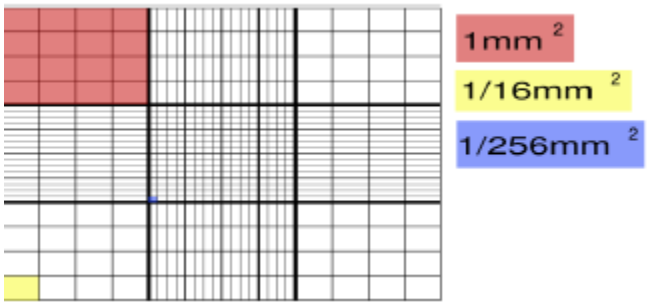
8. Examine the preparation under low power objective, first to see the distribution of the cells, and then count with high power objective.

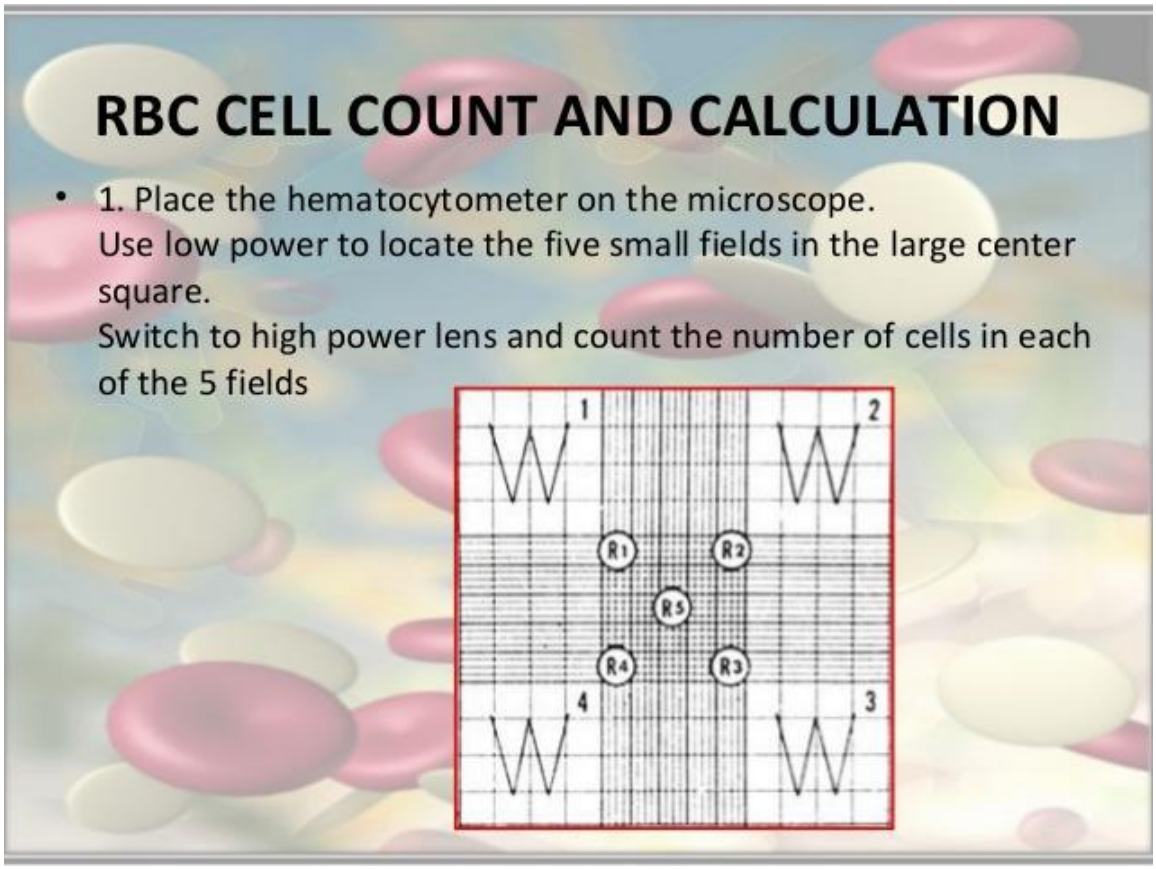
9. Count the total number of red blood cells in 5 intermediate squares; four of them in the corners and one from the center. You will have to count 16 small squares for each intermediate squares (i.e. a total of 80 small squares). All the corpuscles which touch the upper and the left-hand lines of one square are counted, these which touch lower or right hand lines are considered outside the square.

Counted the number of R.B.C. in 80 small squares, each one of these squares has an area of A depth of counting platform chamber is The volume of fluid above each small square is = (X) 1/4000x 80=1/50 If the number of cells in 80 small squares is N, then the number of cells in cubic millimeter of diluted blood is =N x 50 x200

=N x 10000

= ? Cells.


1mm 2
1/16mm 2
1/256mm 2


RBC CELL COUNT AND CALCULATION
1. Place the hematocytometer on the microscope.
Use low power to locate the five small fields in the large center square.
Switch to high power lens and count the number of cells in each of the 5 fields
1
2
R1
R2
R5
R4
R3
4
3

White Blood Cells Count (WBCs)

The Total Leukocyte Count Learning Objective:

1- Know white cell counting method.

2- 2- Identify haemocytometer**.**

White Blood Cells Count

It is counting the number of the different types of leucocyte in one c.mm of circulating blood. These cells are the infection fighting portion of the blood and play a role in inflammation. A low count can indicate bone marrow problems, chemical exposure, autoimmune disease, and problems with the liver or spleen. High levels can indicate the presences of tissue damage (burns), leukemia and infectious disease

Principle: The method consists in the accurate dilution of a measured quantity of blood using a special type of dilution fluid (Turk‹s Fluid).

