SRI SHANMUGHA COLLEGE OF ENGINEERING AND TECHNOLOGY

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BM8313 HUMAN PHYSIOLOGY PRACTICALS

Department of Biomedical Engineering

PREPARED BY

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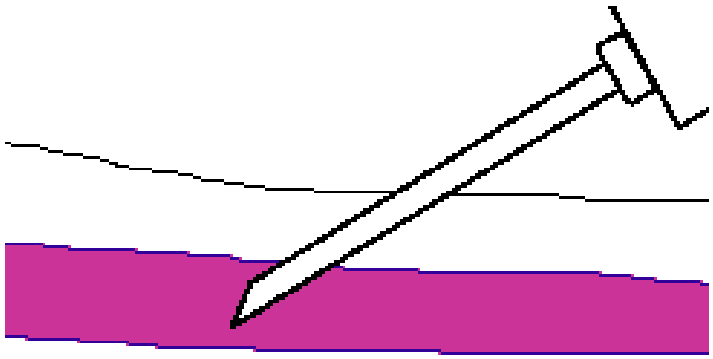
ASP/CHEMISTRY

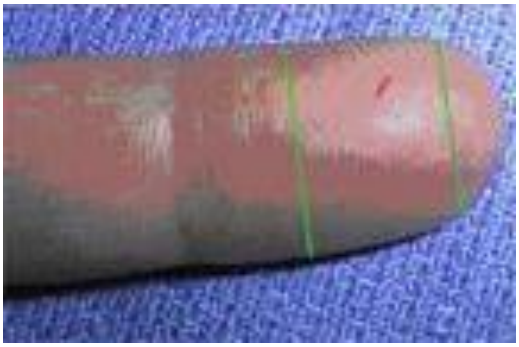


EX.NO: 01 **PROCEDURE FOR COLLECTION OF BLOOD**

DATE: **SAMPLES AND STORAGE**

Venepuncture 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. See below for venipuncture site selection "notes."
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. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. 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.



14. 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.

Fingerstick 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.

Areas to Avoid When Choosing a Site for Blood Draw:

Certain areas are to be avoided when choosing a site for blood draw:

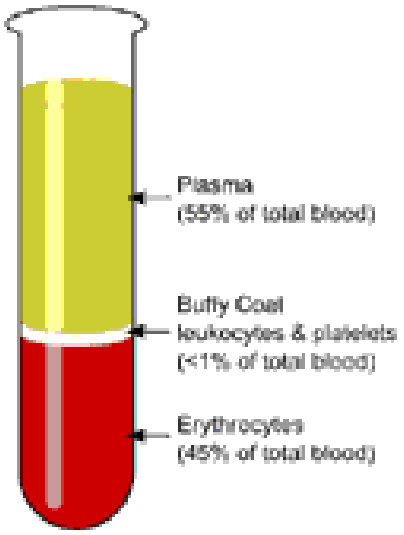
1. Extensive scars from burns and surgery - it is difficult to puncture the scar tissue and obtain a specimen.
2. The upper extremity on the side of a previous mastectomy - test results may be affected because of lymphedema.
3. Hematoma - may cause erroneous test results. If another site is not available, collect the specimen distal to the hematoma.
4. Intravenous therapy (IV) / blood transfusions - fluid may dilute the specimen, so collect from the opposite arm if possible.
5. Cannula/fistula/heparin lock - hospitals have special policies regarding these devices. In general, blood should not be drawn from an arm with a fistula or cannula without consulting the attending physician.
6. Edematous extremities - tissue fluid accumulation alters test results.

Techniques to Prevent Hemolysis (which can interfere with many tests):

1. Mix all tubes with anticoagulant additives gently (vigorous shaking can cause hemolysis) 5-10 times.
2. Avoid drawing blood from a hematoma; select another draw site.
3. If using a needle and syringe, avoid drawing the plunger back too forcefully.
4. Make sure the venipuncture site is dry before proceeding with draw.
5. Avoid a probing, traumatic venipuncture.
6. Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).
7. Avoid massaging, squeezing, or probing a site.
8. Avoid excessive fist clenching.
9. If blood flow into tube slows, adjust needle position to remain in the center of the lumen.

Blood Sample Handling and Processing:

Pre-centrifugation Handling - The first critical step in the lab testing process, after obtaining the sample, is the preparation of the blood samples. Specimen integrity can be maintained by following some basic handling processes:


Plasma
(55% of total blood)
Buffy Coat
leukocytes & platelets
(<1% of total blood)
Erythrocytes
(45% of total blood)

1. Fill tubes to the stated draw volume to ensure the proper blood-to-additive ratio. Allow the tubes to fill until the vacuum is exhausted and blood flow ceases.
2. Vacutainer tubes should be stored at 4-25°C (39-77°F).
3. Tubes should not be used beyond the designated expiration date.
4. Mix all gel barrier and additive tubes by gentle inversion 5 to10 times immediately after the draw. This assists in the clotting process. This also assures homogenous mixing of the additives with the blood in all types of additive tubes.
5. Serum separator tubes should clot for a full 30 minutes in a vertical position prior to centrifugation. Short clotting times can result in fibrin formation, which may interfere with complete gel barrier formation.

