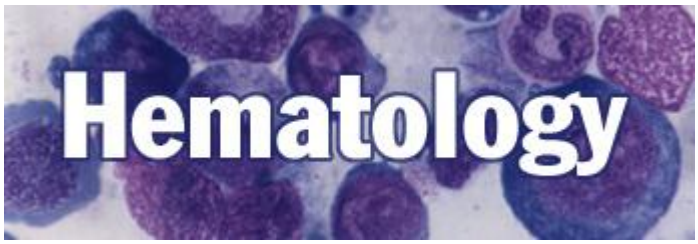
H.S.C (VOCATIONAL)

MEDICAL LABORATORY TECHNICIAN

STD: XII (PAPER-1)

Clinical Hematology, Clinical Pathology and Blood Banking

PRACTICALS



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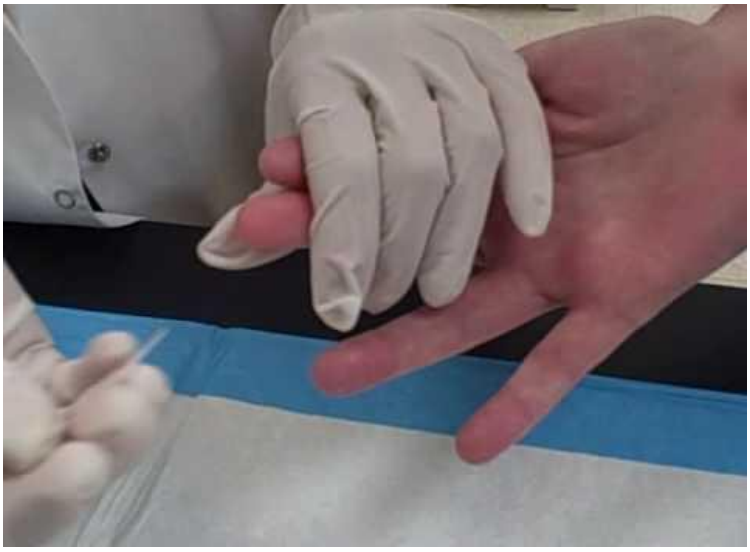
Week no.	1
Practical No.	**1**
Title/ Aim	**Blood collection** To collect blood by capillary Puncture Method
Objective	Students shall be able to collect blood by capillary puncture.
Principle	Capillary puncture is used to collect blood for various tests.
Requirements	1. Disposable needles or lancet, 2. spirit swab 3. Other as per test e.g. Sahli's pipette in case of Hb estimation or slides in case of blood groups. 4. Sites of blood collection • Ball of Finger • Ear lobe • Great toe or heel in case of infants
Environment	MLT laboratory
Procedure	1. First clean the site with 95% alcohol or spirit. Allow it to dry completely. 2. Hold the ball of a finger tightly with the thumb and index finger. 3. With the help of a disposable needle or lancet give a bold prick. (The prick should be sharp and quick to minimize pain. It should be deep enough (2-4 mm). Squeezing tends to stop

	the flow of blood, also adding tissue fluid to the drop of blood, which gives inaccurate count, so squeezing should not be done.) 4. Wipe off the first drop of blood and next is used for testing. After collection place a cotton swab over the punctured site. Apply slight pressure to stop blood flow.
Observations The procedure used to collect blood by capillary puncture is described in the form of images as follows-  Figure 1.1	
Result	Blood is collected and labeled as per requirement.
Clinical significance	• When amount of blood required is small capillary method is used. E. g. Hemoglobin estimation, blood group determination, blood sugar level estimation by Glucometer (in

	emergency cases), etc.
Skills to be achieved	Students will collect blood by capillary puncture.
Skill evaluating criteria	1. Proper preparation and selection of site for capillary puncture- 4marks 2. Correct procedure- 4marks 3. Proper organization of requirements as per test to be carried out e.g. Hb estimation- 2marks
FAQs	1. What are different sites for capillary puncture? 2. Precautions to be taken before collecting capillary blood.
Assignment	Collect blood of 50 different subjects for various tests.
Reference	Chapter 2 Handbook of Haematology, clinical pathology and Blood banking

Week no.	2
Practical No.	**2**
Title/ Aim	**Blood collection** To collect blood by vein puncture method.
Objective	Students shall be able to collect blood by vein puncture.
Principle	Vein puncture is used to collect blood for various tests.
Requirements	1. Disposable syringe (2cc, 5cc, 10cc) and needle 2. Tourniquet 3. Spirit swab 4. Anticoagulant bulbs - ▪ EDTA Bulb – This bulb is used for cell counts, hemoglobin estimation, etc. ▪ Fluoride Bulb – This bulb is used for blood sugar level estimation. ▪ Double Oxalate Bulb – It is also used for hematological studies. 5. Sites of blood collection ▪ Anticubital fossa
Environment	MLT laboratory
Procedure	1. Ask the patient sit or lie down with the forearm rested on the table. 2. Apply tourniquet. 3. Choose one of the veins in anticubital fossa.

	Select a vein which is both visible and palpable and well fixed to surrounding. 4. Now release the tourniquet. 5. Clean the anticubital fossa thoroughly with a spirit swab. 6. Reapply the tourniquet. 7. Ask the patient to close his/her fist by taking thumb inside, so that the veins become prominent. 8. Grasp the patient's forearm with left hand and retract the soft tissues downwards below the site of puncture with the help of thumb. 9. Hold the syringe in the right hand and bring the needle to the skin over vein. (If vein is large and fixed the skin and vein may be punctured with single thrust. If vein is small or slippery then the skin is punctured first and then vein.) Puncture the vein with single thrust. 10. When blood starts flowing into the syringe release the tourniquet. 11. When necessary amount of blood is collected slowly withdraw the needle keeping a cotton swab over the punctured site. 12. Press the punctured site gently for 2minutes. 13. Ask the patient to elevate the arm and give

	pressure for few minutes. 14. Immediately transfer the collected blood in to an appropriate bulb. First remove the needle from the syringe to prevent clotting of blood and then pour the blood in to the bulb.
Observations The procedure used to collect blood by vein is described in the form of images as follows-   Figure2.1Vein puncture method Figure2.2 Transferring blood into container	
Result	Blood is collected and labeled as per requirement.
Clinical significance	• Quantity of blood collected by vein puncture is more than capillary puncture so when numbers of tests to be carried out are more then vein puncture is method of choice. • When serum or plasma is to be separated

	from blood vein puncture is used. • Different tests carried out by vein Punctureare biochemistry- LFT, KFT, Special hematological tests, ESR, PCV,etc.
Skills to be achieved	Students will collect blood by vein puncture.
Skill evaluating criteria	1. Proper preparation of site and selection of vein- 2marks 2. Correct procedure- Proper steps- 4marks 3. Correct quantity- 2marks 4. Proper use of anticoagulant bulbs- 2marks
FAQs	1. What are sites of vein puncture? 2. What are complications of vein puncture?
Assignment	Collect blood of 10 different subjects.
Reference	Chapter 2 Handbook of Haematology, clinical pathology and Blood banking

Week no.	3
Practical No.	**3**
Title/ Aim	Hemoglobin estimation To estimate Hb by cynomethhemoglobin method.
Objective	Students shall carry out hemoglobin estimation by cyanmethhemoglobin method.
Principle	When blood is mixed with Drabkin's reagent containing K cyanide & K ferricyanide hemoglobin reacts with ferricyanide to form methemoglobin which is converted to stable cyanmethemoglobin by cyanide. The intensity of the color is proportional to hemoglobin concentration & is compared with a known cyanmethemoglobin standard at 540nm (green filter).
Requirements	1. Hb-pipette. 2. Test tubes 3. Photometer or spectrophotometer. 4. Drabkin's reagent- • K ferricyanide- 400mg • K dihydrogen phosphate 280mg • K cyanide 100mg • Distilled water- 1000ml This reagent is stable in a polythene container at 2-8 ^{o}C

	5. Cyanmethemoglobin standard (Hb standard)- commercially available. It is directly pipette in a cuvette & optical density measured at 540nm. The reading obtain corresponds to 15 gm% Hb 6. Specimen - • Capillary blood or • Thoroughly mixed anticoagulated (EDTA or double oxalate) venous blood. The Specimen need not be a fasting sample.
Environment	MLT laboratory

Procedure

1. Pipette in the tubes labeled as follows-

	Test	**Blank**
Drabkin's reagent	5.0 ml	5.0ml
Blood	0.02 ml	

2. Mix the contents in the tube labeled as 'Test 'thoroughly and wait for 5 minutes.
3. Measure the absorbance of this solution at 540nm in a colorimeter after adjusting the OD at 0 by using Drabkin's solution as blank.
4. Read absorbencies of standard (15 g/dl) by pipetting it directly in a cuvette.

Observations

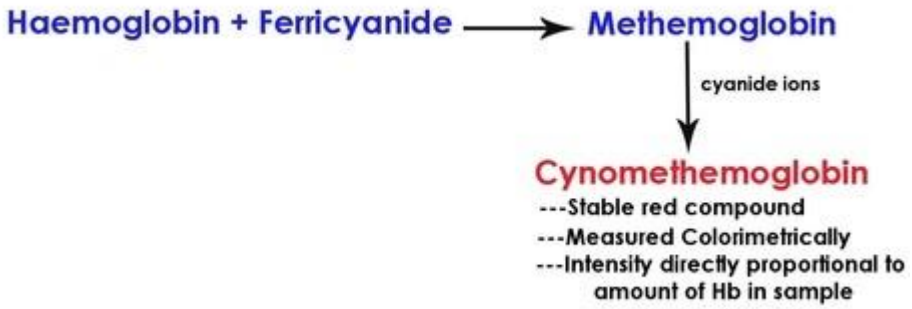


Figure 3.1

Calculation-

Hemoglobin, g/dl = $\frac{\text{O.D. of TEST} \times 15}{\text{O.D. of STD}}$

Result	Normal values Men 14-18gm% Women 12-16gm%
Clinical significance	1. This is the internationally recommended method for determining hemoglobin. 2. This method is highly accurate and is the most direct analysis available for hemoglobin iron. Its disadvantage is the use of cyanide

	compounds, which, if handled carefully, should present little hazard. 3. Hemoglobin estimation is used as a screening test for detecting anemia. 4. It is usually performed as a part of Complete Blood Count (CBC).
Skills to be achieved	Students will pipette exact quantity of blood in sahli's pipette and determine Hemoglobin of patients correctly.
Skill evaluating criteria	• Proper collection of blood- 2marks • Proper mixing of blood sample- 2marks • Correct filling of Sahli's pipette up to mark (20µl)- 2marks • Proper pipetting of Drabkin's reagent- 2marks • Proper adjustment of colorimeter- 2marks
FAQs	1. What is hemoglobin? 2. Explain principle of Drabkin's method. 3. Give normal value of Hb in males and females.
Assignment	Perform Hb of 10 different patients with Drabkin's method.
Reference	Chapter 4 Handbook of Haematology, clinical pathology and Blood banking

Week no.	4
Practical No.	**4**
Title/ Aim	**Red Blood Cell count** To perform RBC count by using Neubaur's chamber.
Objective	Students shall carry out RBC count by manual method.
Principle	The blood specimen is diluted 1:200 with the R.B.C. diluting fluid &cells are counted under high power by using a counting chamber. The number of cells in undiluted blood are calculated & reported as the number of RBC/cumm of whole blood.
Requirements	**1.** Microscope **2.** Improved Neubauer's chamber **3.** RBC pipette **4.** RBC diluting fluid- Sodium citrate 3.0 gm Formalin 1.0ml D/W to 100ml **5. Specimens-** • Double oxalate or EDTA blood • Capillary blood
Environment	MLT laboratory
Procedure	• Mix the anticoagulated blood carefully. • Draw blood up to 0.5 marks.

	• Draw diluting fluid up to 101 marks. • The pipette is rotated rapidly by keeping it horizontal during mixing. • After 5 minutes, by discarding few drops from the pipette & holding it slightly inclined small volume of the fluid is introduced under cover slip on the counting chamber. • Allow the cells to settle for 2 to 3 minutes. • Place the counting chamber on the stage of microscope. • Adjust it under low power objective & locate the large square in the center with 25 small squares. • Now switch to high power objective. • In the central square out of 25 squares count RBCs in 4 corner squares & in Central Square.

Observations

Calculation –

Total RBC/lit of blood=RBCs /cumm x10^6 OR

$$\text{Total RBC/cumm} = \frac{\text{Number of red cells counted} \times \text{dilution factor}}{\text{Area counted} \times \text{depth of chamber}}$$

Where—

Dilution =1:200

Area counted= 80 /400 =1/5 sq.mm.

Depth of chamber =1/10 mm

Number of red cell counted=N

Hence total RBC/cumm = $\frac{N \times 200}{1/5 \times 1/10}$

= N×200×50

= N× 10,000

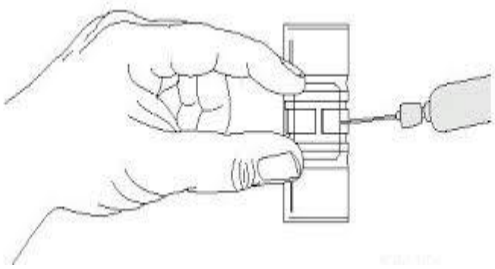


Fig.4.1

Fig. 4.2 RBC pipette

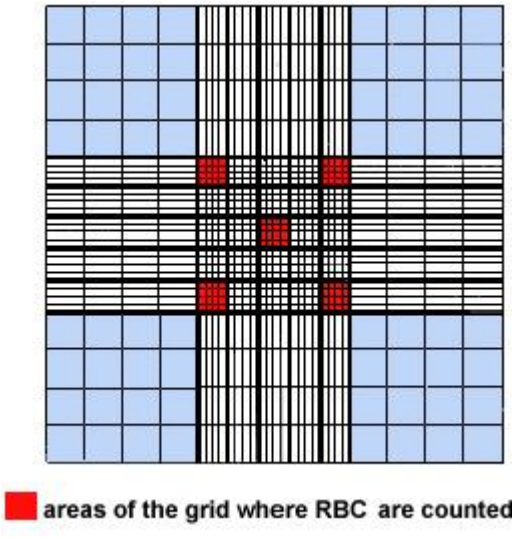


Fig. 4.3

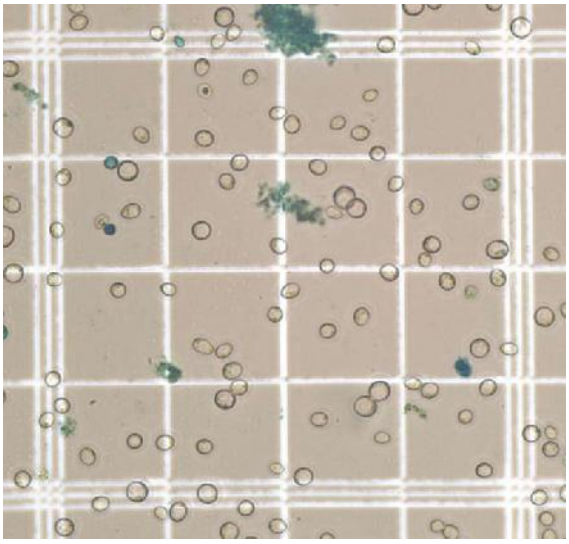


Fig. 4.4

Result	**Normal value-** • Male- 4.5 to 6.0 x10^6cells/cumm • Female- 4.0 to 4.5 x 10^6cells/cumm
Clinical significance	An increase in RBC count is observed in condition such as –

	➢ Haemoconcentration due to burns, cholera. ➢ Central cyanotic stages as seen in chronic heart disease. ➢ Decrease lung function. ➢ Polycythaemia. Decrease in RBC count is observed in condition such as – ➢ Old age ➢ In pregnancy ➢ Different anemias
Skills to be achieved	Students can count RBCs on counting chamber.
Skill evaluating criteria	1. Proper collection of blood (from the area where there is no hemoconcentration or edema)- 2marks 2. Proper pipetting of blood- 2mark 3. Correct adjustment of cover slip and charging of counting chamber- 2marks 4. Use of standardized counting chamber- 2marks 5. Adjustment of Neubaur's chamber -2marks
FAQs	1. Give composition of RBC diluting fluid. 2. Give normal value of RBC count. 3. Describe RBC pipette.
Assignment	Perform RBC count of 10 different subjects.
Reference	Chapter 5 Handbook of Haematology, clinical pathology and Blood banking

Week no.	5
Practical No.	**5**
Title/ Aim	**White Blood Cell count** To perform Total WBC count of given sample.
Objective	Students shall carry out Total WBC count by manual method.
Principle	The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leukocytes. The blood specimen is diluted 1:20 in a WBC pipette with the diluting fluid & cells are counted under low power objective. The number of cells in undiluted blood are calculated & reported as the number of WBC/cumm of whole blood.
Requirements	1. Microscope 2. Improved Neubaur chamber 3. WBC pipette 4. WBC diluting fluid- • Glacial acetic acid- 2.0 ml • 1 % gentian violet- 1.0 ml • Distilled water- 97 ml 5. Specimen- • Double oxalated or EDTA blood • Capillary blood
Environment	MLT laboratory

Procedure	1. Draw blood up to 0.5 mark of W.B.C pipette. 2. Wipe excess blood out site the pipette by using cotton. 3. Draw diluting fluid up to 11 mark. 4. Mix the contents in the pipette. After 5 minutes discard few drops and charge the counting chamber and allow the cells to settle for 2 to 3min. 5. Adjust the chamber by using low power objective. 6. Count the number of cells in large four corner squares. 7. While counting cells attached to left and upper margin are counted and that attached to right and lower margin are not counted.