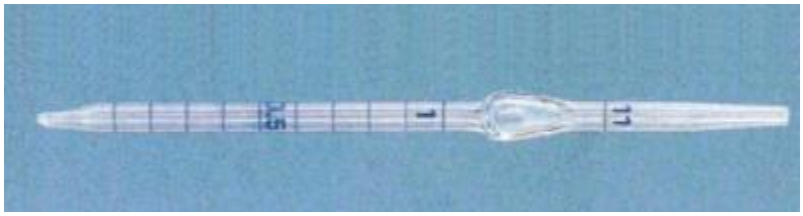
Materials &Instruments :

1- Whole blood

2- Turk‹s diluting fluid :

Reagent of Turk's fluidfunction A – Glacial acetic acid to haemolyse R.B.C. B – Aqueous gentian violet to color the nuclei of W.B.C. C – Distilled water

3-W.B.C. Pipette : consists of capillary tube marked with the figures 0.5 , 1.0 (stem) , and 11 ,with a bulb between the marks 1 and 11 , the bulb contains a small white bead.



4 – Haemocytometer(Neubauer‹s counting chamber), with cover class.

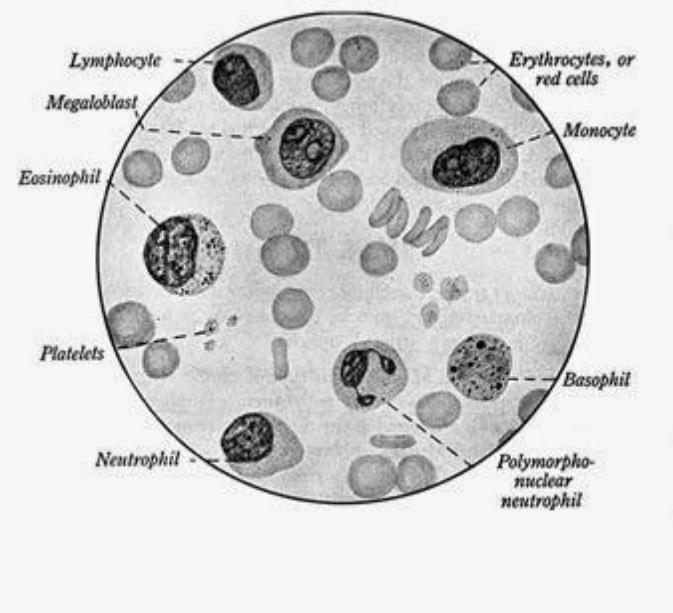
5 – Microscope .

6 – Lancet .

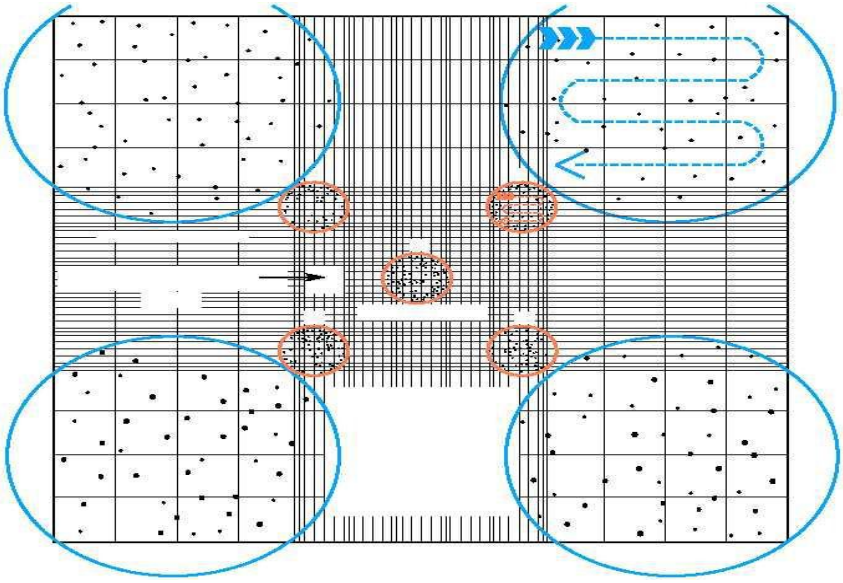
7 – Alcohol .+ cotton

Normal value:
¤**Adult :4000-11000 c/mm.**
¤**Children (1-10 year) : 4500-13000 c/mm.**
Infant : 10000-25000 c/mm.

Procedure:
1- Obtain a drop of blood in the same manner as in R.B.C. count, draw blood from a finger puncture in W.B.C. pipette to the mark 0.5
2- Wipe off quickly any extra blood that may be attached to the outside of the tip of pipette.
3- Dip down the tip of pipette immediately in white cell solution, suck the fluid gently up to mark 11
4- Mix the contents by rotating the pipette between two fingers, the white bead helps in the mixing.
5- Avoid sucking air bubbles throughout the process.
6- The dilution is 1/20
7- Discard the first four drops of the mixture , the pipette is then hold at an angle of 45 , the tip is made to touch the space between the cover slip and counting chamber platform , the fluid will run under the cover slip .
8- Focus the microscope in the centre of the central platform using the low power objective.
9- Count the total number of W.B.C. throughout the one large square



Calculation : * Each large square has an area 1sq mm. * The depth of the chamber is 1/10 mm. Therefore the volume of the fluid covering 1 large square is 1/10 cubic mm. The diluted blood will be =10 x N . The blood is diluted 20 times . Then 1cmm of blood contain N x 10 x 20 . Total Number



HEMACYTOMETER (COUNTING CHAMBER) IMPROVED NEUBAUER RULING A - B - C - D ARE FIELDS USED IN DOING THE WHITE BLOOD CELL COUNT 1 - 2 - 3 - 4 - 5. ARE FIELDS USED IN DOING THE RED BLOOD CELL COUNT. (Letters, numbers, and arrows are not actually seen in the counting chamber. They are for illustration only. Circles depict areas seen through the microscope.) Low power (100X)

Clotting time (C.T.)(Coagulation time)

Learning objectives:

1. Will be able to measure (CT). 2-Learn about clotting time (CT).

C.T. Is the time required for a measured amount of blood to clot under certain specialized condition.