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.

1. 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.
2. 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.
3. NOTE: Gel flow may be impeded if chilled before or after centrifugation.
4. Tubes should remain closed at all times during the centrifugation process.
5. Place the closed tubes in the centrifuge as a “balanced load” noting the following:
6. Opposing tube holders must be identical and contain the same cushion or none at all.
7. Opposing tube holders must be empty or loaded with equally weighted samples (tubes of the same size and equal in fill).
8. 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.

Centrifuge Safety

1. Interference with an activated centrifuge by an impatient employee can result in bodily injury in the form of direct trauma or aerosolization of hazardous droplets.

2. Centrifuges must never be operated without a cover in place.
3. Uncovered specimen tubes must not be centrifuged.
4. Centrifuges must never be slowed down or stopped by grasping part(s) of the device with your hand or by applying another object against the rotating equipment.
5. Be sure the centrifuge is appropriately balanced before activating. If an abnormal noise, vibration, or sound is noted while the centrifuge is in operation, immediately stop the unit (turn off the switch) and check for a possible load imbalance.
6. Clean the centrifuge daily with a disinfectant and paper towel. Broken tubes or liquid spills must be cleaned immediately.

EX.NO: 02 DETERMINATION OF BLOOD GROUP

DATE:

AIM:

To perform a human blood typing experiment to demonstrate the agglutination.

PRINCIPLE:

Although not directly genome to the study of microbiology ABO blood typing is aexcellent demonstration of the stereological principle of agglutination around the turn of the century,it was determined by Carl Landstainer that those for four different immunological blood types. This theory was bound on the fact that two distinct antigen (agglutination) AB could be present on the surface of the blood cells on the present or absent of either or both of these antigens, blood types were established as A, B, AB, or O constitute the ABO.

CLASSIFICATION SYSTEM:

Antigen(agglutinogen)	Plasma antibody	Blood group
A	Anti-B	A
B	Anti-A	B
A+B	None	AB
None	Anti-A & Anti-B	O

ABO- Classification system:

The medical important is a fact that the fluid portion of the blood plasma may contain antibodies (agglutinates). If present, these antibodies are not reactive against the individuals own red blood cells antigen. When mixed with ABO antigen of a different blood type however as during the course of blood transfusion, a violet incompatible reaction may occur.

The ABO blood typing is a routine prerequisite to blood transformation. In the experiment on the ABO blood typing procedure is done by separately mixing a drop of blood with Anti-A and AntiB serum on a glass slide. The determination of blood type was made by observing agglutination on the slide.

Materials:

REAGENTS:

Anti-A & Anti-B blood typing serum of 10% alcohol.

EQUIPMENTS:

Microscope slides, blood lancets, absorbant cotton, wooden applicator sticks, wax pencils.

PROCEDURE:

1. Using a wax pencil, divide the microscopic slide into half-labelled are slide anti-A & antiB on the other side.
2. Place one drop of each antiserum on the approximately labelled section of the slide.
3. Using a piece of absorbent cotton moistened with 70% ethyl alcohol, wipe the tip of the middle finger.
4. Using a sterile blood lanset prick the disinfected area of the finger.
5. Allow one drop of blood to flow in to each drop of blood of antiserum on the slide.
6. With separate applicator sticks, mix each drop of blood and its respective antiserum.
7. Rock the slide between the finger in a bad check and forth motion.
8. Observed both mixtures for one minute for clumping (agglutination) of RBC.

RESULT:

The given blood was found to belong to group_____.

EX.NO: 03 BLEEDING TIME/CLOTTING TIME

DATE:

AIM:

To find out the time for the given blood sample.

PRINCIPLE:

Clotting time is the time interval between the appearance of blood and the clot.

REQUIREMENT:

- Glass slide
- Lancet
- Capillary tube
- Stop watch

CLOTTING TIME:

A deep prick is made in the finger tip. Flow the blood into the capillary tube. Stop watch simultaneously when capture tube is filled place it for 1-2 min after that start breaking tube at ½ min interval until fiber is thread formed so soon between the broken piece of capillary tube. When the finish thread appeared note down the time taken and record it as clotting time of the blood.

BLEEDING TIME:

Clean the finger with the spirit and allow it to dry make a prick. Blood will start flowing note the time for the bleeding taking place by blotting the blood oozing out after half minute. A fresh part of the blotting paper should be used each time to check for bleeding after quite some time it can be noted blood spot on the filter paper get thinner and disappears finally and stoppage of bleeding this is the time taken for the bleeding to seize. The above measure time is noted as bleeding time and is in minutes.

NORMAL VALUE:

BLEEDING TIME	2 ½ minutes
CLOTTING TIME	5-10 minutes

RESULT:

Bleeding time of the given sample_____

Clotting time of the given sample _____

EX.NO: 04

ENUMERATION OF RBCs

DATE:

AIM:

To enumerate the number of RBC present in the sample using haemocytometry.