Observations

Calculations-

$$\text{Total WBC/cumm} = \frac{\text{Number of red cells counted} \times \text{dilution factor}}{\text{Area counted x depth of chamber}}$$

Where-

Dilution =1:20

Area counted= 4

Depth of fluid=1/10 mm

Number of white cells counted=N

$$\text{Hence total WBCs/cumm} = \frac{\text{N x 20}}{\text{4 x 1/10}}$$

$$= N \times 5 \times 10 = N \text{ x}50$$

Fig. 5.1 WBC pipette

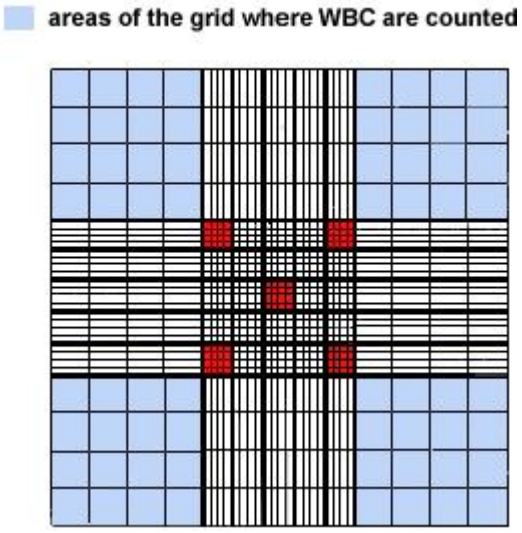


Fig. 5.2

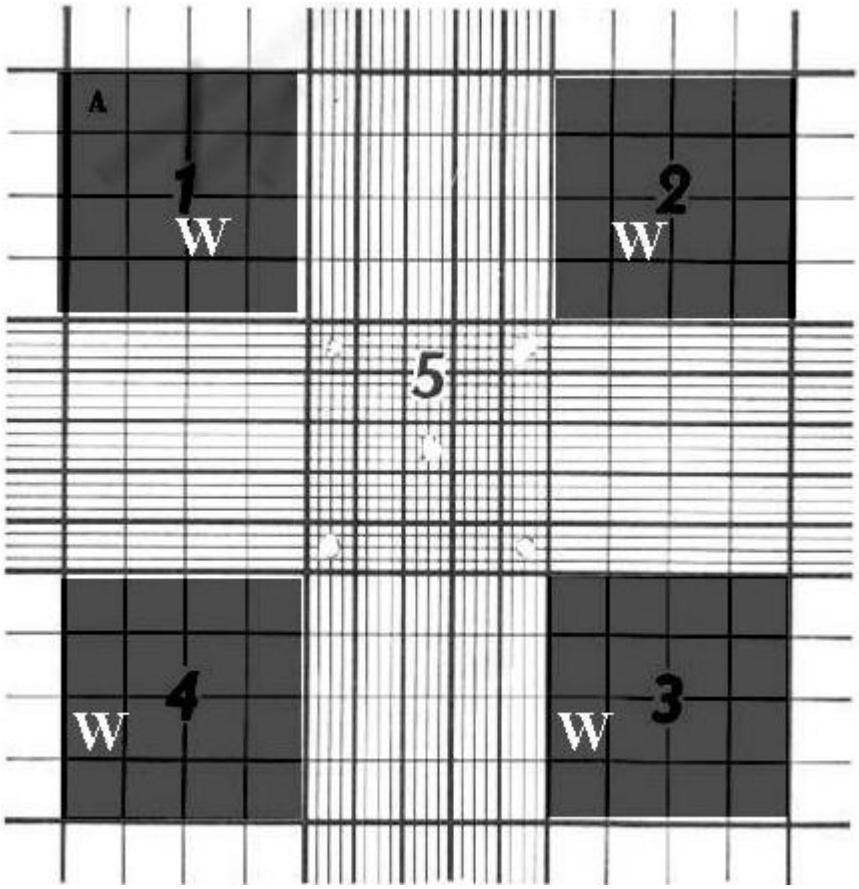


Fig. 5.3

Result

Normal value-

Age	Count
Adults	4000-10,000 cells/mm^3
At birth	10,000-25,000 cells/mm^3
1-3 years	6,000-18,000 cells/mm^3
4-7 years	6,000-15,000 cells/mm^3
8-12 years	44,500-13,500 cells/mm^3

Clinical significance

Increase in total leucocytes count more than 10,000 is called Leucocytosis and decrease in count less than 4,000 is called Leucopenia**.**

Causes of leucocytosis- It may be physiological or pathological.

Physiological

- Age- At birth the total leukocyte count is about 18000/cumm it drops gradually to adult level.
- Pregnancy- At 'full term' the total count tends to be about 12000 to 15000 /cumm. It rises soon after delivery and then gradually returns to normal.
- High temperature
- Severe pain
- Muscular exercise

Pathological

- It is for a transient period in infection.
- The degree of rise in leucocyte depends on

	the type & severity of infection& response of body. • The infection may be bacterial, viral, protozoal and parasitic. • Leucocytosis is also observed in severe hemorrhage. • Leukemia. **Cause of leucopenia** Certain viral and bacterial infections lead to leucopenia. 1. Infections • Bacterial (typhoid, paratyphoid, tuberculosis) • Viral (hepatitis, influenza, measles) • Protozoal (malaria) 2. Some cases of leukemia (Subleukemic leukemia), Anemia (iron deficiency, megaloblastic, Aplastic)
Skills to be achieved	Students can count WBCs using Neubaur's chamber.
Skill evaluating criteria	1. Proper collection of blood (from the area where there is no hemoconcentration or edema)- 2 marks 2. Proper pipetting of blood- 2 Marks 3. Correct adjustment of cover slip and charging of counting chamber- 1 mark

	4. Adjustment of Neubaur's chamber on microscope to visualize counting squares - 1 marks 5. Proper counting of WBC in WBC squares – 2 marks 6. Correct calculation using formula – 2 marks
FAQs	1. Give composition of WBC diluting fluid. 2. Give normal value of WBC count. 3. Describe WBC pipette.
Assignment	Perform WBC count of 10 different subjects.
Reference	Chapter 5 Handbook of Haematology, clinical pathology and Blood banking

Week no.	6
Practical No.	**6**
Title/ Aim	**Differential leukocyte count**
Objective	Students shall prepare peripheral smear and also perform DLC.
Principle	The polychromic staining solution (Wright stain Leishman, Giemsa) contains methylene blue and eosin. The stains are called Romanowasky stains as they are made up of combination of acidic and basic dyes. These basic and acidic dyes induce multiple colors when applied to cells. Methanol acts as a fixative and also acts as a solvent. The basic component of white cells i.e. cytoplasm is stained by acidic dye and described as eosinophilic or acidophilic. The acidic component e.g. nucleus take blue to purple color by basic stain and called as basophilic. The neutral component of the cell is stained by both the dyes. Differential count is percent distribution of various white cells in the peripheral blood.
Requirements	1. Microscope 2. Microscope slides and glass spreader 3. Cedar wood oil 4. Reagents- • Wright stain- 0.2 gm of the powder in 100 ml

	of acetone free methanol. The solution is allowed to stand for few days before using. • Buffer water- a. Sodium dihydrogen phosphate 3.76gms b. Potassium dihydrogen phosphate 2.10gms c. Distilled Water to 1000ml Keep at room temperature ($25^0C \pm 5^0C$) 5. Specimen- • Capillary puncture blood • EDTA blood
Environment	MLT laboratory

Procedure

1. Making of the smear-
   - Take a clean dry grease free slide.
   - Transfer a small drop of blood near the edge of the slide.
   - Place the spreader slide at an angle of 30^0 to 35^0 pull back the spreader until it touches the drop of blood let the blood run along the edge of the spreader.
   - Push the spreader forward at the end of the slide with a smooth movement.
   - Dry the blood smear at RT. Adequate drying is essential to preserve quality of the film.
2. Identification marking-

- By using lead pencil write ID no on the slide

3. Fixing the smear-
    - The slide should be stained after making the smear. Methanol present in the stain fixes the smear.
4. Staining the film-
    - Cover the smear with the staining solution by adding 10 to 15 drops on the smear. Wait exactly for 1 minute.
    - Add equal number of the drops of buffer solution. Mix the reaction mixture adequately by blowing on it through pipette. Wait for 10 to 15 minutes.
    - Wash the smear by running tap water.
    - Allow the slide to dry at RT.
5. Examination of film-
    - First examine the stained smear under the low power. In an ideal smear 3 zones will appear thick area (head), body and thin end of smear (tail).
    - Choose the portion slightly before the tail end where the red cells are beginning to overlap.
    - Place a drop of oil on the smear. Turn the oil immersion objective and increase light by opening the iris diaphragm.
    - Examine the film by moving from one field to next systematically. Record the type the leucocytes seen in each field.
    - Count at least 100 leukocytes.

Observations- Smear preparation and stained smear

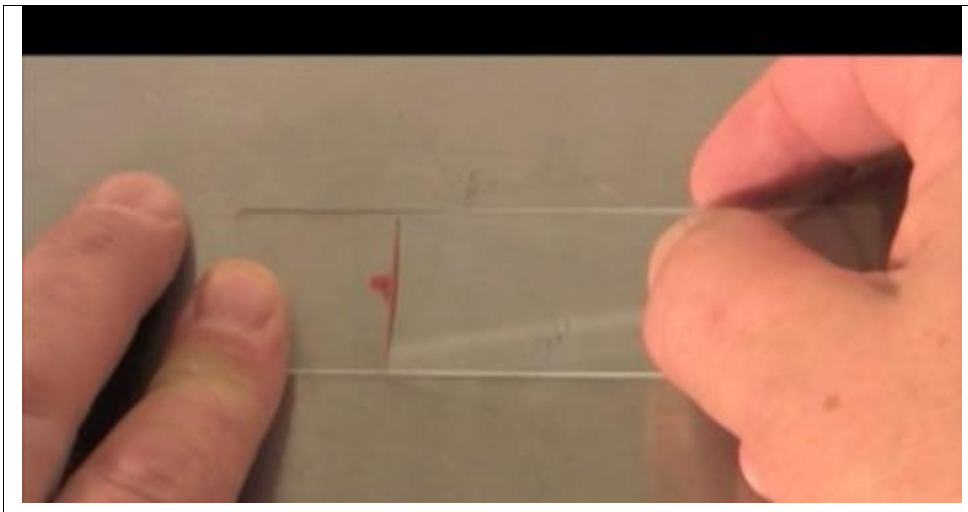


Fig.6.1 Smear preparation

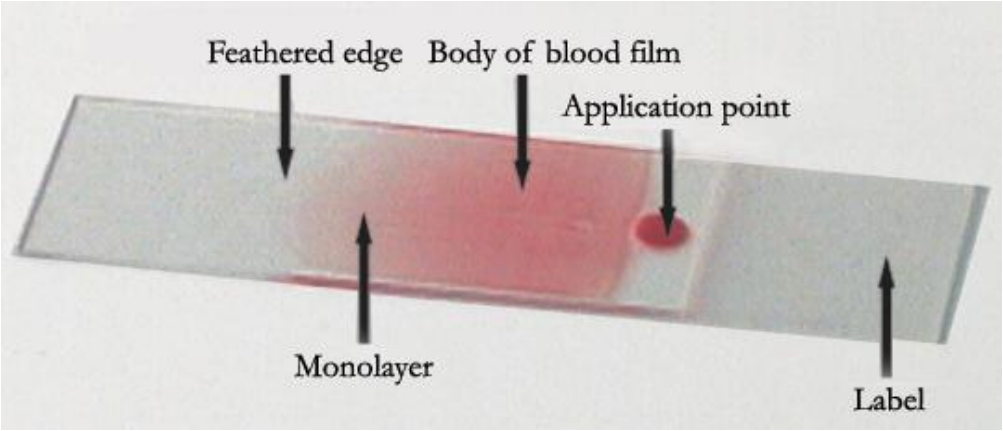


Fig. 6.2 Properly prepared smear

Fig. 6.3 Unstained and stained smear

Result

Normal value-

1. Neutrophils- 40-70%
2. Eosinophils- 1-8%
3. Basophils- 0-1%
4. Lymphocytes- 20-40%
5. Monocytes- 2-10%

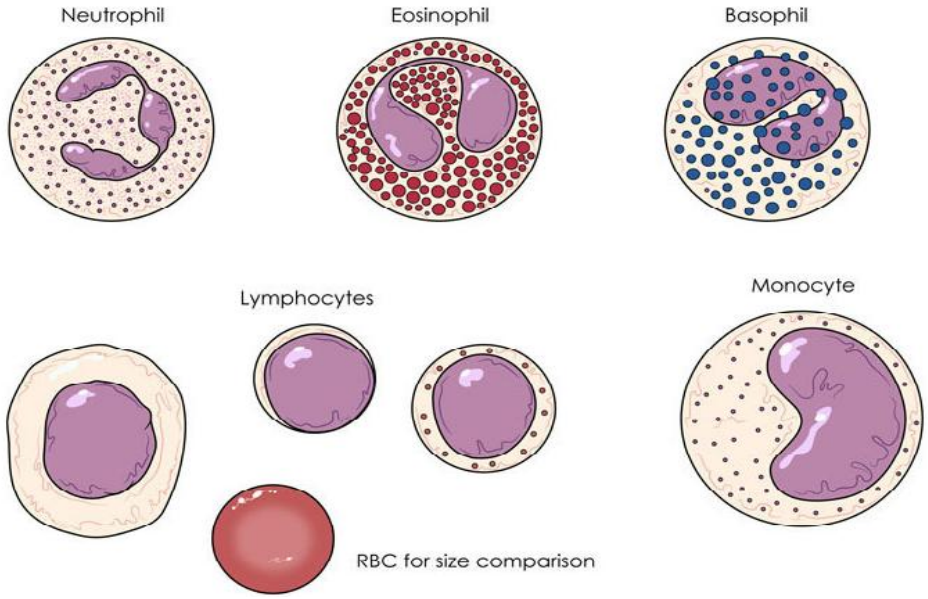


Fig 6.4

Clinical significance	Differential count is useful to identify changes in distribution of white cells which may be related to specific types of disorders. It also gives idea regarding the severity of disease & the degree of response of body. 1. Neutrophilia- Increase in Neutrophil count. • Pyogenic bacterial infection. 2. Neutropenia- Decrease in Neutrophil count. • Bacterial (typhoid, paratyphoid, tuberculosis) • Viral (hepatitis, influenza, measles) • Anaemias 3. Lymphocytosis- Increase in lymphocyte count. It may be relative or absolute. **Relative**- is actual number of lymphocytes remain unchanged but due to decrease in Neutrophils it appears increased. In **Absolute** actual count increases. It is found in Infections like • Mumps, cough, measles, influenza, typhoid • Chronic lymphatic leukemia 4. Lymphopenia-decrease in lymphocyte count. • Acute stage of infection 5. Eosinophilia-increase in Eosinophil count. • Hypersensitivity reaction

	• Parasitic infestation • Chronic inflammatory disease 6. Monocytosis-increase in Monocyte count. • Tuberculosis, • Malaria, • Kala azar 7. Basophilia-increase in basophile count. • Chronic myeloid leukemia
Skills to be achieved	Students can prepare smear, stain it and do the differential count.
Skill evaluating criteria	• Smear preparation technique- 3marks • Correct staining method- 1marks • Staining technique- 3 marks • Proper identification of cells- 3 marks
FAQs	1. How will you prepare smear? 2. What are criteria for a good smear? 3. What are Romanowasky stains? 4. Describe morphology different types of WBCs.
Assignment	Prepare smear and do the differential count of 10 different samples.
Reference	Chapter 6 Handbook of Haematology, clinical pathology and Blood banking

Week no.	7
Practical No.	**7**
Title/ Aim	**Study of morphology of blood cells** To identify various normal and abnormal forms of blood cells.
Objective	Students shall prepare peripheral smear and study the morphology of different types of blood cells.
Principle	Peripheral smear examination allows identification of different types blood cells and their abnormal forms.
Requirements	1. A stained peripheral smear 2. Immersion oil 3. Microscope
Environment	MLT laboratory
Procedure	1. Prepare smear and stain with Wright's or Leishman's stain (Refer practical no.6). 2. Choose the portion of the smear where the red cells are beginning to overlap. 3. Place a drop of immersion oil on the smear. Turn it to the oil immersion objective and examine the film by moving from one field to the next, systematically. 4. Observe different types of blood cells- RBCs, WBCs and platelets. 5. Study the normal and abnormal forms of

	cells.