Two Methods 1- Capillary tube method;

(Dale and laid) method 2- Lee and While method.

1. Capillary method:- Material required: 1- Capillary tube (non-heparinized). 2- Stop-watch. 3- Lancet, cotton and alcohol.

Procedure: 1-Draw blood into capillary tubes (non-heparinized) 2-Start stop watch immediately after puncture happened 3-Examine at 30 second intervals for clotting by break a piece of capillary tube and see for fibrin threads.

Note: the time is when clotting first seen. *In the normal case clotting begins 1- 8* minutes

●Lee and While method:-

Material required:

1-Four test tubesand rack. 2- Water bath. 3- Stop-watch (four). 4- Blood sample. 5- Plastic syringe, cotton and alcohol.

Procedure:-

1-Fill the water bath by D.W. at 37c°.

2- Let the rack with four test tubes in water bath.

3- Draw 4ml of blood by plastic syringe from vein

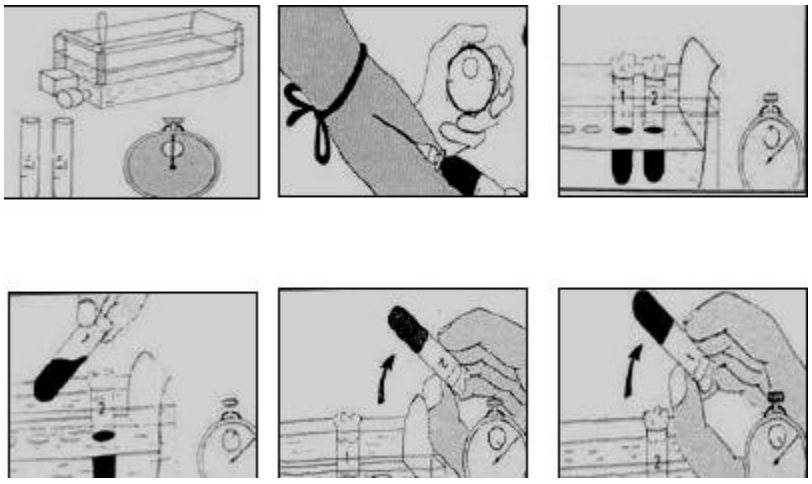
. 4- Start the watch after appearing of blood in the syringe.

5- Transfer 1ml of blood to each test tube.

6- After one minuteremoveone test tube from water bath and see the fibrin formation.If you don't see fibrin, leave all tubes in water bath for 1/2 min and see the clot formation.

7- Stop the watch immediately if you see the fibrin.(clotting)

Normal range of clotting time is 5-11 minutes (usually 6-7 minute at 37oC)



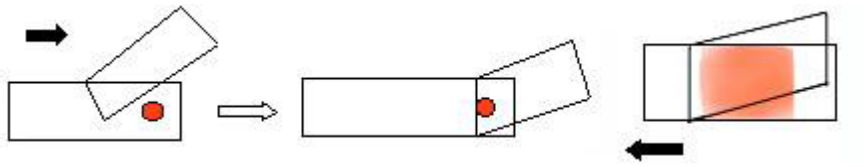
The Blood Film

Learning objectives:

1-How to make and stain the blood film. 2-Examine blood smears. 3-Explain blood film. An increased white count may be due to bacterial infection or an acute or chronic leukemia.

Slide Method of Spreading a Blood Smear

- 2 clean slides, one to cover with blood film & one used as spreader. (Never put your fingers on the surface of the slide).
- Clean the finger with alcohol, allow it dry & then prick it with a disposable lancet to obtain a drop of blood.
- Place a small drop of blood near end of glass slide.
- Place the spreader at approximately 40-45$_o$ in front of the drop and move the spreader back to the right until it just touches the drop. The drop immediately runs along the edge of the spreader. Then move the spreader slide to the left direction.