PRINCIPLE:

The blood specimen is diluted (200 times) with red cell diluting fluid which does not remove the white cells but allows the red cells to be counted under 400x magnification in a known volume of fluid. Finally the number of cells in undiluted blood is calculated and reported as the number of red cells/vL pf whole blood.

PROCEDURE:

1. Label an Erlenmeyer flask (25ml) or test tube.
2. Pipette 4.0ml of diluting fluid in to the container using the 5ml graduated pipette (0.1ml graduation).
3. Draw well mixed anti coagulted blood specimen in to the sahlir pipette (20vL) up to the graduation mark. This yields 0.02ml of the specimen. Do not allow air bubbles to enter. 4. Blow the blood in to the diluting fluid held in the container. The dilution of blood is 4.02/0.02 = 201 times. Leave the pipette inside the flask. This will be used for filling the counting chamber.
4. Take the haemocytometer and place it on the flat surface of the work bench. Place the cover slip on the counting chamber.
5. Mix well the diluted blood and then take a small quantity of diluted blood in sahlir pipette which was left in the flask or test tube.
6. Allow a small drop of diluted blood hanging from the pipette to step in to the counter chamber by capillary action.
7. Rinse the pipette with the diluted blood a few times before filling a halfway.
8. Leave the counting chamber on the stage of the microscope between clips to bold slide.
9. So that the counting chamber can be moved.
10. Switch to low power (10x) objective adjust the light and focus on the walls of the counting chamber on the letters. Then slowly move the stage towards the middle of the slide until the rulings are visible sharpen the focus. Locate the large square in the center with 25 small squares. It must be uniform or else you should reload the chamber.
11. Place the counting chamber on the stage of the microscope between the clips to roll the slide. So that the counting chamber can be moved.
12. Carefully switch into high dry objective (40x) and move the chamber so that the smaller upper left corner square is completely in the field of vision.

13. Count the number of red cells seen on the small squares (0.2 X 0.2 = 0.04sq m.m) of the upper left corner which is divided into 16 smaller squares to facilitate counting.
14. Repeat the counting the four other square are marked by “R”. It is advisable to complete all counts to the corner squares and then move to the fifth square to be counted.
15. Make a total of all the cells countedi in 58 squares. It is recommended that the other side is charged . With a replicatives of the same specimen diluted separately.

RESULT:

The total number of red blood cells in the given serum sample was
cells/sq.mm.

EX.NO : 05 # ENUMERATION OF WBCS

DATE:

AIM:

To enumerate the number of WBCs present in the blood sample using haemocytometer.

PRINCIPLE:

Blood is diluted with acid solution which removes the red blood cells by haemolysis and also accentuates the nuclei of the white cells. Thus the counting of the white cells becomes easy. Counting is done with a microscope under low pressure (100x magnification) and knowing the volume of fluid examined and the dilution of the blood, the number of white cells, per (µ.mm or VL) in undiluted whole blood is calculated.

REAGENTS:

WBC diluting fluid, sahlir pipette (20 ull), Erlen meyer flask (25ml), microscope, haemocytometer and cover slip, rubber tubing for moth pipette surgical gauge, dilution of the blood is (2.03 + 0.002) /002 = 20.

PROCEDURE:

1. Label on Erlen meyer (25ml) or a 10ml test tube held in a rack.
2. Pipette 0.38ml of diluting fluid into the flask using the 1ml graduated pipette (0.01ml graduation)
3. With the help of 20ml sahlir pipette, draw well mixed anti-coagulated venous blood up to the mark.
4. Hold the pipette in the horizontal position and wipe the outside of the pipette with absorbent paper. Check the level of the blood specimen it should still be on the mask. Otherwise repeat step -3.
5. Blow the blood pipette (sahlir, Tc) 3 times and leave it inside the flask. Gently mix the blood well by swifting and wait for 2 minutes for complete haemolysis.
6. Fill the counting chamber in the same way as described under the red cell count.
7. Place the counting chamber on the stage of the microscope, turn the objective to low point and locate the grid.
8. Focus on one of the “W” marked areas. Each “W” marked square has 16 small squares to facilitate counting. The area of the “w” marked square is 18sq.mm.
9. Scroll all the four “W” marked corner squared in order to find out the distribution of cells. The cells are identifies by their blue colour with a dark dot in the centre (nucleus). There will be no red cells.

10. Focus on one of the corner squares and count the white cells. Systematically starting from the upper left small squares each “W” squares. Apply the same margin rule as for the red cell count (i.e) count the cells lying on two adjacent margins and discard these on the other two margins.
11. Repeat the entire white cells count on the other side of the chamber. Make an average of the two side (4.8 sq mm area).

RESULT:

The total number of white blood cells in the given serum sample was
cells/sq.mm.