Observations

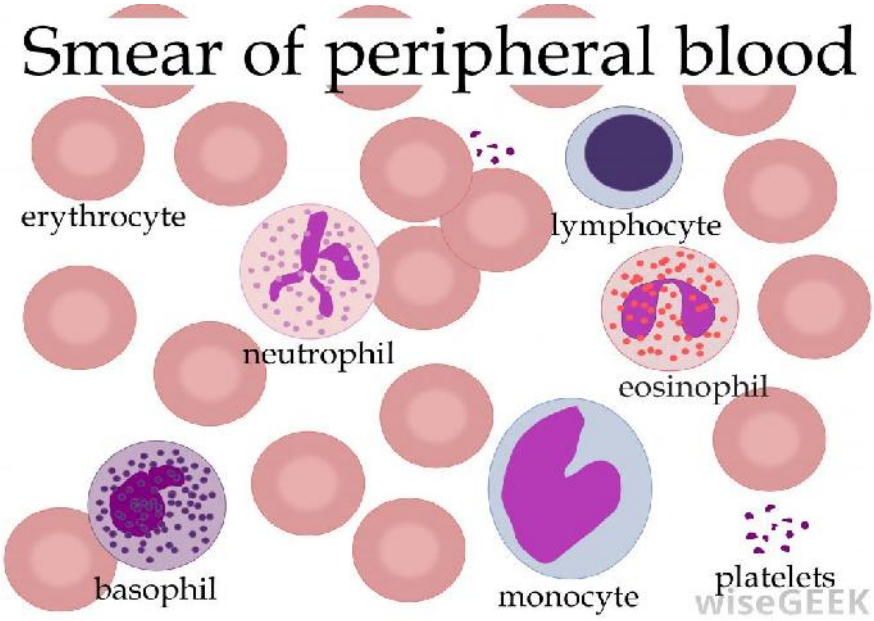


Fig. 7.1

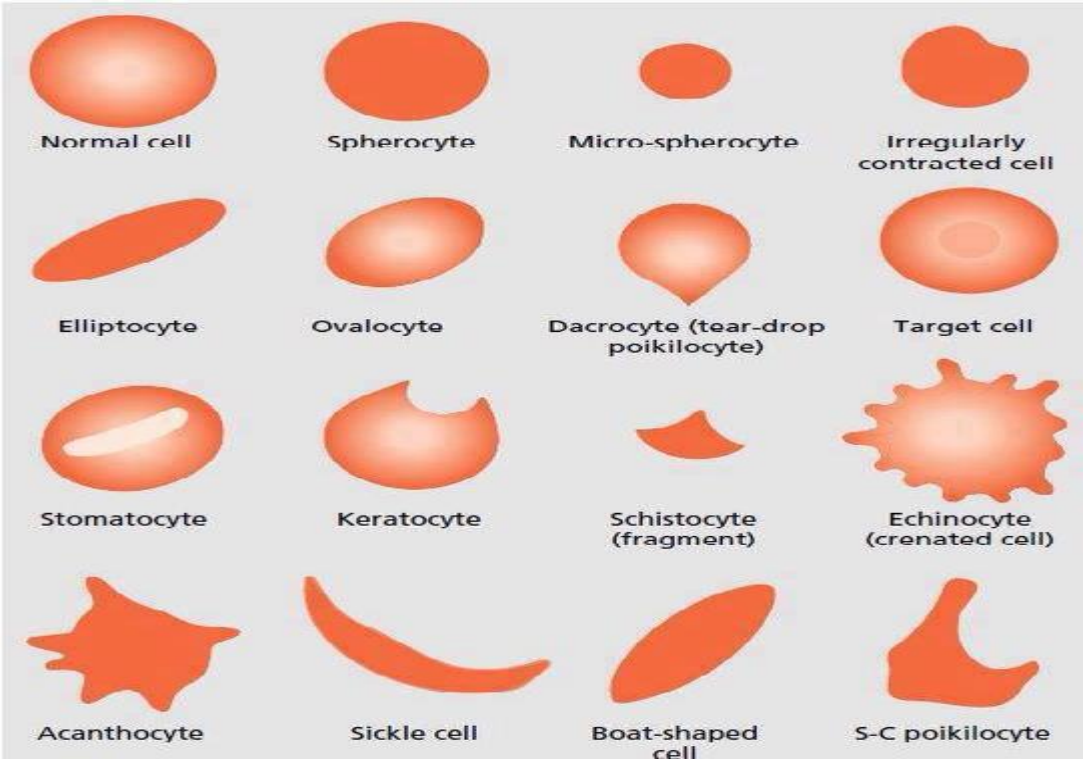


Fig. 7.2

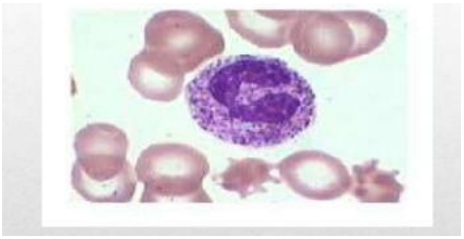


Fig. 7.3 Toxic granules

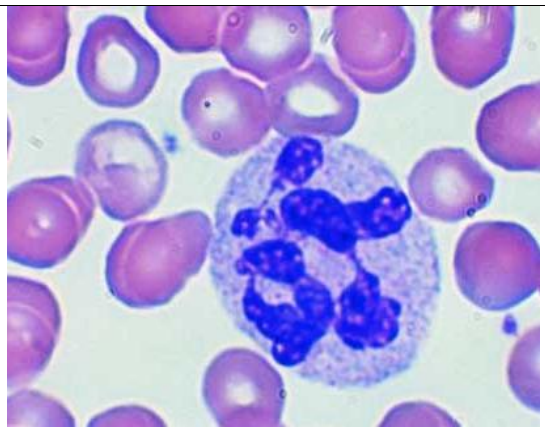


Fig. 7.4 Hyper segmented neutrophil

Result	A good peripheral smear is obtained and cells are observed.
Clinical significance	• Peripheral smear study helps to identify normal and abnormal forms of RBCs. • Also abnormalities of WBCs can be observed. • Approximate platelet count can be estimated.
Skills to be achieved	Students can identify abnormal blood cells.
Skill evaluating criteria	• A good peripheral smear preparation- 5marks • Proper identification of abnormality in blood cells- 5marks
FAQs	1. How will you prepare smear? 2. Which abnormality can you see in WBCs? 3. What are different abnormal forms of RBCs?
Assignment	Study 10 different peripheral smears to identify normal and abnormal blood cells.
Reference	Chapter 6 Handbook of Haematology, clinical pathology and Blood banking.

Week no.	8
Practical No.	**8**
Title/ Aim	**Absolute Eosinophil count**
Objective	Students shall perform absolute eosinophil count and should know its significance.
Principle	Blood is diluted with a special diluting fluid, which removes red cells and stains the Eosinophils red .These cells are then counted under low power in a known volume of fluid by using Neubauer counting chamber.
Requirements	1. Microscope 2. Improved Neubauer chamber 3. Diluting fluid (Hingleman's solution) • Yellow eosin - 0.5 g • 95%phenol -0.5 ml • Formalin - 0.5 ml • D/W - 99 ml 4. Specimen- • E.D.T.A. or Heparinized blood
Environment	MLT laboratory
Procedure	1. Pipette 0.36 ml of diluting fluid in a test tube. 2. By using Hb pipette add 0.04 ml of blood (use twice) 3. Mix and keep for 10 minutes. 4. Mix the diluent and charge the chamber.

	5. Keep in moist Petri dish for 2-3 minutes. 6. Count the cells under low power objective. 7. Count the cells in all nine squares.

Observations

Calculation-

$$\text{Total Eosinophils/cumm} = \frac{\text{Number of cells counted x dilution factor}}{\text{Area counted x depth of fluid}}$$

Where

Dilution- 10

Area- $9mm^2$

Volume of fluid = Area counted x depth

$= 9 \times 0.1$

$= 0.9$

$$\text{Total number of Eosinophil/cumm} = \frac{\text{Number of cells counted x 10}}{0.9}$$

$$= \frac{N \times 10}{0.9}$$

Result	**Normal range-** • 40-440/cumm.
Clinical significance	• Increased Eosinophil count is often associated with allergic reaction, parasitic infection, brucellosis & in certain leukemia. • Decrease Eosinophil count is seen with hyperadrenalism (Increase in the adrenal function).
Skills to be achieved	Students can count eosinophils on neubaur;s

	chamber.
Skill evaluating criteria	1. Adjustment of Neubaur's chamber on microscope- 2 marks 2. Proper identification of Eosinophils- 4 marks 3. Correct calculation- 4 marks
FAQs	1. What is principle of Absolute Eosinophil count? 2. What is formula for counting Absolute eosinophil count?
Assignment	1. Perform Absolute eosinophil count of 10 different samples.
Reference	Chapter 6 Handbook of Haematology, clinical pathology and Blood banking.

Week no.	9
Practical No.	**9**
Title/ Aim	**Reticulocyte count** To determine number of reticulocytes in a given sample of blood.
Objective	**S**tudent shall be able to count Reticulocytes using supravital stain from the given blood sample.
Principle	**S**upravital staining method is used for reticulocyte count. Blood is mixed with brilliant cresol blue stain and the stain enters the cell in living condition. The RNA in the cells is precipitated by staining as dark blue network or reticulum.
Requirements	1. Glass slides 2. Test tube 3. Pasteur pipette 4. Capillary tube 5. Test tube rack 6. Microscope 7. Brilliant cresol blue solution • Brilliant cresol blue 1.0 gm • Sodium citrate 0.4 gm • Sodium chloride 0.85 gm • Distilled water up to 100ml 8. Specimen**:** EDTA anti-coagulated blood

Environment	MLT laboratory
Procedure	1. Filter a small amount of stain (about 5ml). 2. In a test tube, add 2 drops of blood and 2 drops of stain. Mix thoroughly. 3. Cover the tubes with cotton plug and keep at 37^{o}c for 30 minutes. 4. Prepare a thin smear of the stained blood specimen by using a spreader. Air-dry the smear. 5. First examine under low power and then change to the oil immersion objective. 6. Counting is difficult because large number of cells is present in each field and all have to be counted. So to make it easy,field of vision may be cut down by inserting a small circular piece of paper with square cut from its centre in the eye piece. 7. Reticulocytes are identified by fine deep violet filaments and granules arranged in a network. Red cells stain pale blue. 8. Simultaneously count Reticulocytes and red cells in about 15 fields. Or most commonly 500 cells are counted.
Observations- Calculations: Reticulocyte % = $\frac{\text{Number of reticulocytes counted x 100}}{\text{No. of red cells counted}}$	

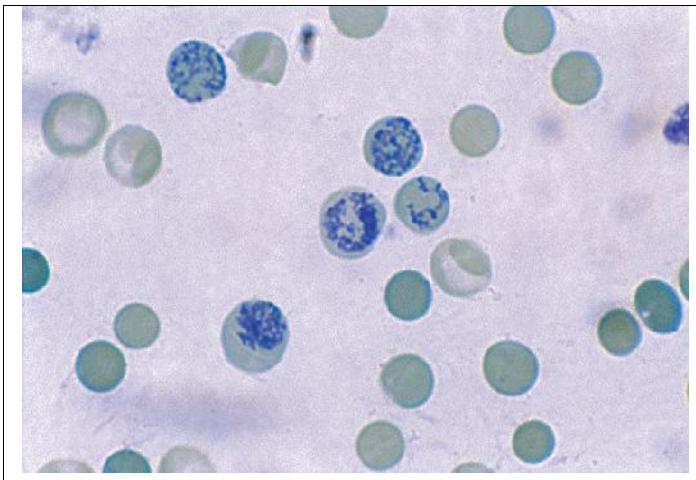


Fig 9.1

Result	**<u>Normal value</u>**: • Adults- 0.2 to 2% • Infants- 2 to 6%
Clinical significance	• The number of reticulocytes in peripheral blood is a reflection of red cell forming activity (Erythropoietic activity) of bone marrow. • Increase in reticulocytes indicates increased activity of bone marrow found in hemolytic anemia or acute blood loss (Reticulosis). • Repeated absence or low counts of reticulocytes indicate bone marrow depression found in aplastic anemia.
Skills to be achieved	Students can identify bone marrow activity by counting reticulocytes.
Skill evaluating criteria	1. Smear preparation – 2 marks 2. Content of diluting fluid – 2 marks 3. Correct staining – 2 marks

	4. Correct cell identification – 2 marks 5. Accurate calculation using formula – 2 marks
FAQs	1. What are reticulocytes? 2. Give its normal range. 3. Describe supravital staining. 4. Principle of supravital staining.
Assignment	Perform reticulocyte count of 10 different samples.
Reference	Chapter 5 Handbook of Haematology, clinical pathology and Blood banking.

Week no.	10
Practical No.	**10**
Title/ Aim	**ESR-Erythrocyte sedimentation rate** To determine ESR by Westergren method.
Objective	Students shall be able to obtain ESR from the collected blood sample by Westergren'sMethod
Principle	When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to bottom. The rate at which red cells fall is known as erythrocyte sedimentation rate. It occurs in three stages • Stage of **aggregation** when the red cells form rouleaux. • Stage of **sedimentation** in which the falling of red cells takes place. Larger the aggregates faster will be sedimentation. • Stage of **packing** when individual cells or aggregates slow down due to crowding.
Requirements	1. Westergren's pipette 2. Westergren's stand 3. Timer or watch. 4. Specimen- Take 0.5ml 3.8% sodium citrate in a test tube and add 1.5ml venous blood. Mix well.
Environment	MLT laboratory

Procedure	1. Fill Westergren's pipette exactly upto the '0' mark with the help of rubber (Avoid air bubbles). 2. Place the tube upright in the stand. Note the time. Allow the tube to stand exactly for 1hour. 3. After 1hour note the level at which the red cell column has fallen. 4. Report the result in terms of mm at the end of 1^{st} hr.

Observations- It will show two layers-

1. Clear plasma at the top
2. Column of red cells at the bottom.

Fig. 10.1 Westergren method

Result Normal Value	Male- 0-15 mm at the end of 1hr Female- 0-20mm at the end of 1hr
Clinical significance	1. Changes in ESR are not diagnostic of any specific disease.

	2. ESR is used to check the progress of the disease. If patient is recovering ESR falls. 3. ESR is increased in all conditions where there is breakdown or entry of foreign proteins in the blood. It increases in Tuberculosis, Myocardial infarction (MI) and Acute Rheumatic fever (ARF) and decreases with recovery. 4. In MI and ARF it is used to decide the moment when rest in bed may be discontinued. As long as ESR is raised bed rest is indicated. 5. ESR is less in polycythaemia vera.
Skills to be achieved	Students can perform ESR correctly.
Skill evaluating criteria	• Proper filling of Westergren's pipette- 3marks • Noting down the exact level of red cell column that has fallen- 3 marks • Use of proper anticoagulant- 2 marks • Correct reporting time- 2 marks
FAQs	1. Define ESR. Explain its steps. 2. What is the normal value of ESR?
Assignment	Perform ESR by Westergren method of 10 different samples.
Reference	Chapter 7 Handbook of Clinical pathology, Hematology and Blood banking.

Week no.	11
Practical No.	**11**
Title/ Aim	**ESR-Erythrocyte sedimentation rate** To determine ESR by Wintrobe's method.
Objective	Students shall be able to obtain ESR from the collected blood sample by Wintrobe'sMethod
Principle	When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to bottom. The rate at which red cells fall is known as erythrocyte sedimentation rate. It occurs in three stages • Stage of **aggregation** when the red cells form rouleaux. • Stage of **sedimentation** in which the falling of red cells takes place. Larger the aggregates faster will be sedimentation. • Stage of **packing** when individual cells or aggregates slow down due to crowding. When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to bottom. The rate at which red cells fall is known as erythrocyte sedimentation rate. It occurs in three stages

	• Stage of **aggregation** when the red cells form rouleaux. • Stage of **sedimentation** in which the falling of red cells takes place. Larger the aggregates faster will be sedimentation. • Stage of **packing** when individual cells or aggregates slow down due to crowding.
Requirements	1. Wintrobe's tube 2. Wintrobe's stand 3. Pasteur pipette 4. Timer or watch 5. Specimen • EDTA blood
Environment	MLT laboratory
Procedure	**1.** Mix the blood sample carefully. 2. Label a Wintrobe's tube. 3. Fill the tube by using a Pasteur pipette upto '0'mark. Avoid trapping of bubbles. 4. Place the tube in exactly vertical position in a Wintrobe's stand. 5. Allow the tube to stand exactly for 1hour. 6. After 1hour observe the level at which the red cell column has fallen. 7. Report the result in terms of mm at the end of 1^{st} hr.
Observations	

It will show two layers:

1. Clear plasma at the top
2. Column of red cells at the bottom.

Fig 11.1

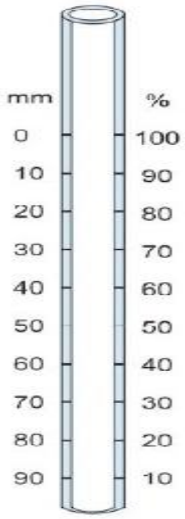


Fig 11.2

Result	Normal value • Male- 0-9 mm at the end of 1hr • Female- 0-20mm at the end of 1hr
Clinical significance	1. Changes in ESR are not diagnostic of any specific disease. 2. ESR is used to check the progress of the disease. If patient is recovering ESR falls. 3. ESR is increased in all conditions where there is breakdown or entry of foreign proteins in the blood. It increases in Tuberculosis, Myocardial infarction (MI) and Acute Rheumatic fever (ARF) and decreases with recovery. 4. In MI and ARF it is used to decide the moment when rest in bed may be

	discontinued. As long as ESR is raised bed rest is indicated. 5. ESR is less in polycythaemia vera.
Skills to be achieved	Students can fill wintrobe's tube correctly and carry out ESR determination.
Skill evaluating criteria	• Proper filling of Wintrobe's pipette- 3marks • Use of an anticoagulant- 2 marks • Noting down the exact level of red cell column that has fallen- 3marks • Correct reporting time- 2 marks
FAQs	1. Define ESR. Explain its steps. 2. What is the normal value of ESR?
Assignment	Perform ESR by Wintrobe's method of 10 different samples.
Reference	Chapter 7 Handbook of Clinical pathology, Hematology and Blood banking.

Week no.	12
Practical No.	**12**
Title/ Aim	**Haematocrit - PCV** (Packed Cell Volume) To determine PCV by Wintrobe's (Macrohaematocrit) method.
Objective	Students shall be able to obtain PCV from the collected blood sample by Wintrobe's method.
Principle	An anticoagulated blood is centrifuged to packed cells to maximum in a Wintrobe's tube at high speed. The volume of packed cells is determined and expressed in percentage.
Requirements	1. Wintrobe's haematocrit tube 2. Centrifuge machine 3. Pasteur pipette 4. Specimen • EDTA or Double oxalated blood.
Environment	MLT laboratory
Procedure	1. Mix the blood sample carefully. 2. Label a Wintrobe's tube. Fill the tube by using a Pasteur pipette or a syringe up to 100 mm mark. Avoid trapping of bubble. 3. Place the tube in a centrifuge machine. Use another Wintrobe's tube filled with another sample for the purpose of balancing. 4. Centrifuge for 30 minutes at 3000 rpm. 5. Note the reading. Multiply by 100 for volume

	Percentage (%).

Observations-When a column of blood is centrifuged it shows three layers.

1. A lowermost layer of packed cells-indicates PCV.
2. A Buffy coat- is a thin layer of WBCs and platelets which is just above the packed cells. It is grayish in color. It is 0.5 to 1mm thick.
3. The uppermost layer of plasma.