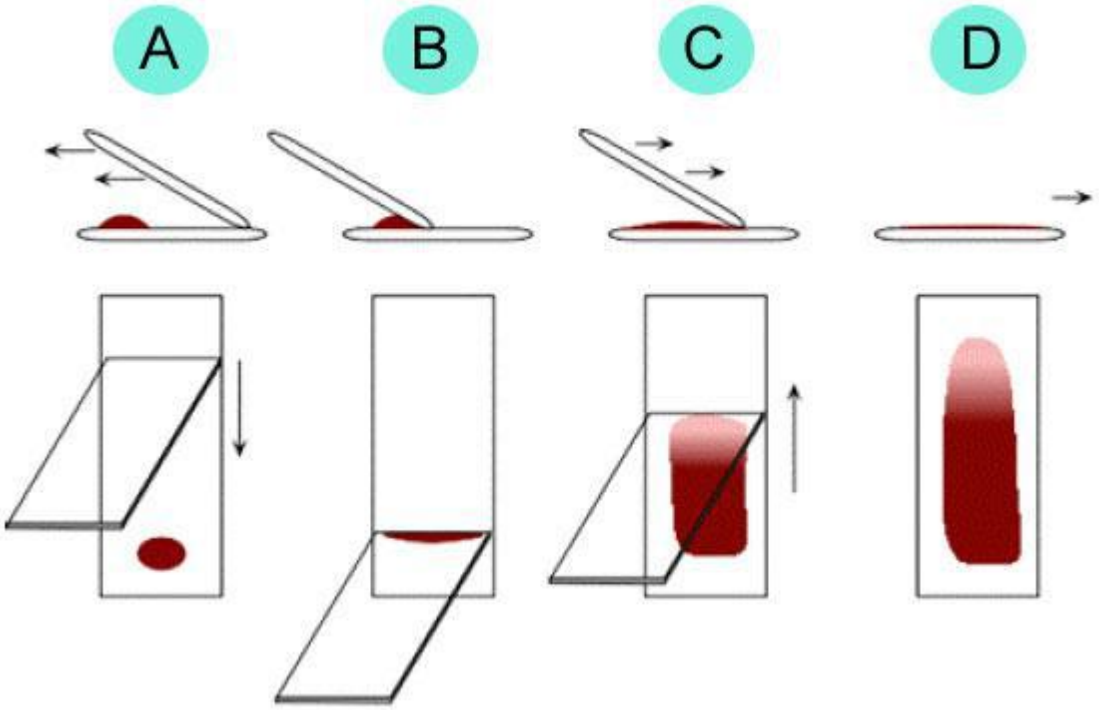


- Thick, viscous, blood should be spread rapidly with the spreader at a lower angle. Thin, anaemic, blood should be spread slowly with the spreader held at a greater angle (up to 50-60^o).
- The finished smear should have a nicely rounded tail.
- Allow the smear to dry in air at room temperature; unheated table fans can be used to hasten the process.
- Stain the smear with Leishman's stain.

Examining blood smear:-

- The examining of a well-made, well stained smear is a very important part of the full blood examination and essential parameter in a hematologic profile.

- Every blood smear should be examined first by low power (X10) objective to find the body of the film and to check the total white cell count to see that there is nothing abnormal or unusual about the red cells, observe abnormalities of red cells such as size, shape, Hb content & inclusions.
- After these observations, rack around to high power oil immersion to confirm findings.



ABO Blood group system

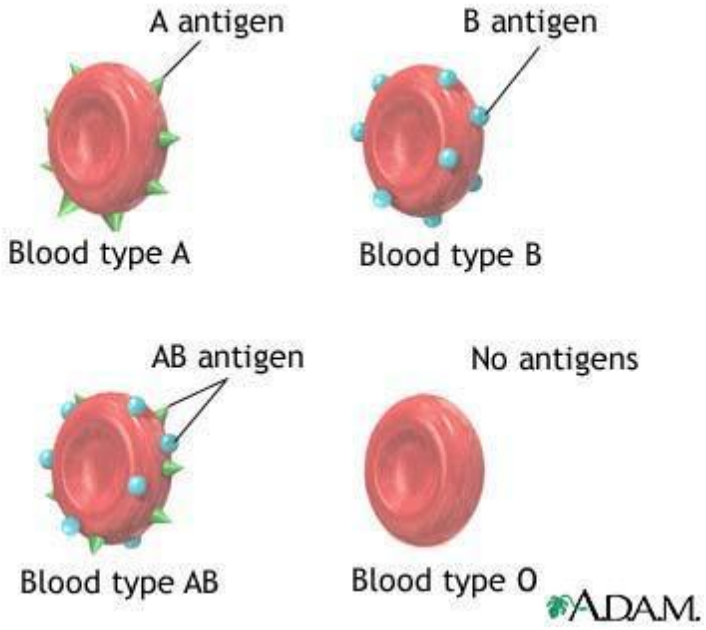
Classification of human blood according to whether red blood cells (erythrocytes) have or lack the inherited antigens called A (including A1 and A2) and B on their surface.

Blood type O (lacking both),

type A (having only A),

type B (having only B)

type AB (having both).



The ABO antigens make certain blood types incompatible for transfusion. They are developed well before birth and remain through life. The frequencies of blood groups vary among different racial groups and in different geographic areas**.**

Recipient	Blood donor			
	O	A	B	AB
O	✓	X	X	X
A	✓	✓	X	X
B	✓	X	✓	X
AB	✓	✓	✓	✓

The antigen and the antibody in different blood groups is as follows:-

Blood group	Antigen agglutinate (in RBC)	Antibody Agglutinin (serum)
A	A	Anti – B
B	B	Anti – A
AB	A + B	(-)
O	–	Anti A + B

There are two methods for blood grouping:-

1-Slide method

2-Tube method

1. The Technique of Slide Method:- In an emergency, ABO grouping may be carried out rapidly on tiles, the method is only a little, satisfactory than the tube method in grouping a patient before transfusion.

It is less satisfactory for determining the exact group of a blood donor.

- Principle :-
40 % of cell suspension is mixed with anti – A and anti – B grouping sera and examined for agglutination (clump).

Blood sample	Anti- A serum	Anti-B (serum)	Group
1	+	-	A
2	-	+	B
3	+	+	AB
4	-	-	O

- In blood sample 3 there is clumping in anti- A and anti – B because the red cell have both A and B antigen .
- In blood sample 4 as there is no antigen in red blood cell, there is no clumping