Table:

Samples	**Absorbance at 540 nm (a.u)**
Blank	
Standard	
Test	

Calculation:

Amount of hemoglobin present in the given sample (mg/dl) = $A_T - A_B$ / $A_S - A_B$ x Con. of Std. x

Vol. of Std. x 100 / Vol. of Serum

=

EX. NO: 06 **ESTIMATION OF HAEMOGLOBIN IN**

DATE: **BLOOD BY WONG'S METHOD**

Aim:

To estimate the amount of iron and haemoglobin present in given blood sample.

Principle:

1.0 ml of blood is digested with concentrated sulphuric acid to get the iron in the free form in the presence of potassium per sulphate. The digest is then deproteinised with sodium tungstate solution, centrifuged and a known amount of the supernatant is treated with potassium thiocyanate. The colour developed is then read in a colorimeter at 540nm.

Reagents:

1. 10% sodium tungstate solution
2. Saturated potassium per sulphate
3. 3N potassium thiocyanate: 140g of potassium thiocyanate was dissolved in 500ml water.
4. Stock standard iron solution: Dissolved 70.2mg of ferrous ammonium sulphate in water and made upto 100ml with the same.1.0 ml of the stock solution contains 100µg of iron.
5. Working standard iron solution: Diluted 10ml of stock standard to 100ml with distilled water. So that 1.0ml of this solution contains 10µg of iron.

PROCEDURE:

1.0ml of the blood was whirled with 4.0ml of concentrated sulphuric acid in a 100ml standard flask for 2 minutes. 4ml of standard potassium per sulphate was added and shook well. To this, 4ml of 10% sodium tungstate solution was added. The flask was cooled and the volume was madeupto the mark. It was shaken well and filtered into a conical flask. This is the experimental solutionI.

To another 100ml standard flask, 4ml saturated potassium per sulphate solution, 4ml of concentrated sulphuric acid and 4ml of sodium tungstate solution were added. The volume was made upto the mark with distilled water. It was filtered into a conical flask. This is experimental solution II.

1.0 to 5.0 ml of working standard solution was taken in different test tubes. Added 0.3ml of concentrated sulphuric acid and 0.4ml of saturated potassium per sulphate to all the tubes. Finally, 1.6ml of 3N potassium thiocyanate solution was added and made up the volume of each tube to 10ml with water. 5ml of the experimental solution I and II, were taken in separate tubes. To each tube added 0.3ml of sulphuric acid, 0.4ml of saturated potassium per sulphate solution and finally added 1.6ml of 3N potassium thiocyanate and made up the volume to 10ml with distilled water. The intensity of the colour developed was read at 540nm in a colorimeter within 10 minutes.

Short procedure:

Samples	**Blank (mL)**	**Standard (mL)**	**Test sample (mL)**
Distilled water	5.0	-	-
Standard Hemoglobin	-	5.0	-
Test sample	-	-	5.0
Con. Sulphuric acid	0.3	0.3	0.3
Potassium per sulphate	0.4	0.4	0.4
3N Potassium thiocyanate	1.6	1.6	1.6
Final volume	10	10	10

RESULT:

The amount of haemoglobin present in 100ml of the given blood sample is_____.

EX. NO: 07 DIFFERENTIAL COUNT OF BLOOD CELLS

DATE:

AIM:

To determine the cellular components of blood under microscopic by preparing a blood smear.

PRINCIPLE:

Microscopic examination of peripheral blood smear is formed as a saline haemotalogical investigation.It is done by preparing staining and exampling a thin flim of blood on glass slide.The blood is often called blood smear.It is used to detect microfilaria.

REQUIREMENTS:

Leishman stain is a mixture of methylene blue in acetone free methyl alcohol.

METHYLENE BLUE (BASIC DIE):

The acid part of cell nuclei DNA and cytoplasm of web granular of basophills.

EOSIN (ACIDIC DYE):

Stain the basic part of the cells eosinophilic granular and haemoglobin od blood cell.

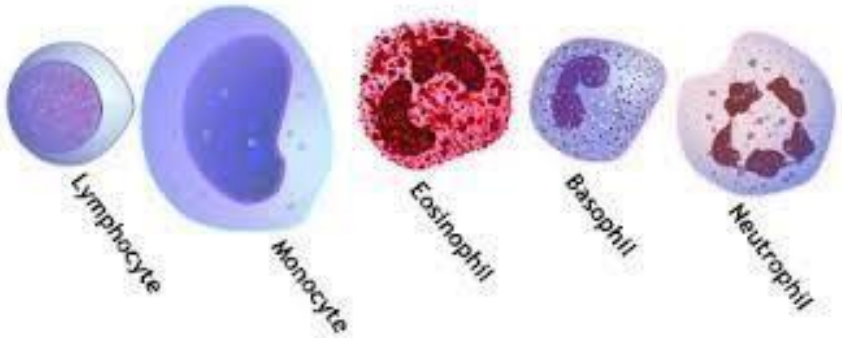
METHYL ALCOHOL:

Fix the smear to the slide.It is because acetone lysis of cell.

What Is a Blood Smear?