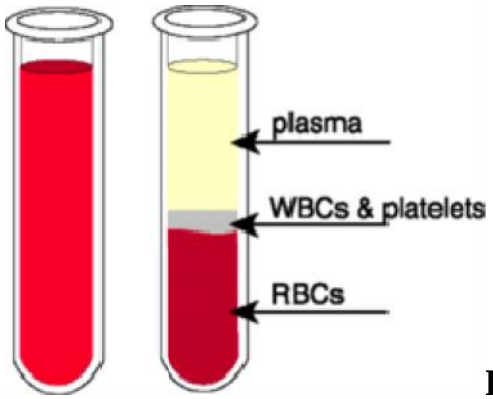


Fig 12.1

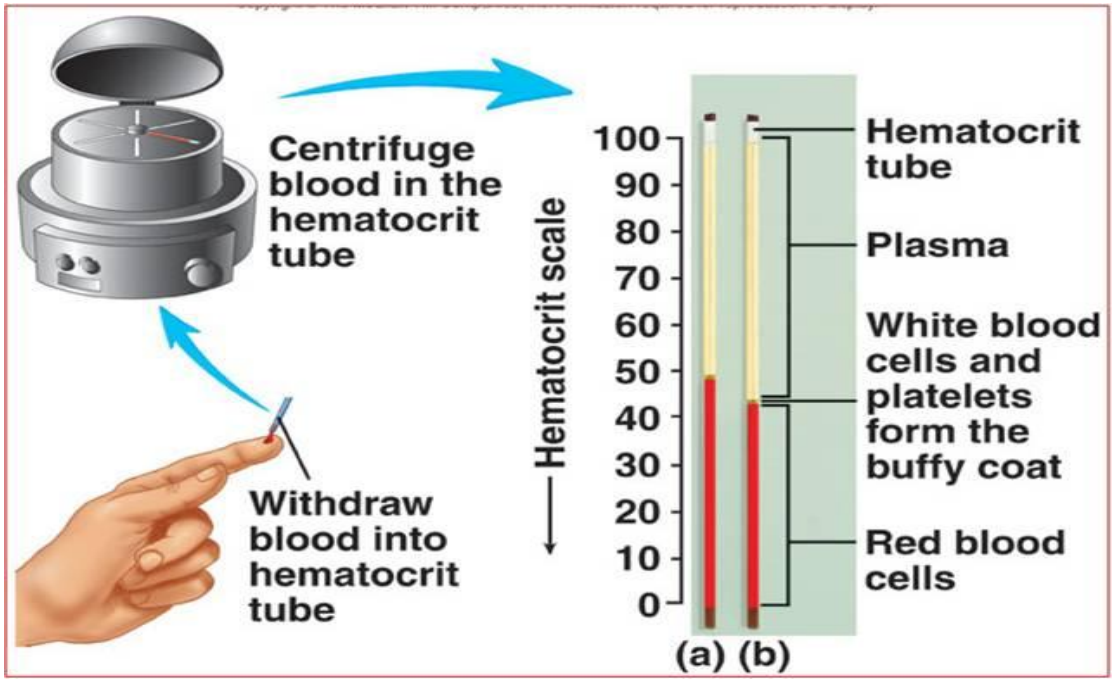


Figure 12.2

Result	**Normal value-** • Males- 40-54% • Females- 36-47%

Clinical significance

When a column of blood is centrifuged it shows three layers.

1. A lowermost layer of packed cells-indicates PCV.

- PCV decreases in Anemia.
- PCV increases in polycythaemia, dehydration, congenital heart disease.

2. A Buffy coat- is a thin layer of WBCs and platelets which is just above the packed cells. It is grayish in color. It is 0.5 to 1mm thick. It gives approximate indication of number of WBCs and platelets. Normally 0.1mm corresponds to 1000WBCs/mm^3. When count gets increased (>30,000/mm3) then 0.1mm ≈ 2000 cells/mm^3. Marked increase in platelets cause increase in Buffy coat. Buffy coat is used in demonstration of LE cell and in cases of sub-leukemic leukemia.
3. The uppermost layer of plasma. The color of plasma indicates certain conditions.

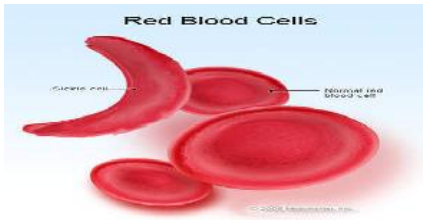
- Pale yellow Normal Yellow Jaundice
- Pink Haemolysis Milky Lipemia
- Brown Meth-haemoglobin
- Cloudy Multiple myeloma

Thus PCV is helpful in finding out information regarding RBCs, WBCs, platelets and plasma.

Skills to be achieved	Students will develop the skill of filling of PCV tube and perform test.

Skill evaluating criteria	• Proper collection of blood- 2marks • Proper filling of Wintrobe's tube- 3marks • Proper use of centrifuge- 2marks • Correct timing- 1marks • Correct reading of Red cell column- 2 marks
FAQs	1. Define PCV. 2. Give normal value. 3. What is the significance of performing PCV?
Assignment	Process any 10 samples for PCV by wintrobe's method.
Reference	Chapter 8 Hand book of Clinical pathology, Hematology and Blood Banking

Week no.	13
Practical No.	**13**
Title/ Aim	**Sickling test** To screen blood smear for sickle cell.
Objective	The student shall be able to screen the given blood sample for sickle cell anemia.
Principle	When whole blood is mixed with sodium metabisulphite which deoxygenates hemoglobin. If RBCs contain Hb-S, they become sickled or half-moon shaped.
Requirements	1. Microscope, 2. Slides, 3. Cover slips, 4. Pasteur pipette, 5. Reagent- Na metabisulphite 2% W/V Na metabisulphite 0.5gms Distilled water 25ml Make fresh solution before use. 6. Specimen**:** EDTA blood
Environment	MLT laboratory
Procedure	1. Place a drop of blood on the glass slide. Add equal drop of sodium metabisulphite. 2. Mix carefully with the corner of a cover slip. Seal edges and place in a Petri dish that has weight filter paper. Incubate for 15 minutes at 37 oc.

	3. Examine the various parts of the preparations under high power of the microscope. 4. If the result is negative re-examine after 2 hours and after 24 hrs.
Observations  **Fig. 13.1**  **Fig. 13.2**	
Clinical significance	1. Sickle cell anemia is due to inheritance of abnormal hemoglobin Hb-S. HbS is insoluble in reduced oxygen tension which makes RBCs susptible to sickling. Sickle cells have tendency to clump together. It can lead to blockage of blood vessels interrupting circulation. 2. The sickle cells get easily hemolysed and lead to chronic hemolytic anemia. 3. When there is homozygous inheritance of the abnormal Hb (HbS-S) leads to sickle cell disease. Clinically it presents as severe hemolytic anemia. 4. When there is heterozygous inheritance of the abnormal HbS (HbS-A) leads to sickle

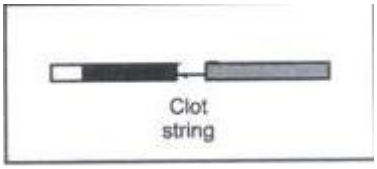
	cell trait. It is mild and asymptomatic.
Skills to be achieved	Students can perform sickle cell screening.
Skill evaluating criteria	• Preparation of Na metabisulphite reagent- 3marks • Smear preparation and proper sealing- 4marks • Identification of sickle cells- 3marks
FAQs	What is sickle cell anemia?
Assignment	Process any 10 samples for sickle cell screening.
Reference	Chapter 10 Handbook of Clinical pathology, Haematology and Blood banking.

Week no.	14
Practical No.	**14**
Title/ Aim	**Bleeding time** To determine bleeding time by **Duke's** method.
Objective	The student shall be able to do bleeding time.
Principle	A standard incision is made on the skin of the patient. The length of time required for bleeding to stop is recorded.
Requirements	1. Lancet or needle 2. Spirit swab 3. Filter paper 4. Stop watch 5. Glass slide.
Environment	MLT laboratory
Procedure	1. Clean the earlobe or a tip of the finger with alcohol and let it dry. 2. For earlobe glass slide is placed behind the earlobe and held firmly in place this provides firmness for incision. 3. Pierce the lobe of the ear by a firm stroke against the glass slide or prick the finger tip . 4. Start the stop watch. 5. Bleeding of the wound should be allowed to proceed without pressure. 6. Note the time for which the puncture bleeds by blotting the drop of blood every 30

	seconds with a filter paper till there is no further staining on the filter paper. 7. Count the number of drops of blotted blood on filter and multiply it with 30 seconds and report with nearest minute.
Observations Bleeding time is_____ minutes and _____ seconds.  Fig. 14.1 Duke's method	
Result	**Normal range:** 1-5 minutes
Clinical significance	• Bleeding time helps to detect vascular defect and platelet disorder. • Prolonged bleeding time is generally associated with thrombocytopenia.
Skills to be achieved	Students can do bleeding time perfectly.
Skill evaluating criteria	• Proper site selection and preparation for pricking- 5marks • Correct time interval for blotting blood drop- 3 marks • Calculation- 2 marks
FAQs	1. Define bleeding time. 2. What are methods of performing bleeding time?

Assignment	Perform bleeding time by Duke's method of 10 different subjects.
Reference	Chapter 11 Handbook of Clinical pathology, Haematology and Blood banking

Week no.	15
Practical No.	**15**
Title/ Aim	**Clotting time** To determine clotting time by capillary method.
Objective	The student shall be able to do clotting time of the given patient.
Principle	Blood is collected in a capillary tube after a finger prick and the stop watch is started. The time required for first appearance of fibrin string is the clotting time.
Requirements	1. Disposable lancet, 2. Capillary tubes (10-15 cms length &diameter 1.5 mm) without anticoagulant.
Procedure	1. Warm up the finger. 2. Make an incision with a sterile lancet, as soon as the blood starts flowing then start the stop watch. 3. Wipe off the first drop of blood and allow the blood to flow through the capillary tube, there must be no air bubble or air gap in the capillary tube. 4. After 2 minutes break off the capillary tubing 1- 2 cm from one end of the capillary tube. 5. When a thin string of fibrin is seen from broken end of the capillary tube stop the stopwatch and record the time.

Environment	MLT laboratory
Observations  **Fig. 15.1**  **Fig. 15.2** Clotting time is_____ minutes & _____seconds.	
Result	**Normal range:** 4 to 9 minutes
Clinical significance	1. Clotting time is performed as a screening test for coagulation disorder. 2. Prior to any major surgery this test is carried out.
Skills to be achieved	Students can do clotting time by capillary method.
Skill evaluating criteria	• Proper site selection and preparation- 3marks • Correct observation for string formation- 4marks • Correct timing- 3marks
FAQs	What is principle of clotting time by capillary method?
Assignment	Perform clotting time on 10 different subjects.
Reference	Chapter 11 Handbook of Clinical pathology, Haematology and Blood banking

Week no.	16
Practical No.	**16**
Title/ Aim	**Clotting time** To determine clotting time by venous **Lee and White** method.
Objective	The student shall be able to do clotting time of the patient.
Principle	Venous blood is collected in a clean and dry test tube without anticoagulant. The time required for clotting is noted.
Requirements	1. Stop watch 2. Disposable syringe 3. Test tubes 4. 37 degrees centigrade water bath 5. Tourniquet 6. Sterile cotton swab 7. Spirit
Environment	MLT laboratory
Procedure	1. Make a venipuncture. 2. Start the stop watch when blood enters in the syringe. 3. Draw 2- 5 ml of blood .Place 1 ml of blood in each test tube. Plug the tube with non absorbent cotton wool. 4. Place the test tube in a stand at 37^0C.

	5. After 3 minutes take the first test tube from the water bath and gently tilt it every 30 seconds at 45 degrees angle. 6. When blood is clotted in 1st tube the same tube can be tilted to an angle of 90 degrees without spilling the contents. 7. As soon as the blood is clotted immediately examine the 2nd tube, usually it clots very soon after the blood in the 1st tube. 8. Stop the stopwatch and note the time. 9. The coagulation time is the clotting time of the second tube.
Observations Clotting time is: First tube: _____minutes and _____ seconds. Second tube: _____minutes and_____ seconds	
Result	**Normal Range:** 5 to 12 minutes.
Clinical significance	1. This method is more accurate than capillary method. 2. Clotting time is performed as a screening test for coagulation disorder. 3. Prior to any major surgery this test is carried out. 4. The clotted blood can be further examined for clot retraction and lyses time.
Skills to be achieved	Students can do clotting time using venous

	blood.
Skill evaluating criteria	1. Correct vein puncture- 3marks 2. Volume of blood should be perfect (about 1ml)- 2mark 3. Accurate incubation temperature- 2marks 4. Temperature of water bath must be precise 37^0 C ± 5^0C- 1mark 5. Correct timing- 2marks
FAQs	1. What is the principle of Lee White clotting time? 2. What are its advantages?
Assignment	Perform clotting time on 10 different subjects
Reference	Chapter 11 Handbook of Clinical pathology, Haematology and Blood banking

Week no.	17
Practical No.	**17**
Title/ Aim	To determine Prothrombin time by Quick's method.
Objective	The student shall be able to find out Prothrombin time of the given blood sample.
Principle	Thrombokinase preparation containing calcium ions are added to citrated plasma. In the presence of factor VII, stage 2 coagulation mechanism is started and the clotting time is recorded after the addition of thrombokinase in the presence of calcium ions.
Requirements	1. 37 ^{o}c water bath, 2. Stop watch, 3. Test tubes, 4. Commercially available Thrombokinase tablet 0.15 grams/dl calcium chloride 5. Specimen**:** citrated plasma
Environment	MLT laboratory
Procedure	1. Place a test tube containing 2 ml of calcium chloride, in 37 ^{0}C water bath. 2. Pipette 0.1 ml of plasma in a small test tub and add 0.1 ml of brain thromboplastin and mix. (Or use thrombokinase tablets as per manufacturer's instructions). 3. Wait for 2 minutes.

	4. Add 0.1 ml of pre warmed calcium chloride solution, mix and start the stopwatch. 5. Observe the test tube in front of a source of light by tilting the tube gently. 6. At the first appearance of a fibrin clot stop the stopwatch. 7. Record the time. Repeat the steps twice to check the reliability of results. 8. Repeat the procedure by using normal plasma. 9. Report prothrombin time in seconds.
Observations  **Fig. 17.1**	
Result	**<u>Normal range:</u>** 14 ± 2 seconds
Clinical significance	1. Prothrombin is synthesized in the liver in the presence of vitamin K. Factor VII is also synthesized in liver which is related to prothrombin. 2. In clotting process in stage II prothrombin is

	converted in to thrombin which converts soluble fibrinogen into insoluble fibrin clot. 3. Prolonged prothrombin time is related to deficiencies of factor II, V, VII and X. 4. PT is used to monitor the coumarin group of drug therapy as excess of it causes hemorrhagic conditions.
Skills to be achieved	Students can do prothrombin time correctly.
Skill evaluating criteria	▪ Separation of plasma- 2marks ▪ Proper anticoagulant- 2marks ▪ Observation for appearance of clot- 2marks ▪ Correct timing- 2marks ▪ Testing normal plasma- 2marks
FAQs	1. What is prothrombin time? 2. What is significance of prothrombin time?
Assignment	Perform prothrombin time of 10 different samples.
Reference	Chapter 11 Handbook of Clinical pathology, Haematology and Blood banking

Week no.	18
Practical No.	**18**
Title/ Aim	**Platelet count** To determine the platelet count of given blood sample.
Objective	Students shall carry out platelet count perfectly.
Principle	Specimen is diluted with platelet diluting fluid which prevents coagulation (Citrate), fixes platelets (formalin) and prevents from clumping. The dye Brilliant cresol blue provides background during counting.
Requirements	1. RBC pipette 2. Improved Neubauer counting chamber 3. Petri dish 4. Microscope 5. Platelet diluting fluid • Tri-sodium citrate 3.8gm • Neutral formalin 0.2ml • Brilliant cresol blue 0.1gm • Distilled water 100ml 6. Specimen • EDTA anticoagulated blood • Capillary blood
Environment	MLT laboratory
Procedure	1. Mix the blood specimen carefully. 2. Draw the blood up to the mark 0.5 in RBC

	pipette. 3. Draw the diluting fluid up to the mark 101. 4. Mix the contents thoroughly. 5. After 5 minutes discard first four drops and load the counting chamber properly. 6. Allow this chamber to stay for 15 minutes in a Petri dish with a moist filter paper. It keeps specimen wet . 7. Keep chamber carefully on the microscope. 8. First focus under low power. 9. Turn under high power and count cells in entire central Square (25 squares with triple lines).
Observations No. of platelets counted = N Dilution = 1 in 200 Volume of fluid = 1 x 0.1 = 0.1 cu mm Platelets count per cu mm (µl) = No. of platelets counted x dilution/volume of fluid = N x 200/0.1 = N x 2000	
Result	Normal value- 1- 4 lakh/mm^3
Clinical significance	1. Decrease in platelet count is called Thrombocytopenia and increase count is called Thrombocytosis. 2. Decrease count indicates aplastic anaemia,

	marrow depression etc. 3. Increase in platelet count indicates polycythemia vera . 4. Decrease platelet count increases risk of bleeding e.g. Dengue fever.
Skills to be achieved	At the end of this practical the student will be able to count the platelets accurately.
Skill evaluating criteria	1. Correct quantity of blood and diluting fluid – 3 marks. 2. Proper selection of squares for the count – 2 marks. 3. Accurate counting- 3 marks 4. Calculations – 2marks.
FAQs	1. Define Thrombocytosis and Thrombocytopenia. 2. What are causes of thrombocytopenia and thrombocytosis? 3. Normal value of Platelet.
Assignment	Perform platelet count of 10 different samples.
Reference	Chapter 11 Handbook of Clinical pathology, Haematology and Blood banking

Week no.	19
Practical No.	**19**
Title/ Aim	Urine analysis To perform physical examination of urine.
Objective	Students shall understand the importance of physical examination of urine.
Requirements	1. Measuring cylinder 2. Urinometer 3. Litmus paper 4. Properly collected urine sample
Environment	MLT laboratory
Procedure	1. Measure the volume of urine sample with the help of measuring cylinder. 2. Examine the urine sample for odor, color and appearance. 3. Note the pH with by strip method or litmus paper. 4. Measure the specific gravity of urine with urinometer. **Procedure:** • Mix the urine and pour into the cylinder. • Carefully float the urinometer by grasping the stem of the urinometer at the top and inserting slowly into the urine. • Read the specific gravity as soon as the urinometer comes to rest.