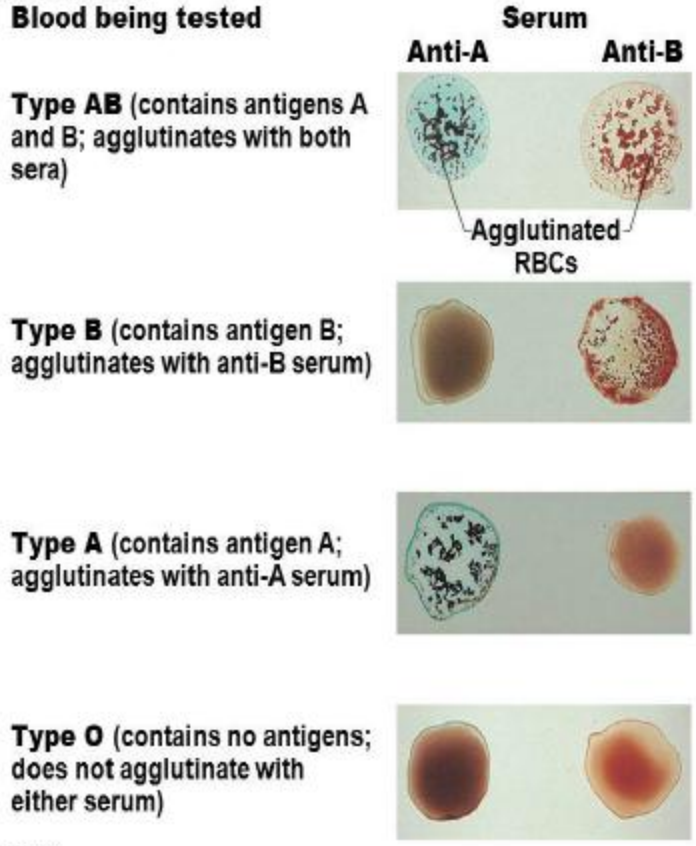
Material required :-

1- clean slides
2- 40% blood cell suspension (washed) 3 times and then 1 volume of cell + volume of saline.
3- Wax pencil
4- Wooden applicator.

Procedure :-

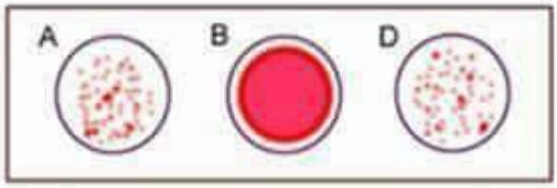
1- Divide slide in 2 portion by the wax pencil on left portion write A and on right write B
2- On A portion take drop of 40% cell suspension and a drop of anti – A group serum.
3- On the other portion, put a drop of 40% cell suspension and drop of anti – B grouping serum.
4- Mix with wooden applicator.
5- Examine for agglutination after 2-3 min.

Figure 10.8 Blood typing of ABO blood groups.

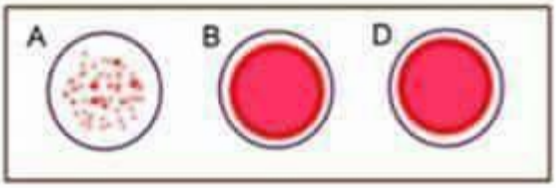


(The Technique of Slide Method For Blood Grouping)

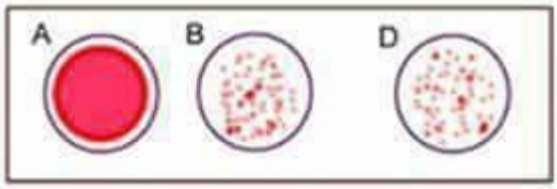
Reading Blood Grouping Results



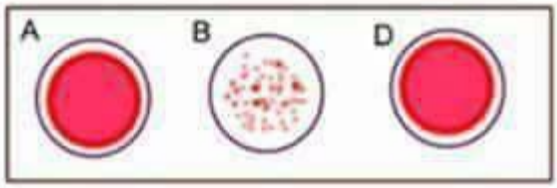
A Positive



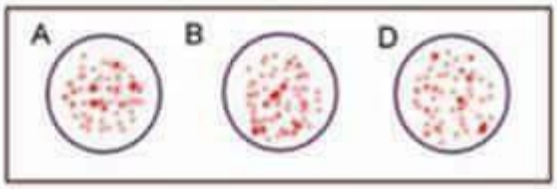
A Negative



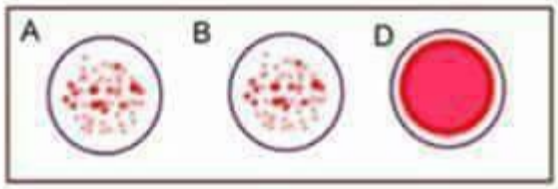
B Positive



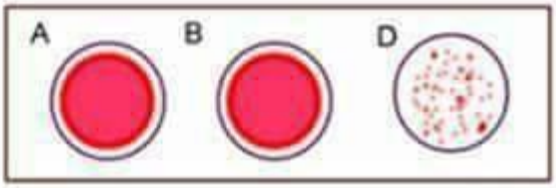
B Negative



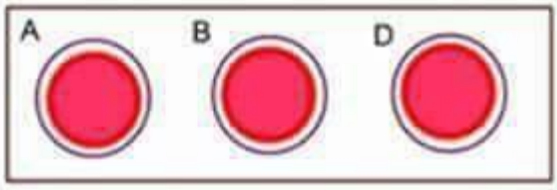
AB Positive



AB Negative



O Positive



O Negative

Rh- Grouping

For most clinical purpose it is sufficient to determine whether a subject is Rh – positive or Rh- negative. This division is made by testing the red cells with the commonest type of Rh antibody known in the fisher nomenclature as Anti- D. In most of the blood transfusion laboratories, Rh (D) grouping is performed along with the ABO grouping and same techniques as used for ABO grouping may also be employed for Rh typing In transfusion centers the red cells of Rh – negative donors are further tested for the presence of the c and E antigens.

- Three Methods used in determine Rh grouping :-

1- Slide Method
2- Sandwitch Method
3- Tube Method

Slide Method:- Principle:-

A (40%) of blood cell suspension is mixed with anti – D grouping serum on a slide. The mixture is left for 1-2 minute and the slide slowly tilted to study the agglutination. The slide is then examined for formation of agglutination.