A blood smear is a diagnostic test used to look for abnormalities within the blood. The cell types are examined under a microscope for unusual shapes or sizes. There are three main cells within the blood that the test focuses on:

- red cells (which carry oxygen throughout the body)
- white cells (which function as part of the body’s immune system)


Lymphocyte
Monocyte
Eosinophil
Basophil
Neutrophil

- platelets (which are important for blood clotting)

Abnormalities in the shape, size, and number of the red blood cells can affect how oxygen travels throughout the blood. These abnormalities are often caused by a mineral or vitamin deficiency, but can be caused by abnormal proteins such as in sickle cell anemia.

White blood cells are an integral part of the body's immune system, a network of tissues and cells that function to keep the body safe from invading microorganisms and eliminate existing infection. Disorders affecting these cells can often result in the body's inability to eliminate or control infections.

Platelet disorders affect blood clotting and are often the result of the body producing the wrong amount of platelets needed (either too many or too few).

PROCEDURE:

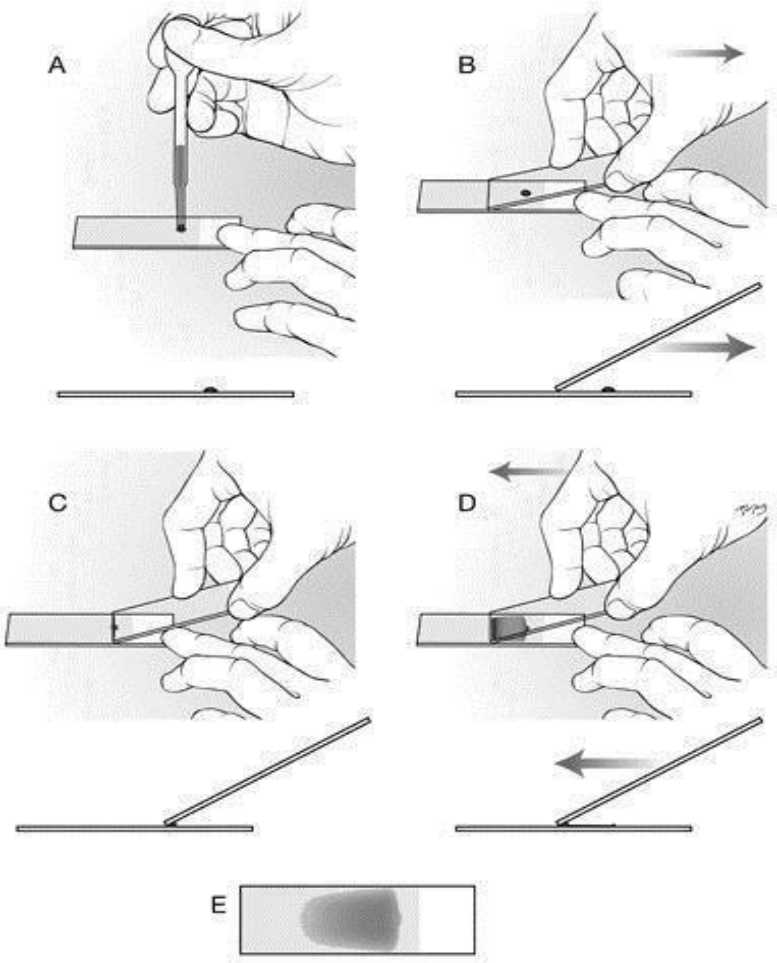
- METHOD OF PREPARING OF SMEAR:
- The blood smear is preparing in2 steps
- Making a blood smear
- Fixing and staining of blood smear.

(ii) MAKING OF BLOOD SMEAR

- Cover glass method.
- Glass slide(wedge method).
- Centrifuge method in common.

- GLASS SLIDE (WEDGE METHOD):
- Take a few cleaned grease free glass slide and one as spreader.
- Clean the tip of middle finger with alcohol and allow the finger to dry and pick fingertip with lancet.
- Discard the first drop of blood.
- Place a drop of blood on specimen slide on flat surface of table and hold it in a position of slide with medium finger and thumb of the left hand.
- Plane the smooth clean edge of the spreader slide in the specimen slide just in front of the drop of the blood.