	• For accurate result do calibration and temperature correction. • Urinometers are usually calibrated for 20˚c. So the observed figure has to be corrected for the difference between this temperature and room temperature in the proportion of 0.001 to every 3˚c.

Observations / Result

Physical criteria	Significance
Volume • Normal- 1200-1500ml	
1. Polyuria- >2000ml	1. Diabetes mellitus and Diabetes insipidus
2. Oliguria- <400ml	2. Diseases of kidney, dehydration from any cause, fever and CCF.
3. Anuria- < 100ml	3. Mismatched blood transfusion, Acute glomerulonephritis, surgical shock,etc
Color: • Normal freshly voided urine - colorless to dark yellow or straw-colored.	
1. Pale	1. Due to very dilute urine. Found in Diabetes insipidus, chronic renal failure.

2. Reddish 3. Brownish yellow or green with yellow foam 4. Brown-Black 5. Milky white	2. Presence of blood in urine (Hemoglobinuria and hematuria) 3. Presence of bile pigments, associated with jaundice. 4. Associated with poisoning (Lead, mercury, and phenol), Hemorrhages, and melanotic tumours, alkaptonuria. 5. Presence of fat globules (chyluria) due to filariasis.
Odor: Freshly passed normal urine has a characteristic **aromatic** odor 1. A foul or a putrid smell 2. A fruity smell 3. Musty odor.	1. Urinary tract infection. 2. With diabetic ketosis. 3. The urine of phenylketonuria
Appearance: Normal urine is clear in appearance. • Turbidity and cloudiness of urine and a mucoid, whitish sediment.	• Turbid on cooling due to the precipitation of mucin from the urinary tract or precipitation of calcium phosphate. The turbidity

• Red smoky appearance.	may disappear with the addition of dilute acetic acid to neutral or alkaline urine or heating of acidic urine. • Presence of fat and chyle. • Presence of pus cells, bacteria & mucous • Presence of blood.
Reaction &pH: The freshly voided normal urine is usually **slightly acidic.** In health normal pH vary from **5 to 8**, with average around **6.0.** 1. Acidic urine: 2. Alkaline urine:	• Diet high in meat protein, • Respiratory acidosis (Retention of carbon dioxide), • Metabolic acidosis (uremia, renal tubular acidosis, diabetic ketoacidosis, starvation, severe diarrhea) • Diet rich in vegetables, citrus fruits like lemon,

	• Post prandial urine, • Respiratory alkalosis (Hyperventilation), • Metabolic alkalosis (severe vomiting), • Urinary infections caused by Proteus and Pseudomonas.
Specific gravity: • The specific gravity of normal urine (24 hour collection) ranges from **1.015 to 1.025.**	• The specific gravity of urine is subjected to wide variation. Excessive water intake will dilute the urine and the specific gravity will be less than 1.015. • Profuse perspiration and poor water intake will raise the specific gravity above 1.025. • If the kidney is functioning properly, the concentrated first morning specimen of urine should have a specific gravity greater than 1.020. • Usually, increased discharge of urine accompanied by decreased specific gravity (as low as 1.010) is typical of diabetes insipidus. • Presence of protein and sugar causes increase in specific gravity.
Skills to be achieved	Students can measure specific gravity of urine,

	volume and other parameters correctly.
Skill evaluating criteria	• Proper collection of urine- 2marks • Volume measurement- 2marks • Correct measurement of specific gravity- 4marks • Correct measuring pH- 2marks
FAQs	1. Define polyuria, oliguria and anuria. 2. What is specific gravity? 3. Describe urinometer. 4. How pH is measured? 5. What is significance of volume and specific gravity measurement?
Assignment	Perform physical examination of 10 different urine samples.
Reference	Chapter 13 Handbook of Clinical pathology, Haematology and Blood banking.

Week no.	20
Practical No.	**20**
Title/ Aim	Urine analysis To perform chemical examination of urine.
Objective	Students shall understand the importance of detecting various abnormal constituents in urine like proteins, sugar, etc.
Requirements	1. Urine sample 2. Reagents • Benedict's reagent • Sulphosalycylic acid • Na-nitroprusside • Ammonium sulphate • Liquor ammonia • Benzidine reagent • H_2O_2 • Glacial Acetic acid • Sulphur powder • Barium chloride • Fouchet's reagent
Environment	MLT laboratory
Procedure	1. **Proteins**- Sulphosalicylic acid test

	Principle – When urine sample is mixed with Sulphosalicylic acid if it shows turbidity indicates proteins are present in urine. Procedure**:** • Take 2 ml of centrifuged (clear) urine specimen in a test tube. Add equal amount of sulphosalicylic acid reagent. • Shake the test tube gently and let it stand for 10 minutes. • Note the degree of turbidity by looking at the illuminated tube against a dark background. 2. **Sugar-** Benedict's qualitative test Principle**:** When Benedict's qualitative reagent is heated with urine, glucose present in urine reduces cupric ions to cuprous ions. Alkaline medium is provided to the reaction by sodium carbonate present in the reagent. The original color of Benedict's reagent is blue. It changes to green, yellow, orange or red, according to concentration of glucose present in urine. **Note:** The test is nonspecific for glucose since the reaction may be brought by other carbohydrates such as fructose, galactose , lactose & pentose and also by noncarbohydrates such as ascorbic acid, salicylates, creatine and uric acid. Hence if Benedicts test is positive it is

	necessary to perform glucose oxidase (uristix) test to confirm whether it is due to only glucose. **Procedure**: • Take 5 ml of Benedict's qualitative reagent in a test tube. • Add 8 drops of urine. • Heat carefully on spirit lamp or burner. • Allow to cool & observe color. **3. Ketone bodies**- Rothera's test Principa**l:** Nitroprusside used in this test reacts with both acetone & acetoacetic acid in the presence of alkali (ammonium hydroxide) to produce a purple colored compound. Procedure**:** • Take 5ml of urine in a test tube. • Saturate it with ammonium sulphate. • Add one crystal of sodium nitroprusside. • Let liquor ammonia run carefully down side of the test tube so it forms a layer on the top of the urine. • If ketone bodies are present, there is formation of pink – purple color ring at the junction of two layers. 4. **Blood**- Benzidine test Principle**:** Haemoglobin is a complex substance & one of its constituents is 'Haem' which acts as

	a catalyst when hydrogen peroxide is mixed with substances like benzidine. Procedure: • Dissolve a small amount (knife- point full) of benzidine powder in 2ml of glacial acetic acid & add an equal volume of 3% hydrogen peroxide. • Add 2ml of urine & mix. • The appearance of green or blue color indicates presence of haemoglobin. **5. Bile-** **a. Bile pigments**- Fouchet's test • Barium chloride solution is added in the urine. • A precipitate of barium sulphate is produced onto which bile pigments are adsorbed. • Filter this solution and obtain precipitate on filter paper. • Or alternatively centrifuge the mixture, pour off supernatant & obtain precipitate in the test tube. • Add 2-3 drops of Fouchet's reagent on the precipitate on filter paper or sediment in the test tube. • If color changes to green, bile pigments are present.

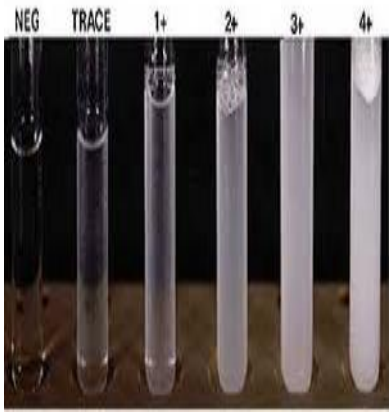
b. **Bile salts**- Hay's sulphur test

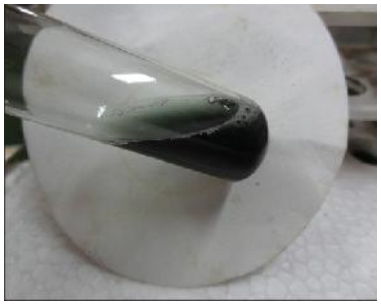
Principle**:** Bile salts when present lower the surface tension of the urine.

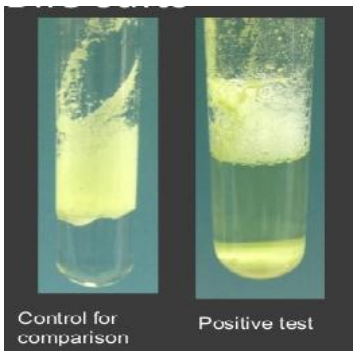
Method

- Take urine sample in the test tube.
- Sprinkle the sulphur powder on the surface of urine.
- If bile salts are present they sink to the bottom.
- In case of normal urine, sulphur particles float on the surface of urine.

Observations

Abnormal constituents present	**Observation**
Proteins  Figure 20.1	Negative- No turbidity or no increase in turbidity (0.005g/dl or less). Trace- Faint turbidity (0.010g/dl) 1+ Distinct turbidity but no granulation (0.050g/dl) 2+ Turbidity with granulation but no flocculation (0.20g/dl) 3+ Turbidity with granulation and flocculation (0.50g/dl) 4+ Clumps of precipitate (1.0g/dl)
Sugar	**Color** / **Conclusion** Blue / Sugar Absent

<table>
<tr>
<td> 
Figure 30.2</td>
<td>
<table>
<tr><td>Green & slight yellow precipitate</td><td>Sugar present 0.5%</td></tr>
<tr><td>Green and thick yellow precipitate</td><td>Sugar present 1%</td></tr>
<tr><td>Yellow and orange precipitate</td><td>Sugar present 1.5%</td></tr>
<tr><td>Orange and orange to red precipitate</td><td>Sugar present 2% and above</td></tr>
</table>
</td>
</tr>
<tr>
<td>Ketone bodies
 
Figure 40.3</td>
<td>Presence of ketone bodies shows formation of pink – purple color ring at the junction of two layers.</td>
</tr>
<tr>
<td>Blood
 
Figure 50.4</td>
<td>The appearance of green or blue color indicates presence of blood.</td>
</tr>
<tr>
<td>Bile pigments
 
Figure 60.5</td>
<td>If color changes to green, bile pigments are present.</td>
</tr>
</table>

Bile salts  Figure 70.6	Normal urine- Sulphur particles float on the Surface of urine. Presence of bile salts- Sulphur powder sink to the bottom.

Result/ clinical significance

Abnormal constituent present	Significance
Proteins	**Pathological proteinuria** It is of three types- Pre-renal- Due to secondary effects on kidney. Due to impairment of renal circulation. Found in dehydration, heart disease, fever, severe anaemia, etc. Renal- Due to diseases of kidney like Inflammation of kidney (nephritis), urinary tract infection, degenerative diseases of kidney(nephrosis), Post renal- Lesions in renal pelvis, bladder, prostatic urethra, etc.
Sugar	Various clinical conditions related to glycosuria are as follows: • Diabetes mellitus is the most common cause

	of hyperglycemia. • Hyperthyroidism, hyperpituitarism & hyperadrenalism. • In renal glycosuria, due to defect in the reabsorptive ability of the renal tubules • Non pathological cases of transitory hyperglycemia which will result into glycosuria are ▪ Pregnancy: Glycosuria is due to lowered renal threshold. ▪ Stress and anxiety: Hyperglycemia due to an increased output of epinephrine and glucocorticoids.
Ketone bodies	• Diabetes mellitus is the most important disorder in which ketonuria occurs. • Ketonuria also accompanies the other conditions such as anorexia, fasting, starvation, fever and prolonged vomiting.
Blood Haematuria Haemoglobinuria	➢ Renal calculi, acute infections, nephritic syndrome, trauma to kidney, tumours of kidney, etc. ➢ Thrombocytopenia, coagulation factor deficiency. ➢ Haemolytic anaemias

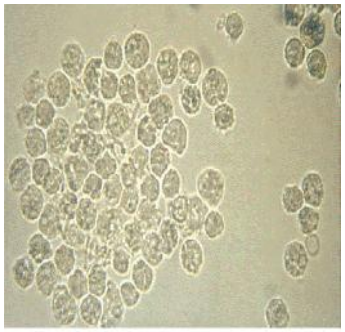
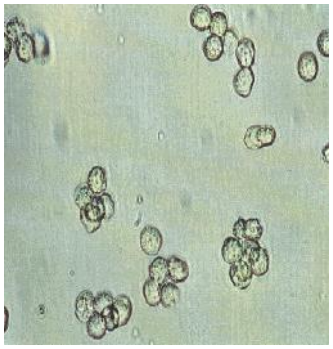
	➢ Complications of falciparum malaria ➢ Severe burns ➢ Bacterial toxins, ➢ Mushroom poisoning ➢ Poisoning from snake venom
Bile pigments Bile salts	Seen in cases of obstructive jaundice.
Skills to be achieved	Students can detect different abnormal components perfectly.
Skill evaluating criteria	• Preparation of benzidine reagent- 1mark • Correct technique of tests- 6marks (1mark of each test) • Correct interpretation of result- 3marks
FAQs	• Give principle of a. Benedict's test b. Sulphosalycylic acid test c. Rothera's test d. Benzidine test e. Fouchet's test f. Hay's sulphur test • Give significance of each constituent in urine.
Assignment	Perform chemical tests on 10 different samples.
Reference	Chapter 13 Handbook of Clinical pathology, Haematology and Blood banking

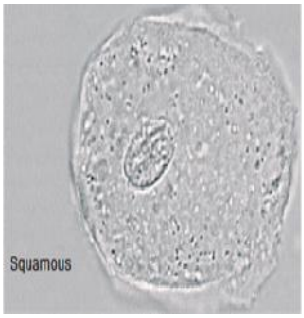
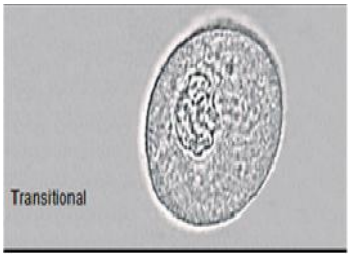
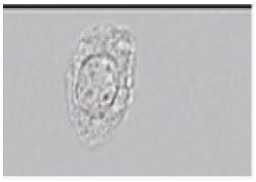
Week no.	21
Practical No.	**21**
Title/ Aim	Urine analysis To perform microscopic examination of urine.
Objective	Students shall understand the importance of various casts, crystals and cells found on microscopic examination of urine.
Principle	The microscopic elements present in urine are collected in the form of deposit by

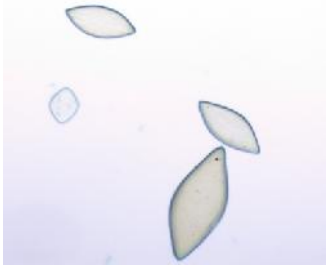
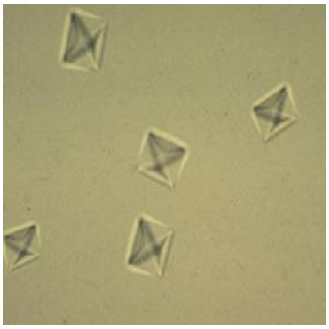
	centrifugation. A small drop of the sediment is examined by making a cover slip preparation under microscope.
Requirements	1. Urine sample 2. Centrifuge 3. Glass slide 4. Cover slip
Environment	MLT laboratory
Procedure	1. Centrifuge 5-10ml of fresh urine sample. 2. Pour off the supernatant fluid. 3. Mix the deposit with remaining few drops of urine. 4. Transfer a small quantity on a clean glass slide. 5. Apply a cover slip. Air bubbles should not be there. 6. Examine under microscope.

Microscopic constituent	**Morphology**	**significance**

Observations / Result

Cells <u>Leucocytes</u>: Pus cells • Normal urine can contain 2-3 pus cells/hpf  Figure 21.1 <u>Erythrocytes:</u> -  Figure 21.2 <u>Epithelial cells:</u> Normally few cells3 to 5/ hpf	Granular spheres 12µm in diameter. Nuclear segments appear as small, round discrete nuclei. In fresh urine these cells have a normal, pale or yellow appearance. They appear smooth, biconcave discs about 7 µ diameters and 2 µ thick. They do not contain nuclei.	• Presence of >50/hpf – Acute infection of urinary tract infection. • Moderate no of leucocytes along with leucocyte casts- Bacterial or non-bacterial renal disease. • Increase count- Cystitis, bladder tumor Prostatitis. Renal calculi, acute infections, nephritic syndrome, trauma to kidney, tumours of kidney, etc.

Three main types 1. Squamous  Figure 21.3 2. Transitional  Figure 21.4 3. Tubular  Figure 21.5	1. Large flat cells with abundant cytoplasm with small round nuclei. Margins are folded. 2. Round or pear shape. Round central nuclei. 2-3 times of leukocytes. 3. Single large oblong or egg shape with coarsely granular eosinophilic cytoplasm. Multiple nuclei.	• Acute tubular necrosis • Ischaemic injuries to kidneys • Renal transplant rejection
Crystals **Crystals found in acidic urine**. 1. Uric acid crystals	Diamond rhombic or rosette form. These are usually stained	Presence of uric acids can be a normal occurrence.