- **Material Required**:

1- Anti – D grouping serum.
2- 2- 40% patients blood cell suspension.
3- Slide
4- 4- Wooden applicator

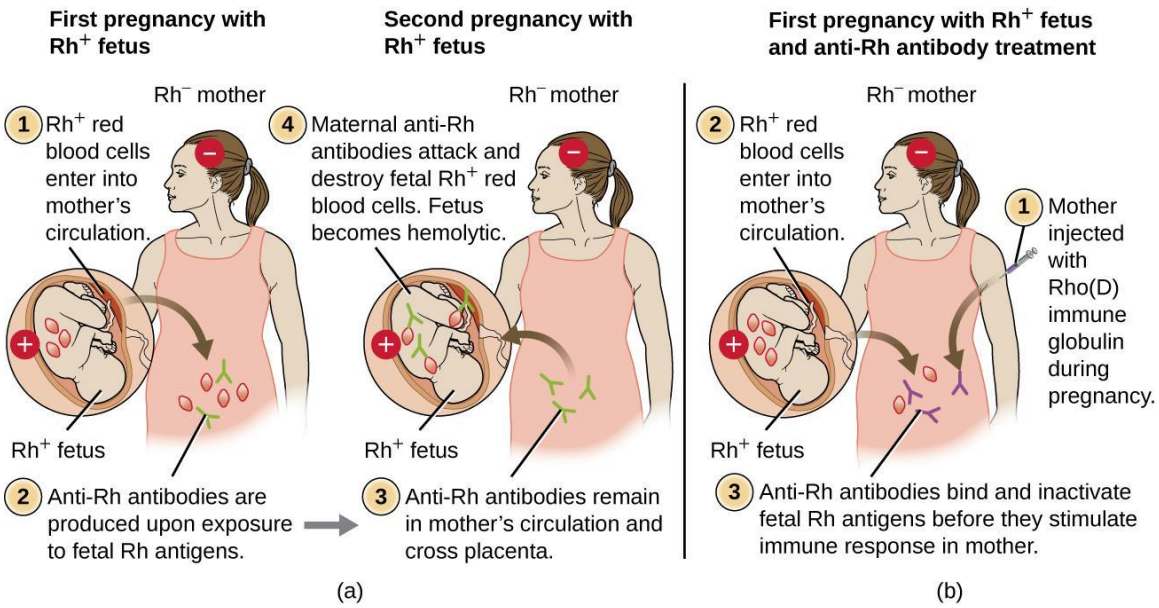
- Procedure:-

1- One drop of anti D grouping sera is added to one drop of 40% patient's cell suspension.
2- Mix with wooden applicator
3- Examine after 2-3 min for agglutination.

Agglutination---------- Rh +

No Agglutination---------- Rh

R*@296JilrlC(zlRXvr8n@&W



Rh Factor

- The Rh factor was first identified in the blood of a rhesus monkey. Also called **Rhesus factor.**
- If an Rh- person receives Rh+ blood, hemolysis and anemia occur.
- **Rh** factor is very important, specially in pregnancy.
- If mother is Rh- and the fetus is Rh+, A condition called **Erythroblastosis Fetalis** occurs, which can cause fetal death.

Blood pressure

What is the high blood pressure? High blood pressure (HBP) is a serious condition that can lead to coronary heart disease (also called coronary artery disease) , heart failure, stroke kidney failure , and other health problem. Blood pressure; is the force of blood pushing against the walls of the arteries as the heart pumps blood . if this pressure rises and stays high over time.

Blood pressure numbers :

Blood pressure is measured as systolic and diastolic pressure (systolic) refers to blood pressure when the heart beats while pumping blood . (diastolic) refers to blood pressure when the heart is at rest between beats. You most often will see blood pressure number written with the systolic number above or before the diastolic number , such as 120/80 mmHg. (the mmHg is millimeters of mercury_ the units used to measure blood pressure) The table below shows normal blood pressure numbers for adults . It also shows which number put you at greater risk for health problems,

category	**Systolic (top number)**		**Diastolic (bottom number)**
normal	Less than 120	and	Less than 80
prehypertention	120_ 139	or	80-89
High blood pressure			
Stage 1	140- 159	or	90-99
Stage 2	160 or higher	or	100 or higher

The condition and blood pressure Blood pressure doesn't stay the same all the time , it lowers as you sleep and rises when you wake up. Blood pressure

also rises when you're excited, nervous, or active. If your number stay above normal most of the time, you're at risk for health problems. All levels above 120/80 mmHg raise your risk , and the risk grows as blood pressure number rise , "prehypertension" means you're likely to end up with HBP, unless you take steps to prevent it . Your systolic and diastolic number may not be in the same blood pressure category In this case , the more sever category is the one you're in , for example If your systolic number is 160 and your diastolic number is 80 , you have stage 2 HBP . if your systolic number is 120 and your diastolic is 95 , you have stage 1 HBP, if you have diabetes or chronic kidney disease . HBP is defined as 130/80 mmHg or higher . HBP numbers also differ for children and teens. **Causes of hypertension :** Hypertension occurs when blood forced the arteries at an increased pressure. The hypertension is considered to be present when a person systolic blood pressure is consistently(140 mmHg) or greater and their diastolic blood pressure is consistently (90mmHg) or greater for more than 90% of people with high blood pressure the cause is unknown . this called primary or essential hypertension .the remaining 10% is an underlying cause, this called secondary hypertension . -some of the main causes for secondary hypertension:- 1- chronic kidney disease. 2-disease in the arteries supplying the kidney 3-chronic alcohol abuse
4-hormonal disturbance 5-endocrine tumors

Factor of essential hypertension which increase risk of hypertension;

1- A tendency in the family (genetics)

2- Obesity

3- Smoking

The rules should followed before blood test :-

1- Don’t smoking before testing for 30 minute and don't take any drink contain caffeine

2- The person should have a rest for 5 minute after coming

3- Don’t chewing gum , and don’t speak while doing test

4- You should repeat the test to ensure of it .