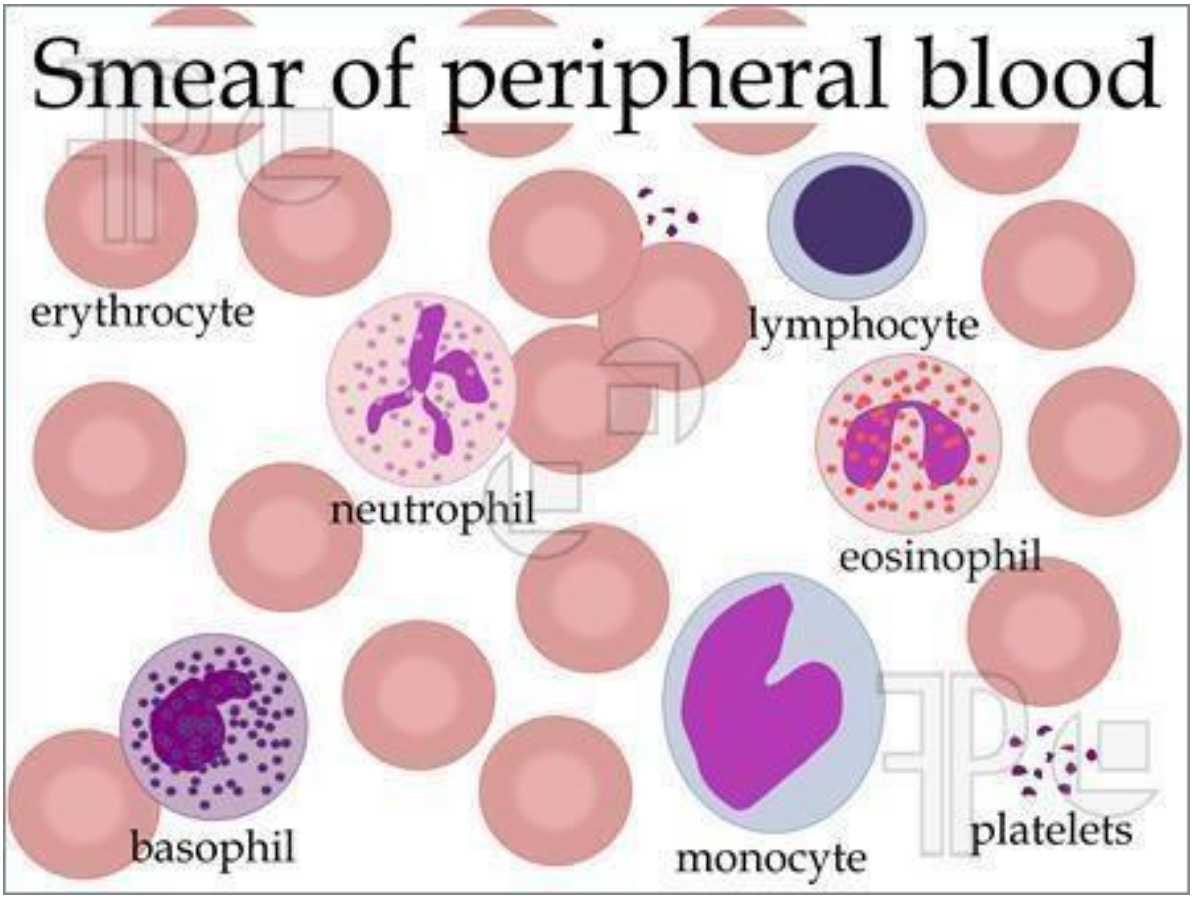
Stepwise procedure:



- Using the right hand draw the spreader back until it touches the drop of blood let the blood run along the edge of the spreader.
- When the blood has spread evenly across the edge of spreader slide push the spreader to other end of slide with a smooth quick and controlled movement.
- Twin the spreader slide over and prepare a second blood film using the same procedure.
- Dry the blood smear quickly by using air.

FIXING AND STAINING THE SMEAR:

- Place the slide on the rack with blood smear pour 8 to 12 drops of Leishman stain on the slide.
- Note the time and leave it for 1-10 minutes.
- Add the double amount of water on the smear.
- Mix the stain and water evenly by blowing gently the air through the pipette.
- Note the time and leave it for 1-10 minutes.
- Pour of the stain of wash the slide gently with water.
- Then the blood smear is examined under the microscope of morphology of the blood cells are sustained.


Smear of peripheral blood
erythrocyte
lymphocyte
neutrophil
eosinophil
basophil
monocyte
platelets

RESULT OF DISCUSSION:

RBC APPEARANCE:

RBC appears as a round body contains no nuclear granules of discrete material.

STAINING:

Stain orange red the red color is darker at the edge of the cells than in the center (giving the appearance of the center hollow). The variation carried by biconcave shaped cells which contain less haemoglobin.

PLATELET APPEARANCE:

Under high power they look dusty of deposite under oil immersion, they look thin heads. This contains nonucleic or fine adjustment platlet feature to distinguish them from deposited stained platelet containing pink.

DISTRIBUTION:

They are present in group or aggregate (many platlets close to each other).

SIZE:

They are smallest cell in the peripheral smear. The size of the platlet is 2-4 diameter.

CELL DESCRIPTION:

LEUKOCYTE(WBC):

NEUTOPHIL:

- Size (10-14)µm.
- Multi lobed (2-3) lobes are connected by thin strands in hands from. The nucleus is sausage shaped also called slab form.

CYTOPLASM:

- Look like pink color containing fine pink granules.

BASOPHILS:

- Size(10-14)µm.
- Nucleus bi lobed or trilobed are usually distinctly visible because the call is studded with granules.

LARGE LYMPHOCYTE:

- Size (10-14)µm.

SMALL LYMPHOCYTE:

- Size same as or slightly larger than RBC.

NUCLEUS:

- Occupies almost the whole cell. It is homogenous of compact.

MONOCYTE:

- Size (12-24)µm. it is largest.