 Figure 21.6	with urinary pigments as yellow or red brown	Pathological conditions: Gout, chronic nephritis, acute febrile conditions.
2. Calcium oxalate crystals  Figure 21.7	• Colorless and octahedral or 'envelope' shaped. • Also appear as oval spheres or biconcave disks which have a dumbbell shape (when viewed from the side).	• Found in urine after the ingestion of tomatoes, spinach, garlic, oranges, asparagus and vitamin C. • Increased number of calcium oxalates in freshly voided urine suggests the possibility of oxalate calculi. • They can be present in increased number in diabetes mellitus & liver diseases.
3. Sodium urates	• Present as amorphous or as crystals.	• Sodium urates have no clinical

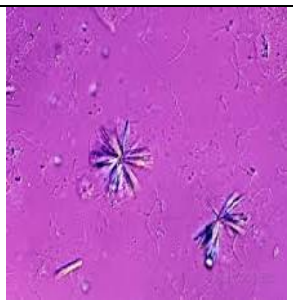
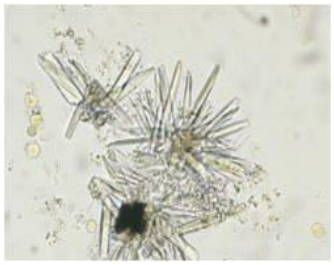
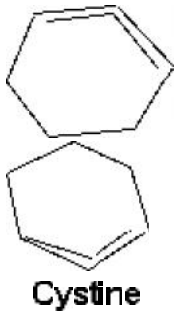
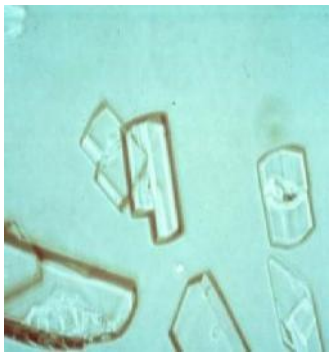
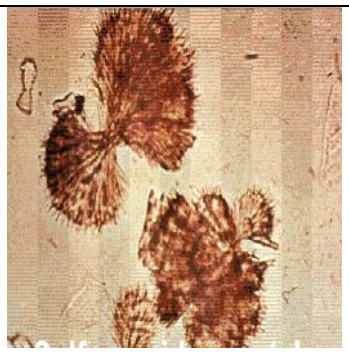
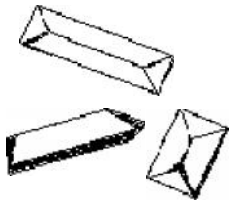
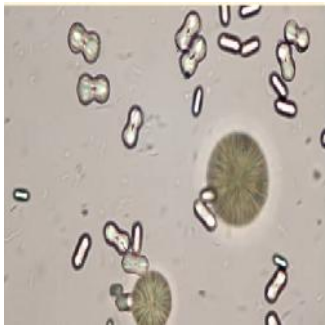
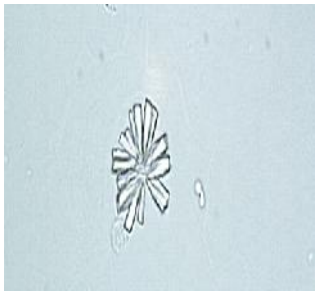
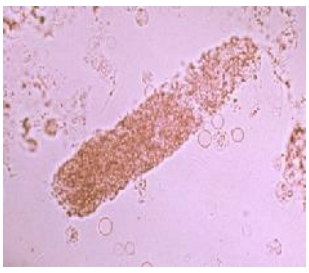
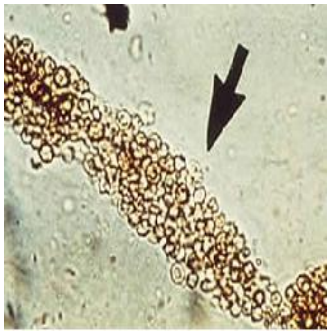
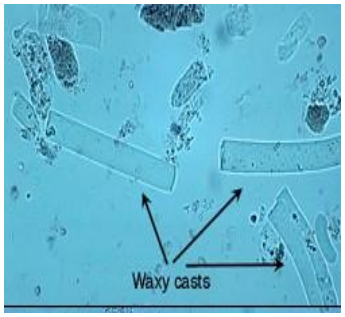
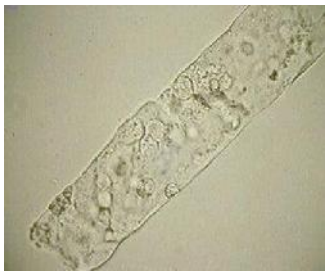
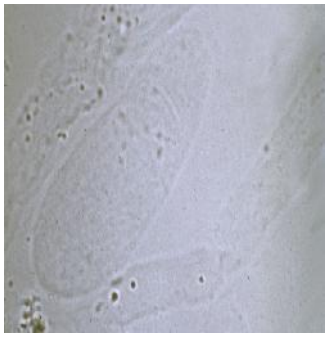
 Figure 21.8	• Crystals are colorless or yellowish needles occurring in clusters.	significance.
4. Calcium Sulphate crystals  Figure 21.9	Long, thin and colorless needles or prisms.	• Calcium sulphate crystals are rarely seen in the urine and they have no clinical significance.
5. Cystine crystals  Figure 21.10	Colorless, refractile, hexagonal plates with equal or unequal sides	• Present either in congenital cystinosis or congenital cystinuria. • They can form calculi.
6. Tyrosine	Appear in the form of	• Severe liver

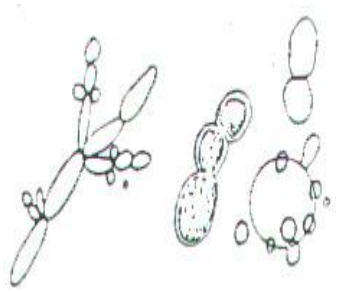
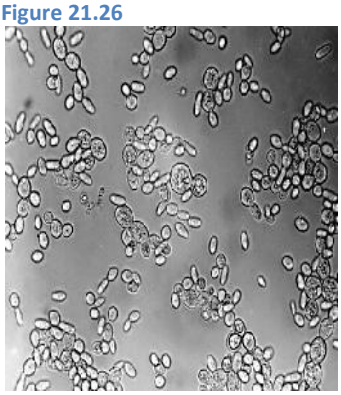
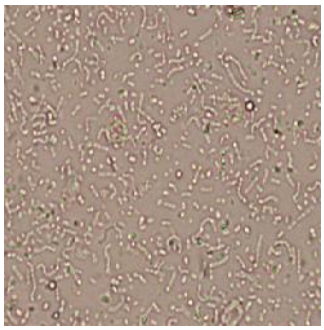
 Figure 21.11	fine, refractile needles, occurring in clusters.	disease and tyrosinosis.
7. Leucine  Figure 21.12	Appear as oily, highly refractile spheroids with redial and concentric striations. They have yellow or brown color.	• Pathological conditions: Severe hepatitis, acute yellow atrophy and maple syrup urine disease.
8. Cholesterol  Figure 21.13	Large, flat and in the form of transparent plates with notched corners.	• Nephritis, • Nephrotic conditions, chyluria , • Excessive tissue breaks down.
9. Sulpha crystals	Most of the sulpha drugs precipitate out as needles. They may	• Sulpha drugs

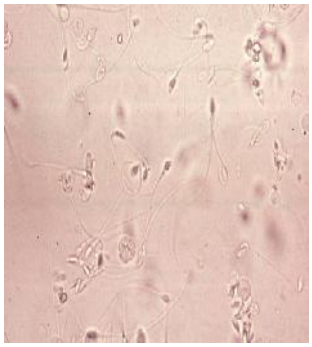
 Figure 21.14	clear or brown in color and usually appear with eccentric binding.	
Crystals found in alkaline urine: **1.** Triple Phosphates: (Ammonium magnesium phosphates)  Triple Phosphate Figure 21.15	Colorless prisms with three to six sides and frequently with oblique end.	• Frequently found in normal urine. • Can form calculi. • Pathological conditions- Chronic cystitis, chronic pyelitis Enlarged prostate.
2.. Calcium carbonate  Figure 21.16	Small, colorless and in the form of spherical, dumbbell shape or as granular type.	They have no clinical significance.

4. Calcium phosphate  Figure 21.17	Long, thin and colorless. The appearance is like prisms with one pointed end, arranged as rosettes or stars or appearing as needles. They may also appear as irregular, granular plates.	• They may be present in normal urine. • They may also form calculi.
5. Ammonium biurates  Figure 21.18	Yellow brown spherical bodies with or without long, irregular spicules.	• Presence of ammonium biurates is abnormal if they are found in fresh urine.
Casts <u>Granular casts</u>  Figure 21.19	Contain closely packed granules	i.Seen in Renal allograft rejection. ii. Accompany pyelonephritis, viral infection.

Red cell casts:  Figure 21.20	The casts may contain only a few RBCs in protein matrix or there may be many cells packed close together with no visible matrix.	• Seen in acute glomerulonephritis, sub acute endocarditis and severe pyelonephritis and in renal infarction.
White cell casts:  Figure 21.21	Granular spheres about 12µm in diameter. Nuclear segments appear as small,round, discrete nuclei.	• Seen in renal infections & noninfectious inflammations.
Epithelial cell cast:  Figure 21.22	The epithelial cells may be arranged haphazardly and vary in size and shape.	• Presence of these casts indicates tubular degeneration and necrosis. • These casts can also be present in severe chronic renal disease.

Waxy cast:  Figure 21.23	• They have very high refractive index. • These are yellow grey or colourless and have a homogeneous appearance.	• Seen in Tubular inflammation and degeneration • Chronic renal failure • Acute and chronic renal allograft rejection.
Fatty casts  Figure 21.24	Fatty casts are formed by incorporated free fat droplets or oval fat bodies.	• Seen in Nephrotic syndrome.
Hyaline casts:  Figure 21.25	• They are colorless homogenous, transparent & with rounded ends.	• A few hyaline casts may be present in the normal urine. • These casts can be seen in increased number even in the mildest kind of renal disease.
Mucus threads	These are long, thin waxy threads of	▪ These may be present in normal

	ribbon like structures.	urine ▪ Found in high proportion in the presence of inflammation or irritation of the urinary tract.
<u>Yeast cells:</u>  Figure 21.26  Figure 21.27	▪ Smooth, colorless and usually ovoid cells. ▪ Can vary in size and have doubly refractile walls. ▪ Often show budding.	Yeast cells may be found in urinary tract infections (mainly in diabetic patients). They may be present in urine as a result of skin or vaginal contamination.
<u>Bacteria</u>**:**  Figure 21.28		▪ Freshly voided normal urine is generally free of bacteria. ▪ Presence of large number of bacteria with many pus

		cells indicates urinary tract infection.
Spermatozoa**:**  Figure 21.29	Oval bodies and long, thin and delicate tails.	They may be present in urine of man in diseases of genital organs. After coitus they may be present in the urine of both the sexes.

Clinical significance	The microscopic examination is a valuable diagnostic tool for detection and evaluation of renal and urinary tract disorders and other systemic diseases.
Skills to be achieved	Students can prepare cover slip preparation of urine sample for microscopic examination and can handle centrifuge perfectly.
Skill evaluating criteria	• Handling of centrifuge- 2marks • Preparation of cover slip preparation- 4marks • Knowing morphology of different- 4marks cells, crystals and casts.
FAQs	1. How slide for microscopic examination is prepared? 2. Name different cells, crystals and casts found

	in urine.
Assignment	Prepare slide for microscopic examination from 10 different urine samples.
Reference	Chapter 13 Handbook of Clinical pathology, Haematology and Blood banking.

Week no.	22
Practical No.	**22**
Title/ Aim	Stool examination- Physical examination chemical examination and microscopic examination.
Objective	Students shall perform routine analysis of stool. They shall identify common parasites found in stool sample.
Requirements	1. Stool sample collected in proper container. 2. Other as per test.
Environment	MLT laboratory

Procedure / Observations

Physical examination

1. **Consistency and form**- Normally stools are soft and well formed. Abnormally stools are

Abnormal consistency	**Reason**
Watery	Diarrhoea- Bacterial infections, use of purgatives
Large amount of mushy, foul smelling bulky gray stools float on water	Steatorrhoea
Little firm spherical masses	Constipation
Narrow ribbon like	Spastic bowel or rectal narrowing

Rice water stools- Copious, thin with white flakes	Cholera	
Semisolid	Mild diarrhea Digestive upset After taking laxative	

2. **Parasite**- Normally absent. In parasitic infestation presence of adult worm like round worm, pin worm, whip worm, hook worm and Tape worm is seen.

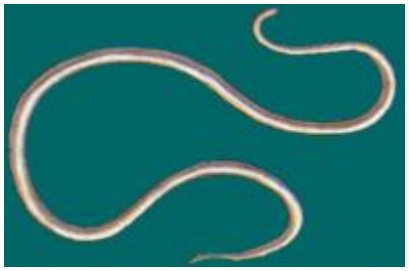


Fig.22.1 Round worm

3. **Color-** Normally feces are light to dark brown in color due to presence of bile pigments. Abnormal colors will be

Abnormal color	Reason
Clay	Obstructive jaundice Presence of $BaSo_4$
Bright red	Blood from lower GIT Beet consumption Contamination with menstrual blood
Black tarry	Bleeding from upper GIT Iron, Bismuth, charcoal consumption
Green	Ingestion of spinach Presence of biliverdin

	Oral antibiotics	

4. **Odour**- Normal feces may smell offensive but not foul smelling. In infections other odors like foul, putrid, sour, etc. can occur.
5. **Pus**- Not normally present. Found in chronic ulcerative colitis and chronic bacillary dysentery. Also in localized abscess or fistulas communicating with sigmoid colon or rectum. It is confirmed by microscopic examination of stool.
6. **Blood** - Normal stool does not contain any blood. Causes are as follows-
    - Peptic ulcer Esophagitis
    - Polyps Infective diarrhoeas
    - Inflammatory bowel syndrome (Crohn's Disease, Ulcerative colitis)
    - Carcinoma
    - Hemorrhoids Ano-rectal fissure
    - Apart from this certain drugs like steroids can lead to appearance of blood in stool.
7. **Blood and mucus**- Not present in normal stool. It is seen with amoebic and bacillary dysentery.

Chemical examination

1. **Occult blood**- It is a hidden blood. It is neither seen by naked eyes nor by microscopic examination. It is detected by chemical test. Most commonly used test is **Benzidine test.**

Principle- Peroxidase type action of hemoglobin in the blood converts

H_2O_2 to water and nascent oxygen. This Oxygen oxidizes benzidine (in acidic medium) to form green to blue color complex.

Requirements

1. Glass slides
2. Applicator stick or nicrome wire loop
3. Benzidine reagent-
    - Take a pinch of Benzidine powder in a test tube.
    - Acidify it with 2 to3 drops of glacial acetic acid & mix well.
    - Add about 1 ml of H_2O_2 & mix well.
4. Specimen-
    - Stool

Method-

1. Place small quantity of stool on clean dry slide.
2. Add 2 drops of benzidine reagent.
3. Observe color.

Result-

- No change in color-Negative i.e. Occult blood is absent.
- Green to blue color- Positive i.e. Occult blood is present.

Note-

- It is very sensitive test and can give positive result with 50-70% of normal adults whose diet contains abundant meat (False positive). False positive result can be overcome by boiling emulsion of feces for 1-2 minutes and then repeating the test.
- In case of a negative result check the reliability of reagent by treating with drop of blood sample.
- In case of positive result check the reliability of cleanliness of the slide

by placing a drop of the reagent mixture on the plain part of the slide. It should not produce color.

- Now a days all occult blood tests are packaged into kit forms. Principle remains same.

Significance- Occult blood is tested to diagnose

a) Iron deficiency anemia,
b) Bacillary dysentery
c) Cancer of GIT
d) Amoebiasis,
e) Peptic ulceration

Microscopic examination

Wet mount is usually carried out for microscopic examination.

It is of two types-Saline and Iodine preparation

Method	Importance
Saline preparation- • Add a drop of normal saline on a glass slide. • Take a little fecal materials by using an applicator stick & mix with a drop of normal saline. • Place a cover slip over it. Avoid formation of air bubble.	• Preliminary microscopic examination of feces to detect protozoan trophozoites and cysts and helminth larvae and eggs. • Characteristic motility of parasite. • Excessive cellular exudates in the form of pus cells or blood cells, macrophages or any other significant material can be detected.
Iodine preparation-	• Iodine stains cysts particularly

• Add a drop of lugol‘s iodine on a other side of the glass slide. • Take a little fecal materials by using an applicator stick& mix with a drop of lugol‘s iodine. • Place a cover slip over it. Avoid formation of air bubble.	nuclear structures and glycogen. • Motility cannot be detected as parasites are not motile in iodine.