Blood Pressure Test: Procedure :

- The patient should sit at chair with comfortably and butting the hand at same level with heart

- Linked to the belt on hand (above the elbow) so that it is a good side when the belt line that appears when the elbow joint

- Place the handset (**stethoscope**) below the belt and established gently – the best place to be heard over the artery

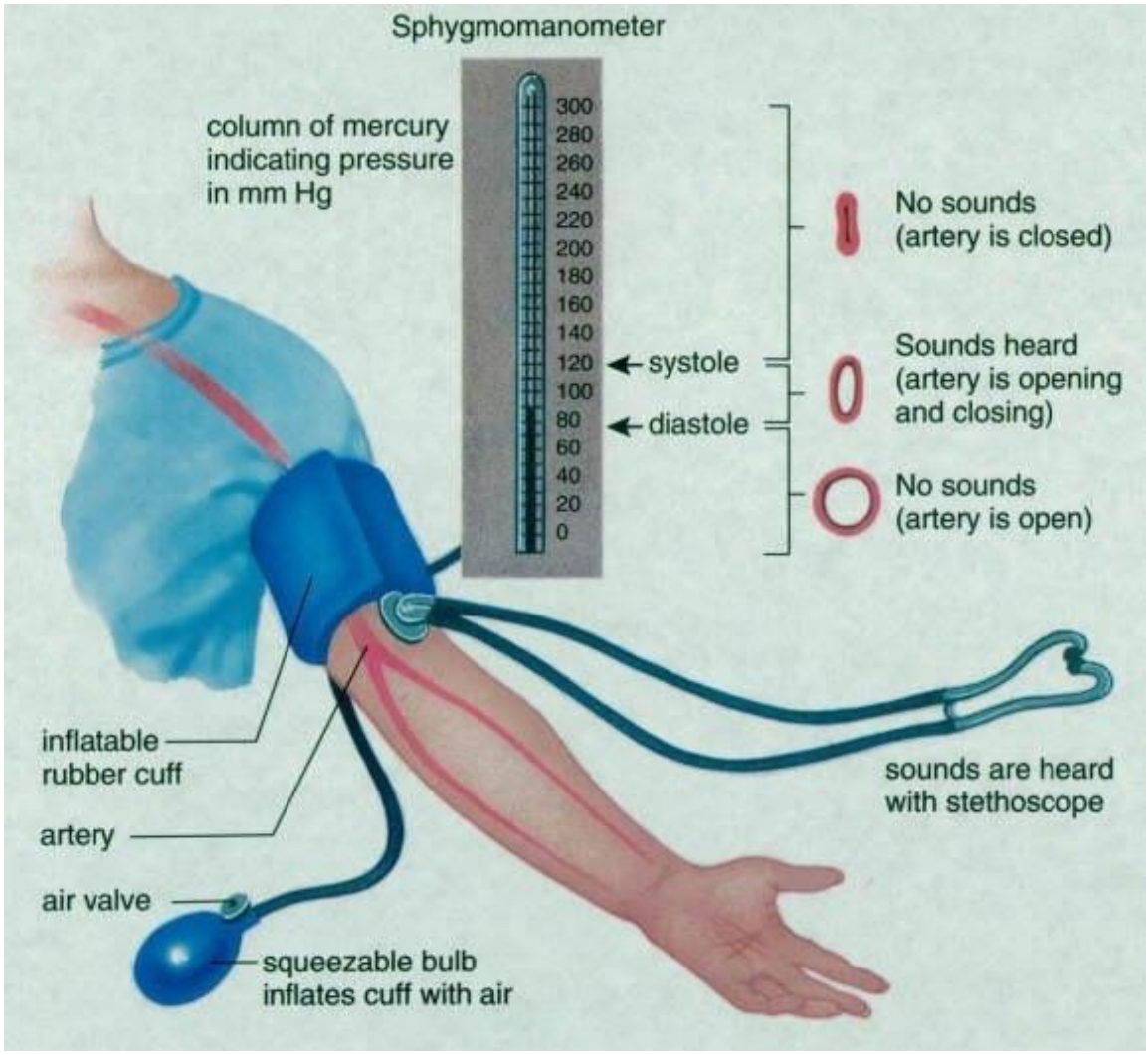
- Close the air valve of air bladder

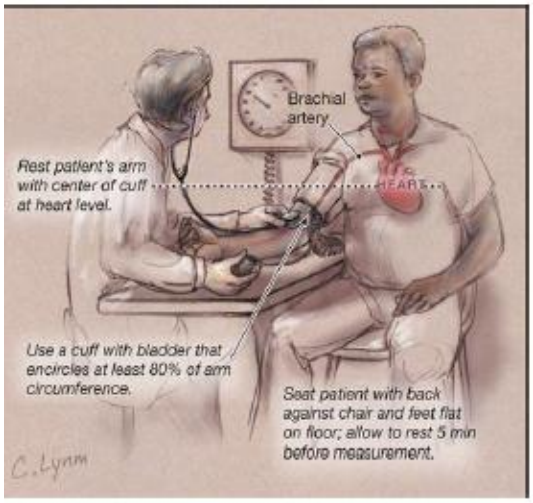
- Then full up the bladder air of pressure measurement instrument (**sphygmomanometer)** until it stops blood from flowing here and not here any sound of blood in the handset (stethoscope)

- Open the valve of air bladder gently so the blood will go on it way and then you can hear the sound of blood (select this point : this is systolic pressure)

- Continuously open the valve until you are don't hear any sound

(select this point : this is diastolic pressure




Brachial artery
Rest patient's arm with center of cuff at heart level.
HEART
Use a cuff with bladder that encircles at least 80% of arm circumference.
Seat patient with back against chair and feet flat on floor; allow to rest 5 min before measurement.
C.Lynn