NUCLEUS:

- Occupy 80% of the cell centrally or slightly eccentrically.

CYTOPLASM:

- Read glass in appearance contains no granules.

RESULT:

Thus the cellular components of the blood under microscope by preparing blood smear was examined successfully.

EX. NO: 08 # PACKED CELL VOLUME/ESR

DATE:

AIM:

To estimate the erythrocyte sedimentation rate (ESR) from the collected blood sample

PRINCIPLE:

The **erythrocyte sedimentation rate (ESR)** is a common hematological test for nonspecific detection of inflammation that may be caused by infection, some cancers and certain autoimmune diseases. It can be defined as the rate at which Red Blood Cells (RBCs) **sediment in a period of one hour.**

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for a period of time, the RBCs – under the influence of gravity- settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour(mm/hr). This mechanism involves three stages:

- **Stage of aggregation**: It is the initial stage in which piling up of RBCs takes place. The phenomenon is known as Rouleaux formation. It occurs in the first 10-15 minutes.
- **Stage of sedimentation**: It is the stage of actual falling of RBCs in which sedimentation occurs at constant rate. This occurs in 30-40 minutes out of 1 hour, depending upon the length of the tube used.
- **Stage of packing**: This is the final stage and is also known as stationary phase. In this, there is a slower rate of falling during which packing of sedimented RBCs in column occurs due to overcrowding. It occurs in final 10 minutes in 1 hour.

WINTROBE'S METHOD

This method uses Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a **length of 11 cm** and **internal diameter of 2.5 mm**. It contains 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking is 0 at the top and 10 at the bottom for ESR. This tube can also be used for PCV. The marking is 10 at the top and 0 at the bottom for PCV.

REQUIREMENTS:

- Anticoagulated blood (EDTA, double oxalate)
- Pasteur pipette
- Timer
- Wintrobe's tube

- Wintrobe's stand

PROCEDURE:

1. Mix the anticoagulated blood thoroughly.
2. By using Pasteur pipette, fill the Wintrobe's tube upto '0' mark. There should be no bubbles in the blood.
3. Place the tube vertically in ESR stand and leave undisturbed for 1 hour.
4. At the end of 1 hour, read the result.

NORMAL VALUE:

For males: 0-9 mm/hr
For females: 0-20 mm/hr

Clinical Significance of ESR

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

Causes of a significantly raised ESR:

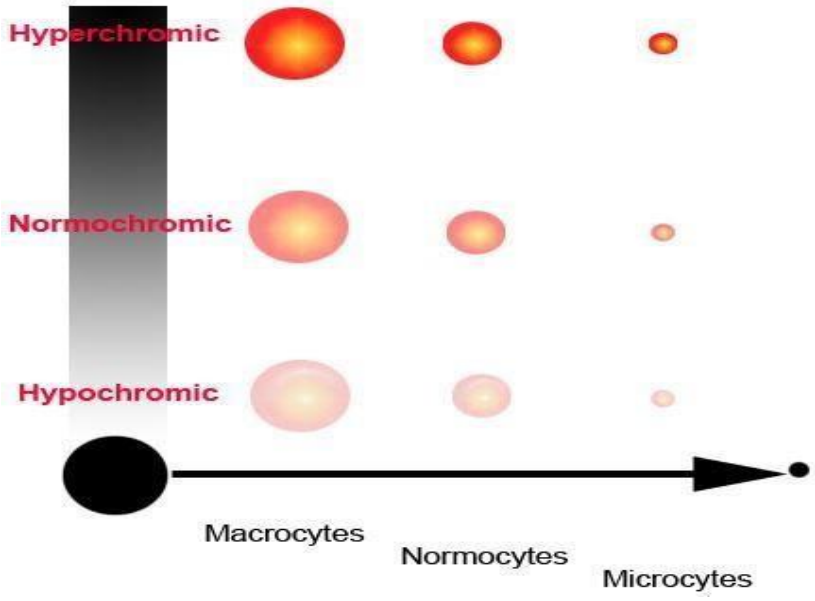
- All types of anemias except sickle cell anemia
- Acute and chronic inflammatory conditions and infections including:
  - HIV disease, Tuberculosis, Acute viral hepatitis, Arthritis, Bacterial endocarditis, Pelvic inflammatory disease, Ruptured ectopic pregnancy, Systemic lupus erythematosus.
- African trypanosomiasis (rises rapidly)
- Visceral leishmaniasis
- Myelomatosis, lymphoma, Hodgkins disease, some tumours
- Drugs, including oral contraceptives

Causes of Reduced ESR:

- Polycythaemia
- Poikilocytosis
- Newborn infants
- Dehydration
- Dengue haemorrhagic fever
- and other conditions associated with haemoconcentration

RESULTS:

The collected blood sample contains_____mm/hr of sedimented Erythrocytes.