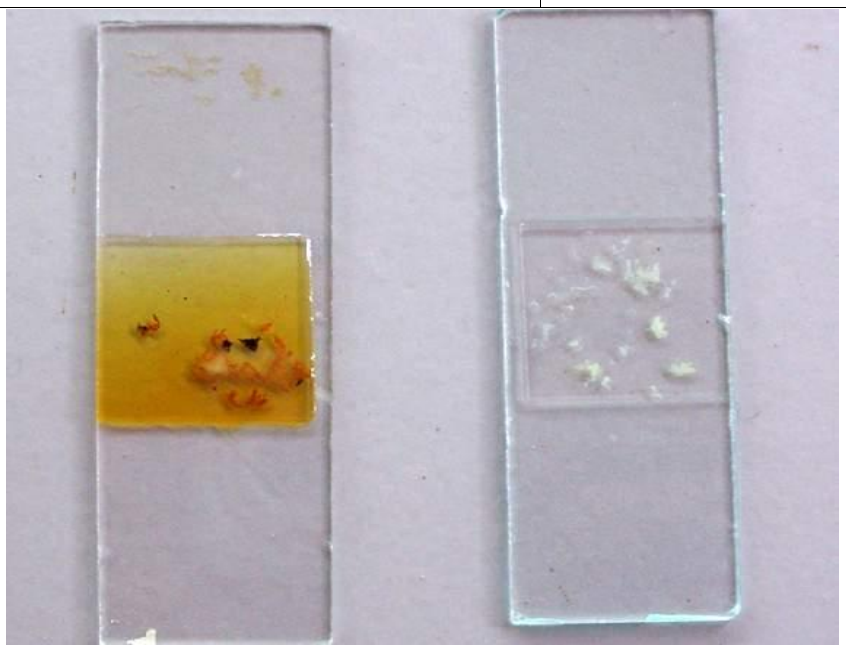


Fig. 22.1

Fig.22.2 Adult worms

Egg Whipworm Egg Hookworm Fig. 22.3

Ascaris lumbricoidis

Fig. 22.4 Unfertilised egg

Fig.22.5 fertlised egg

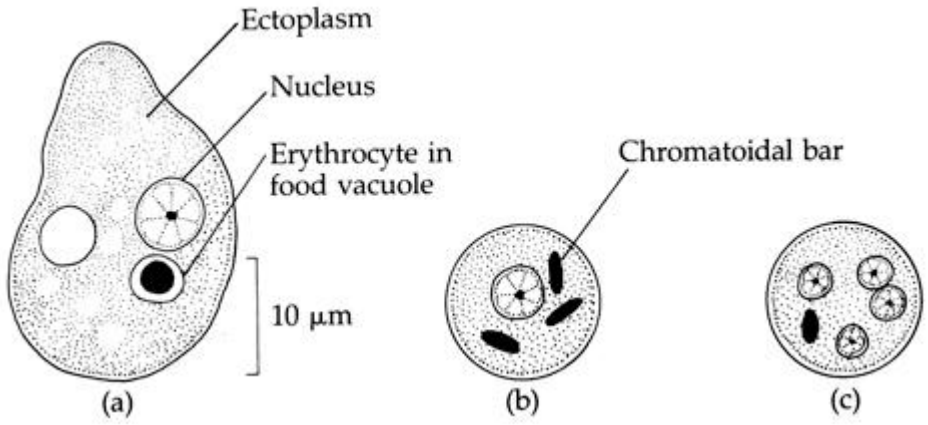
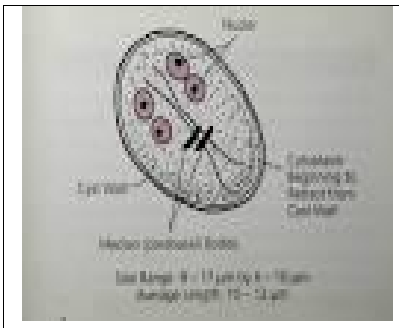


Fig.22.6 Entamoeba histolytica

 Fig.22.7 Giardia lamblia	
Clinical significance	1. Detection of bacterial and parasitic infections of the intestine. 2. Cultural examination will detect bacterial infections like enteric fever, bacillary dysentery, cholera, etc. 3. Common intestinal parasites like hook worm, tape worm, thread worm, etc are detected. 4. Chemical examination helps to detect defects in absorption, deranged liver and pancreatic functions, etc.
Skills to be achieved	Students can prepare wet mount slides for microscopic examination of stool and can test occult blood properly.
Skill evaluating criteria	• Proper collection of stool sample- 2marks • Proper preparation of benzidine reagent- 1mark • Correct interpretation of occult blood test result- 1mark • Proper preparation of wet mount- 4marks • Identification of common parasites- 2marks
FAQs	1. Precautions to be taken before and after

	collection of stool sample. 2. What are causes for blood in stool? 3. What is occult blood? 4. How benzidine reagent is prepared? 5. How wet mount preparation is done?
Assignment	Perform routine examination of 10 different stool samples.
Reference	Chapter 14- Stool examination -Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	23
Practical No.	**23**
Title/ Aim	Routine examination of semen
Objective	The student shall be able to examine semen specimen in the laboratory.
Requirements	1. Glass slides, 2. Cover slips, 3. Test tubes, 4. Pasteur pipette, 5. Neubaur's chamber, 6. WBC pipette, 7. pH paper 8. Reagents • Resorcinol reagent • Leishman's stain or 0.21 gms/dl aqueous basic fuchsine 9. Semen diluting fluid • Sodium bicarbonate- 5.0 gms • Formalin- 1 ml • Distilled water-99 ml **10.** Specimen: • Freshly collected semen
Environment	MLT laboratory

Procedure

Physical examination of semen-

1. Color- Normal colour of semen sample is greyish white, viscid and

opaque.

2. **Volume**- Normal male ejects 2 to 5 ml of semen. Less than 1.5 ml is considered as abnormal. Mostly males associated with infertile marriages tend to have an increased rather than a decreased semen volume.
3. **Viscosity- T**he specimen can be poured drop by drop with a Pasteur pipette is considered as normal.
4. **Coagulation and liquefaction-** Freshly collected semen sample first coagulates. Spontaneous liquefaction occurs within 10 to 20 minutes.

Chemical examination of semen-

1. **Determination of pH-** Dip the pH paper in sample. Note the result. The pH of semen is between 7.2 to 7.8.
2. **Determination of fructose-**

Take 0.5 ml of resorcinol reagent in a test tube. Add 0.5 ml of semen sample.

Mix and heat for 5 minutes.

Observation-

No change in colour- Fructose absent.

Red colour precipitate in 30 seconds- Fructose present.

Microscopic examination of semen-

1. **Study of motility of sperms-**

- Place a drop of liquefied semen on a slide.
- Put cover slip.
- Examine under 40x with reduced light.

- Count the number of actively motile sperms out of the total count of 200.
- Calculate the approx. % of sperms showing actual progressive motion.
- Examine the slide after 2 hrs. 3 hrs. And 6 hrs. (To prevent the drying, place the preparation under a petri dish with a wet filter paper).

2. To determine sperm count-

- After liquefaction, mix the specimen.
- And draw up to the 0.5 mark of a WBC pipette.
- Draw in the semen diluting fluid up to the mark 11 and mix well.
- Load the Neubaur's chamber.
- Allow the sperms to settle down for about 5 minutes.
- Count sperms in the four corner squares as in WBC count.

Calculation-

$$\text{Sperms/ml} = \frac{\text{Sperms counted} \times 10 \times 20 \times 1000}{4}$$

$$= \text{Sperms counted} \times 10 \times 5 \times 1000$$

$$= \text{Sperms counted} \times 50000$$

3. **To study the morphology of sperms-**

- Make a thin smear of liquefied semen on a glass slide. Air-dry it and then heat fix very gently.
- Remove the mucus by dipping in semen diluting fluid.
- Stain the smear by using Leishman's stain or 0.25 gms/dl aqueous basic fuchsin for 5 minutes.
- Observe under low power and then switch to oil immersion objective.

Observations / Result

Charteristic	Normal value	Significance
Volume	2-5 ml	• <1.5 ml result in poor penetration of the cervical mucus by the sperms. • Excess volume i.e. >5ml is associated with decreased motility.
Viscosity	Semen falls drop by drop	• Increased viscosity may inhibit sperm motility.
Liquefaction time	Self liquefaction should be completed by 30 minutes.	• Unliquefied semen or delayed liquefaction time (> 1 hour) is considered as abnormal. It interferes with motility and sperm count.
Color	Freshly ejaculated normal semen is opaque, white or gray-white coagulum. Any abnormal color - yellow, brown or blood stained.	• Presence of pus, blood, etc in the semen.
Fructose	Present	• Absence of fructose results in completely immotile sperms.

Sperm count	60-150 millions/ml	• Count less than normal- Oligospermia • Absence of sperms- Azospermia • Both lead to infertility.
Sperm motility	80% are actively motile 20% sluggishly motile or nonmotile.	• Asthenozoospermia- Decreased motility or nonmotility leads to infertility. • Oligoasthenozoospermia • Abnormalities of sperm movement associated with reduction in sperm number.
Sperm morphology	80% are normal sperms. 20% show abnormal morphology.	Abnormal forms lead to infertility.

Normal sperm:- head cap- light blue ,nuclear posterior- dark blue, body and tail- red or pink spermatozoa size- 50 to 70 µ

Abnormal sperms:- Giant head, minute head, double heads, rudimentary tail, double tailed, without tail, middle section- absent, bifurcated or swollen, abnormally shaped head.

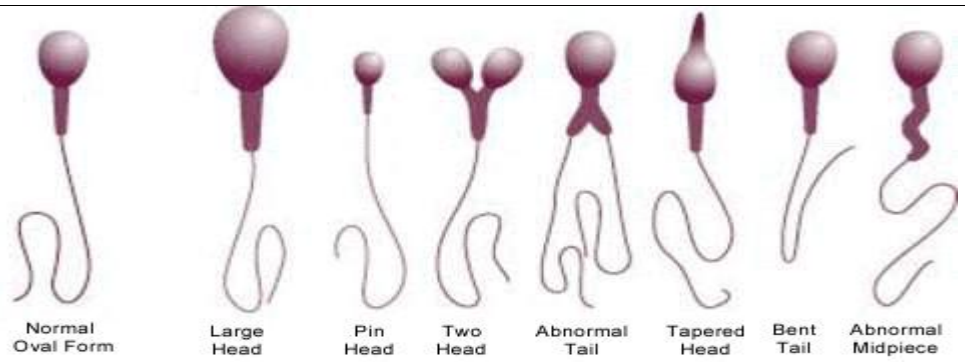


Fig 16.1 Normal and Abnormal sperms

Clinical significance	➢ Evaluation of infertility ➢ Routine follow up of the patients who have undergone vasectomy. ➢ Artificial insemination
Skills to be achieved	Students can study semen sample correctly.
Skill evaluating criteria	• Proper instructions for collection- 4 marks • Noting proper liquefaction and its time- 2marks • Proper dilution for count- 2 marks • Proper smear preparation and staining- 2marks.
FAQs	1. Give composition of diluting fluid. 2. Describe morphology of normal sperm. 3. What are different abnormal forms of sperm?
Assignment	Process 10 samples for routine semen examination.
Reference	Chapter 15- Semen Analysis -Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	24
Practical No.	**24**
Title/ Aim	Sputum examination
Objective	The student shall be able to do routine examination of sputum. They shall do AFB staining.
Requirements	1. Glass slides 2. Cover slips, 3. Pasteur pipette, 4. Gram staining reagent, 5. AFB staining reagent, 6. Wright stain, 7. Microscope 8. Specimen • Early morning specimen or • The entire 24 hrs specimen (Instruction given to the patient - a. The mouth should be rinsed well by using water. b. Ask the patient to do the act of coughing and then collect sample).
Environment	MLT laboratory
Procedure	**Physical examination:** **1.** Quantity- Amount of cough varies with different diseases. >100ml is seen with lung abscess and

	bronchiactasis. **2.** Appearance and consistency- • Watery frothy sputum - Pulmonary edema • Tenacious rusty • (blood stained)- Lobar pneumonia • Purulent - Lung abscess • Frank blood – TB, Pneumonia, malignant tumors of lungs and bronchi • Anchovy sauce – Rupture of amoebic liver abscess in to lungs. 3. Odour- • Acute infection – Odourless • Chronic infection – Foul odour • Bronchiactasis – Sweet smell **Microscopic examination** Unstained and Stained preparation. **1) Unstained** • Place a drop of well mixed sputum on glass slide and place a cover slip on it. • Observe first under low power and afterwards under high power objective. **2) Stained** • Make 3 to 4 smear of the sputum, dry at RT. • Fix by gentle heating on a flame. • Stain smear by using

	➢ Wright / Leishmann / Giemsa stain – For blood cells ➢ Gram stain – For organisms ➢ Z-N stain – For TB bacilli

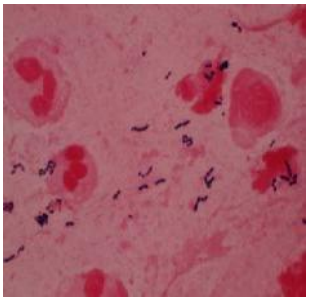
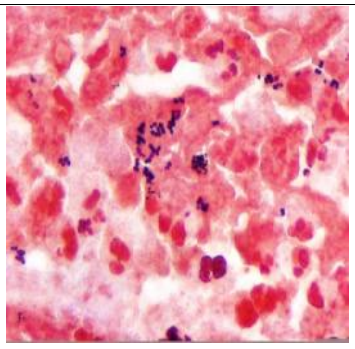
Observations / Result

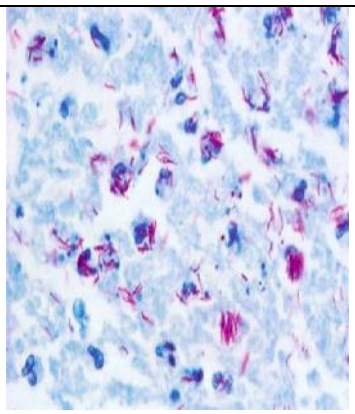
Unstained smear

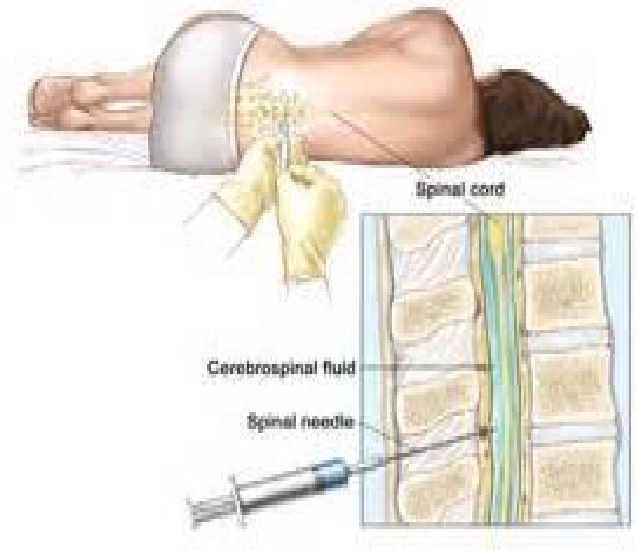
Structure	Description	Significance
Elastic fibres	Slender curled highly refractile fibres	Seen in TB, Abscess, bronchiactasis and carcinoma
Curschmann's spirals	Spirally twisted mucoid strands enclosing epithelial cells and leukocytes (Eosinophils)	Found in Bronchial Asthma
Charcot Leyden Crystals	Colorless pointed hexagons or needle like	Found in Bronchial Asthma Parasitic infection of lung with E. histolytica
Fungi	Yeast like cells Branching or nonbranching double contoured filament or Ray fungus.	Contaminants from mucus membrane of upper respiratory tract
Parasite	-	EH seen with rupture

Rarely seen. Entamoeba histolytica Ova of P. westermani		of amoebic liver abscess in to lungs.
Cells	RBCs, Pus cells and haemosiderin containing macrophages.	Congestive cardiac failure

Stained sample

Cell / organisms	**Description**	**Significance**
Wright's stain Neutrophils (PMN) Lymphocytes Eosinophils RBCs		Pyogenic infection of respiratory tract Early TB Bronchial asthma Hemorrage in to lungs
Epithelial cells		Derived from alveoli of lung or from bronchial tree
Gram stain Gram positive or gram negative organisms		Seen in pyogenic infection of lungs

Z-N stain TB bacilli		Pulmonary TB
Clinical significance	Examination of sputum is valuable in finding out diseases of respiratory tract.	
Skills to be achieved	Students can prepare smear and stain with different stains correctly to identify cells and organisms.	
Skill evaluating criteria	• Proper collection of sputum- 2marks • Wet preparation technique- 2 marks • Smear preparation and staining- 4marks • Identification of cells and bacteria- 2marks	
FAQs	1. What is sputum? 2. What instructions are given to patient before collecting sputum sample? 3. Describe wet preparation. 4. How AFB staining is done?	
Assignment	Prepare smear from 10 different sputum samples and stain with Z-N stain.	
Reference	Practical 5 Staining of bacteria from XI std practical manual of microbiology.	

Week no.	25
Practical No.	**25**
Title/ Aim	Routine examination of CSF
Objective	The student shall be able to examine CSF specimen in the laboratory.
Requirements	1. CSF sample- • CSF is collected by lumber puncture under strict aseptic condition. • It is collected from space between 4th and 5th lumber vertebra to a depth of 4-5 cms. • The fluid is collected in 2 sterile test tubes. The specimen is centrifuged • The supernatant is used for biochemical tests and the sediment is used for weight mount preparation, Gram's staining, India ink preparation etc.  Figure 25.1 2. Other as per test.

Environment	MLT laboratory

Procedure

Physical Examination:

- **Color and appearance:**

➢ Normal CSF is crystal clear and colorless.

➢ The appearance may be cloudy in bacterial infection or xanthochromic in cerebral haemorrhage.

➢ In pathological conditions, presence of blood cells, pus cells, and bacteria may be seen in CSF.

- **Presence of clot or fibrin web:**

➢ Normal CSF does not clot.

➢ The clot formation indicates abnormality. It is associated with increased protein concentration.

➢ A delicate fine clot is seen in Tuberculous Meningitis.

➢ A large clot is seen in purulent Meningitis.

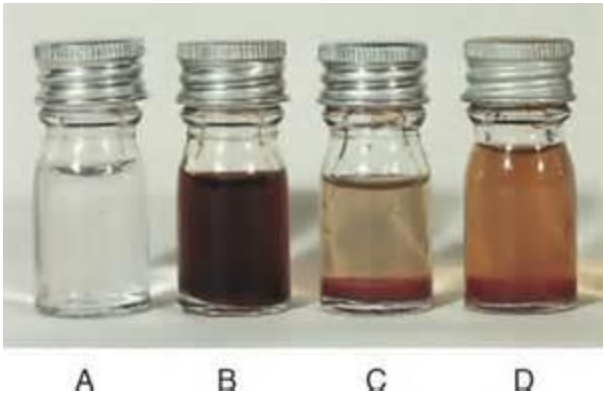


Figure 25.2

- **pH determination:**
  - Normal CSF pH ranges from 2-10.5

Microscopic examination:

Requirements:

1. Glass slides
2. Improved Neubaur's chamber
3. Centrifuge
4. Pasteur pipette
5. Leishman / Gram / Acid fast stain
6. Microscope.

Wet mount preparation:

- Place a drop of CSF sediment on a clean slide.
- Put cover slip and observe under 40x.

Wet mount of CSF is done in suspected cases of Trypanosomiasis and cryptococcosis.

(Trpanosomes are motile and flagellate and, if found in CSF indicates late stage of the disease).

Total leukocyte count:

1. Mix the sample carefully.
2. Fill the counting chamber.
3. If the sample is clear, use undiluted.
4. If sample is cloudy, make 1:20 dilution by using WBC pipette (draw CSF up to 0.5 mark and dilute with the CSF diluting fluid up to 11 mark). Mix well.
5. Load the chamber.
6. Leave for 5 minutes to allow the cells to settle. Count the cells in all nine squares.

Calculation:

WBC in CSF/ mm^3 = no. of cells counted

Area x depths of fluids

= no. of cells counted x 10

9

If CSF specimen is diluted 1:20 then,

WBC in CSF/ mm^3= no. of cells counted

Area x depths of fluids

= no. of cells counted x 10 x 20

9

Note: perform gram's staining, acid fast staining and India ink preparation (refer to XI microbiology section)

Differential count:

- Take a clean dry slide.
- Prepare a thin smear.
- Allow the smear to dry.
- Add 10-15 drops of Leishman's stain.
- Wait for 1 min.
- Add equal amount of buffer's solution (pH 7).
- Mix well and keep for 10 min.
- Wash, dry and observe under oil immersion objective.