Sphygmomanometer
This gives the blood pressure readings
Stethoscope
Inflatable cuff

Measurement of Blood Pressure

Most people have blood pressure taken at some time. It is a simple and painless procedure that gives a lot of useful information about the heart and the condition of the blood vessels. **Blood pressure:** refer to force exerted by circulating blood on the walls of blood vessels. Systolic arterial pressure : defined as the peak beginning of the cardiac cycle. (ventricle construction) Diastolic arterial pressure: is the lowest pressure at the resting phase of the cardiac cycle . -normal blood pressure :-
120 /80 mm/Hg Less 120 Systolic Less 80 Diastolic mmHg__ millimeters of mercury. **How is blood pressure measured:**
=

1- A cuff that inflates is wrapped around your upper arm and kept in place with Velcro . A tube leads out of the cuff to a rubber bulb.

2- Another tube leads from the cuff to a reservoir of mercury at the bottom of a vertical glass column . whatever pressure is in the cuff is shown on the mercury column . the mercury is held within a sealed system - only air travel in the rubber tubing and the cuff .

3- Air is the blown into the cuff and increasing pressure and tightening is felt on the upper arm

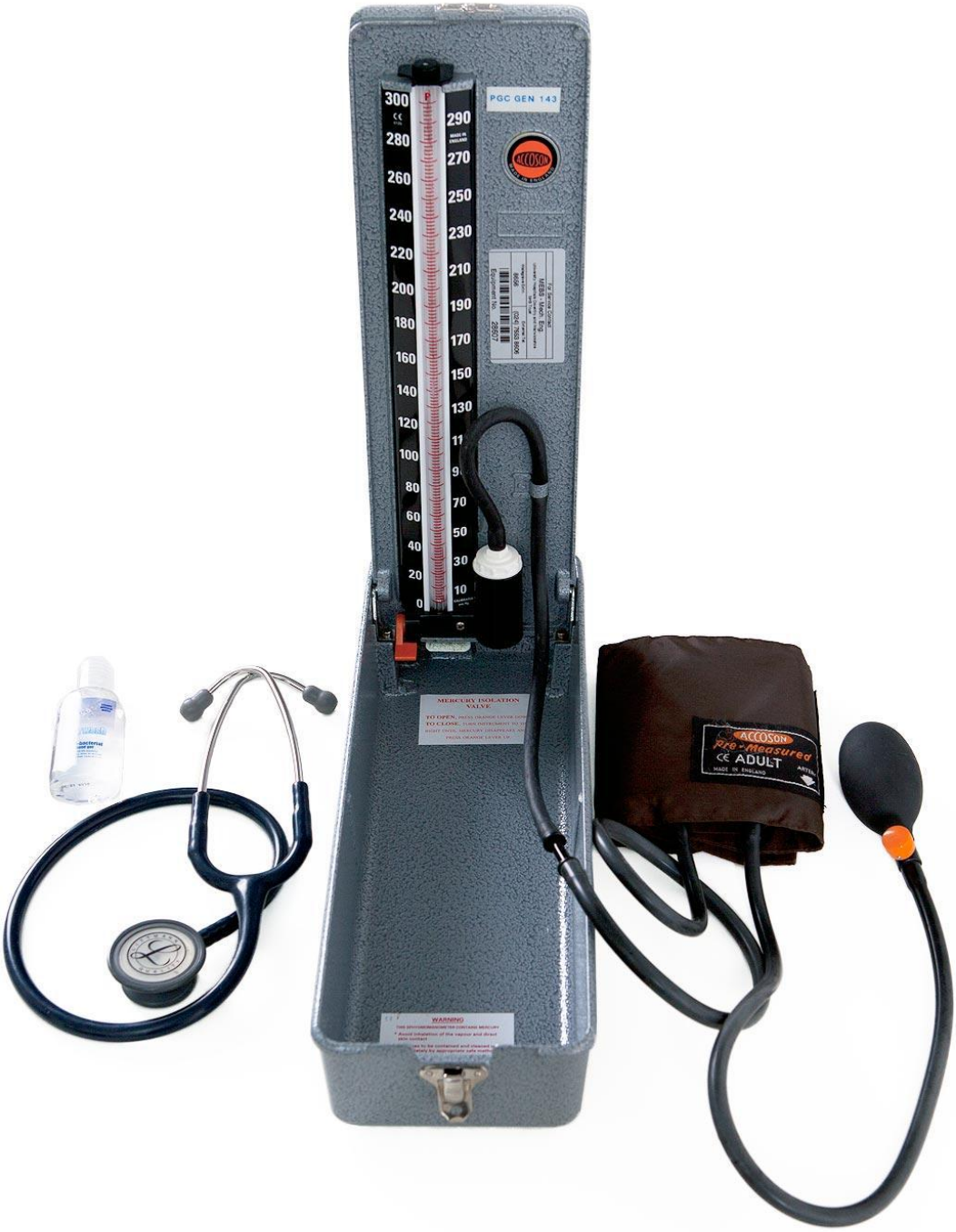
4- The doctor puts a stethoscope to your arm and listens to the pulse while the air is slowly late out again .

5- The systolic pressure is measured when the doctor first hears the pulse

6- This sound will slowly become more distant and finally disappear

7- The diastolic pressure is measured from the moment the doctor Is enable to hear the sound of the pulse

8- The blood pressure is measured in terms of millimeters of mercury (mmHg).


PGC GEN 143
ACCOSON
MADE IN ENGLAND
MERCURY ISOLATION VALVE
WARNING
ACCOSON
Pre-Measured
CE ADULT
MADE IN ENGLAND