Hyperchromic
Normochromic
Hypochromic
Macrocytes
Normocytes
Microcytes

EX NO:09 ESTIMATION OF PCV, MCH, MCV, MCHC

DATE:

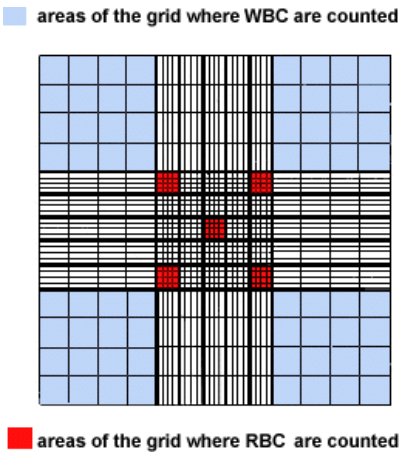
AIM:

To Diagnose the type of anemia by PCV,MCH,MCV,MCHC measurements.

PRINCIPLE:

Diagnose the type of anemia may be assisted by relating the measurements of RBC count,, hematocrit and haemoglobin to drive the PCV,MCH,MCV,MCHC

- Erythrocytes that have a normal size or volume (normal MCV) are called **normocytic**,
- When the MCV is high, they are called **macrocytic**.
- When the MCV is low, they are termed **microcytic**.
- Erythrocytes containing the normal amount of hemoglobin (normal MCHC) are called **normochromic**.
- When the MCHC is abnormally low they are called **hypochromic**, and when the MCHC is abnormally high, **hyperchromic**



The expected range for white cell count in both males and females is as follows:

4,000-11,000 /µl

PROCEDURE:
To calculate the MCV, expressed in femtoliters (fl, or 10^{-15}L), the following formula is used:

$$\text{MCV} = \frac{\text{hematocrit(\%) x 10}}{\text{RBC count(millions / mm}^3\text{ blood)}}$$

(normal range : 80 - 95 gHb/100ml pg)

To calculate the MCHC, expressed as grams of hemoglobin per 100 ml packed cells, the following formula is used:

$$\text{MCHC} = \frac{\text{hemoglobin(g / 100ml) x 100}}{\text{hematocrit(\%)}}$$

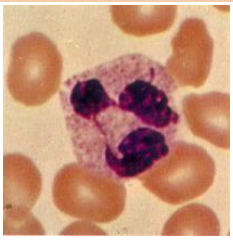
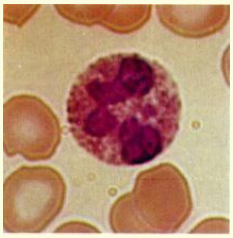
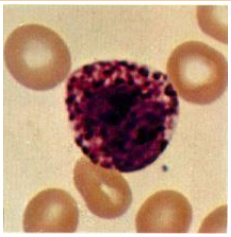
(normal range : 30 – 34 gHb/100ml pg)

To calculate the MCH, expressed as grams of hemoglobin per 100 ml packed cells, the following formula is used:

MCH = Hb in gm% * 10 / RBC count.

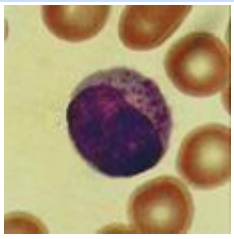
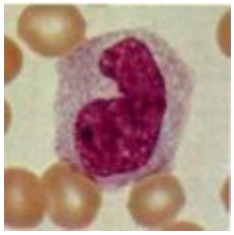
(normal range : 27 – 32 pg)

Granulocytes

	**Neutrophils** have nuclei with several lobes and fine purple granules in their cytoplasm. Their are termed neutrophil, because their granules are not very amenable to staining with either acidic or basic dyes. They are the most numerous of the leukocytes, making up 50-70%.
	**Eosinophils** feature large granules that can be stained bright red with an acidic dye - e.g. eosin. They most often have bi-lobed nuclei. They make up about 1-4% of the leukocytes
	**Basophils** have multi-lobulated nuclei and large granules which stain blue with basic dyes. They occur quite seldomly (less than .1%).

Deviations of different white cell counts from the normal values often indicate a diseased state.**Neutrophilia** (high neutrophil count) often signals localized infections such as appendicitis;**eosinophilia** (high eosinophil count) may indicate allergic conditions or invasion by parasites such as Trichinella; **lymphocytosis** (high lymphocyte count) may be seen in some viral infections; **neutropenia**(low neutrophil count) occurs in typhoid fever, measles or infectious hepatitis, and **eosinopenia** (low eosinophil count) may be produced by an elevated secretion of corticosteroids (in states of stress).

Agranulocytes

	**Lymphocytes** can be large or small. They are spherical and have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules. Lymphocytes represent 20-40% of all leukocytes.
	**Monocytes** are large cells. They have large indented nuclei, often kidney-shaped and very fine cytoplasmic granules. They make up 2-8% of the leukocytes.

Neutrophils, eosinophils and basophils are described collectively as **granulocytes**. They are distinguished by the nature of the granules in their cytoplasm, and generally have small, multilobed nuclei. The 2 other types of white cells are lymphocytes and monocytes and are described collectively as **agranulocytes**.

RESULT:

The collected blood sample contains_____of RBC and WBC.