Chemical examination:

1. **Determination of glucose from CSF:** (Refer to biochemistry section)

Normal value- CSF 40-80 mg/dl

2. Determination of proteins from CSF:

Method-Turbidimetry

Principle- The protein is denatured by the action of sulphosalicylic acid.

Due to the denaturation, the specific internal structure of the protein is lost. And the solubility of the globular protein is changed to an insoluble protein form which is exhibited by the formation of turbidity.

Requirement:

- Normal saline,
- 3 gms/dl sulphosalicylic acid,
- 6 gms/ dl stock protein standard.
- Preparation of working standard: mix 0.1 ml of stock standard and 9.9 ml of normal saline

Procedure:

	Test	**STD**	**Blank**
3 gms per dl sulphosalicylicacid	4 ml	4ml	4ml
CSF	1 ml	-	-
Working standard 60 mg per dl	-	1ml	-
Distilled water	-	-	1ml

Mix and keep at room temperature for 5 min.

Measure the turbidity against blank at 640 nm (red filter)

Calculation:

$$\text{CSF protein mg per dl} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 60$$

3. Determination of chlorides from CSF:

Method: Schales and Schales

Principle: The CSF specimen is titrated with mercuric nitrate in presence

of diphenylcarbazone as indicator. The free mercuric ions combine with chloride ions to form mercuric chloride. The excess mercuric ions combine with the indicator diphenylcarbazone to form blue violet coloured complex.

Requirements:

1. Test tubes
2. Mercuric nitrate reagent
3. Diphenylcarbazone indicator
4. Chloride standard 100meq/l.

Procedure:

1. Take 1.8 ml distilled water in the test tube.
2. Add 0.2 ml of CSF.
3. Add 1 drop of indicator.
4. Mix and titrate against mercuric nitrate reagent till the colour changes to violet blue.
5. Note the titration reading(X ml).
6. Take 1.8 ml distilled water in the test tube.
7. Add 0.2 ml of Chloride standard 100mEq/l.
8. Add 1 drop of indicator.
9. Mix and titrate against mercuric nitrate reagent till the colour changes to violet blue.
10. Note the titration reading(Y ml).

Calculation:

$$\text{CSF chloride mEq/l} = \frac{\text{X ml}}{\text{Y ml}} \times 100$$

Normal value: 700-750 mEq/l

Observations/ Result

Diseases → Charcters ↓	**Normal**	**Purulent meningitis**	**TB meningitis**	**Viral meningitis**	**Syphilitic meningitis**	**Brain tumour**	**Cerebral haemorrage**
Appearance	Clear and colorless	Turbid and big clot	Opalescent and cobweb	Clear and opalescent	Clear and opalescent	Clear	Clear or haemorragic
Proteins mg/100 ml	20-40	500-1500	45-50	20-200	45-500	20-300	20-2000
Sugar mg/100 ml	40-70	Decreased or absent	Decreased	Normal	Decreased	Normal	Normal
Chlorides mg/100 ml	700-750	650-700	500-600	650-700	700-750	700-750	700-750
Total cells/mm^3 Predominant cell	0-5 cells Lymphocytes	5-20000 Neutrophil	25-500 Lymphocyte	10-1000 Lymphocyte	20-500 Lymphocyte	Normal or 10-200 Lymphocyte	Full of red cells
Other finding	---	Gram staining	Cobweb for M. tuberculosis	---	wasserman test	--	--

Clinical significance	CSF examination is carried out for diagnosis of diseases of meninges, brain and spinal cord.
Skills to be achieved	Students can study physical, chemical and microscopic examination correctly.
Skill evaluating criteria	• Properly collected sample- 2marks • Correct technique- 2marks • Proper dilution for count- 2marks • Proper smear preparation and staining-

	2marks • Perfect reading and calculations- 2marks
FAQs	1. What is CSF? What is site and method of collection? 2. Give normal values for CSF proteins, sugar and chlorides.
Assignment	Process 10 samples for routine CSF examination.
Reference	Chapter 16- CSF examination -Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	26
Practical No.	**26**
Title/ Aim	ABO grouping and Rh typing Slide method
Objective	The student shall be able to determine ABO and Rh blood group of the given sample by slide method.
Principle	The procedure using the antisera is based on the principle of agglutination. Normal human red cell possessing antigen will clump in the presence of corresponding antibody.
Requirements	1. Lancet or needle, 2. spirit swab, 3. slide or proclaim tile with cavity, 4. mixing sticks, 5. Pasteur pipettes. 6. Reagents: ▪ Normal Saline ▪ Antisera A(blue color) ▪ Antisera B(yellow color) ▪ Antisera D (Colorless) 7. Specimen: ▪ Anticoagulated blood sample, OR ▪ Capillary blood sample

Environment	MLT laboratory
Procedure	**A**. Preparation of 10% RBC suspension : 1. Mix 5 drops (0.05 ml each) of sediment red cells with 2 ml normal saline (10 drops of sediment with 90 drops of normal saline). 2. Centrifuge at 1500 rpm for 1to 2 minutes. Discard the supernatant. Wash 3 times with normal saline. 3. Add 2 ml of normal saline to sedimented red blood cells. **B**. Procedure for blood grouping 1. Prepare a10% suspension of red blood cells in normal saline. 2. On the blood grouping slides mark the cavities like A, B and D. 3. Put one drop of anti sera A marked A in the cavity, put one drop of anti sera B marked B in the cavity put anti sera D in the cavity marked D. 4. Using a Pasteur pipette add one drop of the 10% RBC suspension in each of the cavity. 5. With separate applicator stick mix each cell and anti sera mixture well. 6. Tilt the slide back and forth and observe for agglutination. ****** Test that shows no agglutination within 2

	minutes is considered negative. Do not interpret peripheral drying or fibrin strands as agglutination.

Observations

Antisera A	Antisera B	Blood group
+	-	**A**
-	+	**B**
+	+	**AB**
-	-	**O**

Antisera D	Blood group
+	Rh positive
-	Rh negative

Result

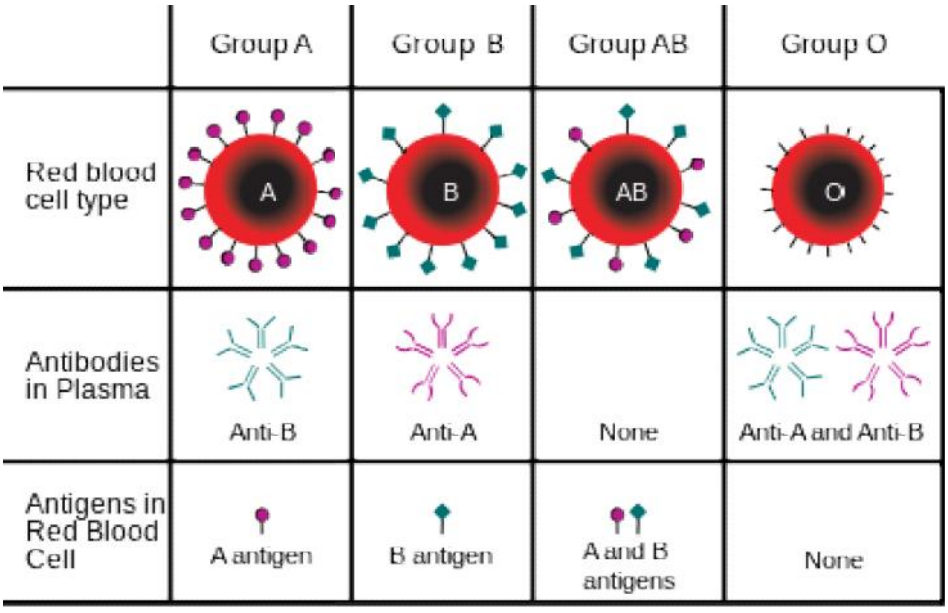


Figure 26.1

ABO blood group of given sample is _____

Rh blood group of given sample is _____.	
Clinical significance	ABO grouping and Rh typing is important in 1. Blood transfusion 2. To check the identity of birth parents. For example, in a paternity case. 3. Before a person donates blood. 4. Before a person donates an organ for transplantation. 5. Before surgery. 6. To show whether two people could be blood relatives. 7. To check the identity of a person suspected of committing a crime.
Skills to be achieved	Students can detect blood group correctly.
Skill evaluating criteria	1. Preparation of RBC suspension- 2 Marks 2. Correct technique- 4 Marks 3. Correct timing- 1 Mark 4. Observation for agglutination- 3 Marks
FAQs	1. What are blood groups? 2. How to preserve antisera? 3. What is agglutination? 4. Principle of blood group determination.
Assignment	Perform blood grouping and Rh typing of 25 different samples.
Reference	Chapter 17 and 18 Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	27
Practical No.	**27**
Title/ Aim	ABO grouping & Rh typing Tube method
Objective	The student shall be able to determine ABO and Rh blood group of the given sample by tube method.
Principle	The procedure using the antisera is based on the principle of agglutination. Normal human red cell possessing antigen will clump in the presence of corresponding antibody.
Requirements	1. Blood grouping tubes. 2. Pasteur pipettes 3. Centrifuge 4. Reagents • Anti-A antisera • Anti-B anti-sera • Anti-D antisera • Normal Saline 5. Specimen • EDTA blood
Environment	MLT laboratory
Procedure	1. Prepare 5% Red cell suspension • Mix 5 drops (0.05 ml each) of sediment red cells with 2 ml normal saline (10 drops of sediment with 90 drops of normal saline).

	• Centrifuge at 1500 rpm for 1to 2 minutes. Discard the supernatant. Wash 3 times with normal saline. • Add 4 ml of normal saline to sedimented red blood cells. 2. Take 3test tubes. Label them as “A”, “B” and “D”. 3. Place 1 drop of anti-A into the “A” tube, 1 drop of anti-B into the “B” tube and 1drop of Anti-D in to “D: tube. 4. Add 1 drop of the RBC suspension to each tube. 5. Gently shake each tube to mix the contents, and then centrifuge tubes at 1500 rpm for 1 minute. The RBCs will form a button or pellet at the bottom of each test tube. 6. Gently resuspend the RBC button and examine for agglutination macro and microscopically. ** Do not interpret peripheral drying or fibrin strands as agglutination.

Observations

Antisera A	Antisera B	Blood group
+	-	**A**
-	+	**B**
+	+	**AB**

-	-	**O**

Antisera D	**Blood group**
+	Rh positive
-	Rh negative

Result

Fig. 27.1

ABO blood group of given sample is _____

Rh blood group of given sample is _____.

Clinical significance	ABO grouping and Rh typing is important in 1. Blood transfusion. 2. To check the identity of birth parents. For example, in a paternity case. 3. Before a person donates blood. 4. Before a person donates an organ for transplantation. 5. Before surgery.

	6. To show whether two people could be blood relatives. 7. To check the identity of a person suspected of committing a crime.
Skills to be achieved	Students can perform blood grouping by tube method perfectly.
Skill evaluating criteria	1. Preparation of RBC suspension- 2 Marks 2. Correct technique- 4 Marks 3. Correct timing- 1 Mark 4. Observation for agglutination- 3 Marks
FAQs	1. How to preserve antisera? 2. What is agglutination? 3. How to prepare 10% red cell suspension?
Assignment	Perform blood grouping and Rh typing of 25 different samples.
Reference	Chapter 17 and 18 Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	28
Practical No.	**28**
Title/ Aim	Cross matching
Objective	Students shall be able to detect any incompatibility between patient's (Recipient) blood and donor's blood.
Principle	Serum of the recipient is tested against the red cells of donor under different conditions in order to find out their compatibility. Agglutination in any one of the condition makes blood unsuitable for transfusion.
Requirements	1. Small test tubes(10x75 mm) 2. Pasteur pipettes 3. Normal saline 4. Centrifuge 5. Specimen • Patient's blood and serum • Donor's blood and serum.
Environment	MLT laboratory
Procedure	1. Prepare 5% cell suspensions of patient's(P) and donor's (D) blood in two separate tubes 2. To the patient's tube, add two drops of patient's serum and one drop of donor's cell suspension.(Major side) 3. To the donor's tube add two drops of donor's

	serum and one drop of patient's cell suspension (minor side). 4. Mix the contents of both the tubes gently and keep the tubes at room temp for 30 minutes. Centrifuge at 1500 rpm for one minute. 5. Examine for agglutination both macroscopically and microscopically. 6. If no agglutination then add two drops of 22% bovine albumin and incubate the tubes at 37 deg for 15 minutes. Centrifuge at 1500 rpm for one minute. Examine for agglutination both macroscopically and microscopically.

Observations-

Recipient's blood			Reactions with donor's red blood cells			
ABO antigens	ABO antibodies	ABO blood type	Donor type O cells	Donor type A cells	Donor type B cells	Donor type AB cells
None	Anti-A Anti-B	O				
A	Anti-B	A				
B	Anti-A	B				
A & B	None	AB				

Table 28.1

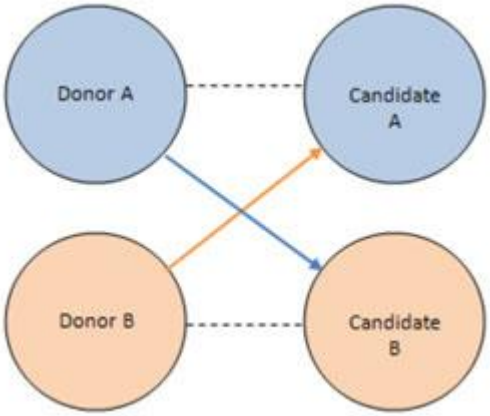


Fig. 28.1

Result

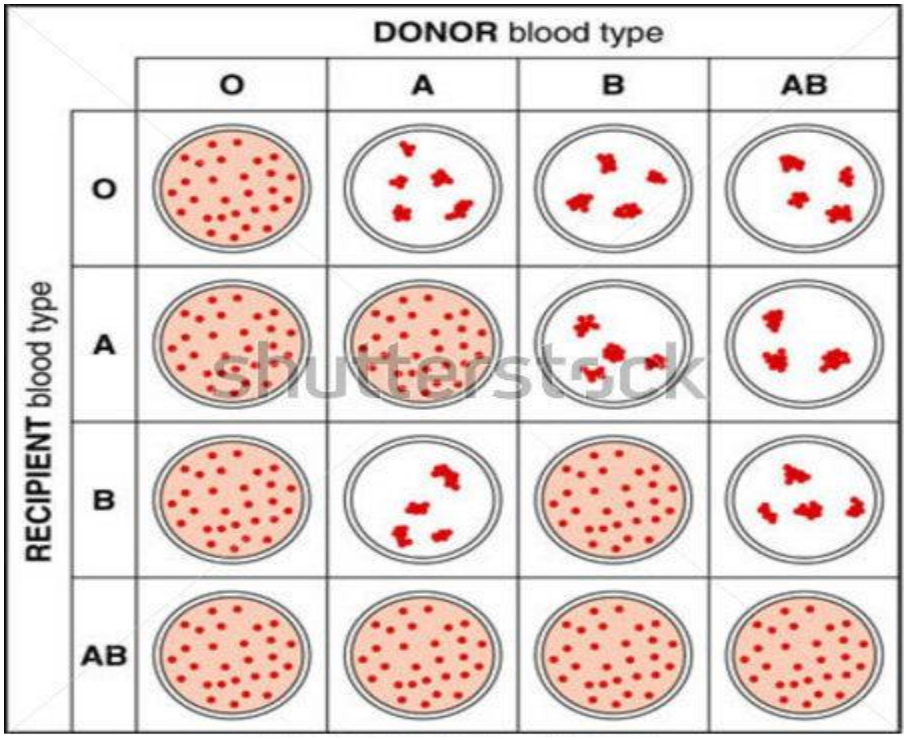


Table 28.2

Agglutination- Incompatible

No agglutination- Compatible

Clinical significance	• Cross matching is the final criterion to detect whether donor's blood is suitable for transfusion to a patient. • Blood showing major incompatibility should

	never be transfused. • Minor cross match results are also important. But in an emergency minor incompatible can be used. • When titer of antibodies is high and multiple transfusions are required then minor incompatible blood cannot be used.
Skills to be achieved	Students can perform cross matching correctly.
Skill evaluating criteria	1. Blood collection- 2marks 2. Separation of serum- 2 marks 3. Preparation of 5% red cell suspension- 2marks 4. Correct technique- 2marks 5. Result interpretation- 2marks
FAQs	1. Define cross matching. 2. What is major and minor cross match? 3. Why cross matching is done?
Assignment	Perform cross matching of 10 different samples.
Reference	Chapter 17 and 18 Hand book of clinical pathology, Hematology and Blood Banking.

Week no.	29
Practical No.	**29**
Title/ Aim	Indirect Coombs (Antihuman Globulin-AHG) test.
Objective	Students shall be able to do Indirect coombs test.
Principle	AHG test detects “sensitized red cells” where red cells are coated with antibody or globulin but do not agglutinate. When the sensitized red cells come in contact with AHG reagent they agglutinate.
Requirements	1. Test tubes 2. Pasteur pipettes 3. Incubator 4. Centrifuge 5. Reagents • AHG serum • Anti-D serum 6. Specimen • Serum 7. Coomb’s control cells- Make pooled “o” positive cells from atleast three different “O” positive samples. Wash these cells three times with normal saline. Prepare 5% saline suspension.
Environment	MLT laboratory

Procedure	1. Take three test tubes. Label them as'T' (test serum), 'P' (positive control) and 'N' (negative control). 2. In tube 'T' add 2 drops of test serum. 3. In tube 'P' add one drop of Anti-D serum. 4. In tube 'N' add one drop of saline. 5. Add one drop of 5% saline suspension of pooled 'O' positive cells in all three tubes. 6. Incubate them at 37^0 for one hour. 7. Wash the cells three times in normal saline to remove excess serum. 8. Add two drops of AHG (coomb's) serum in all tubes. 9. Keep for 5 minutes at room temperature and then centrifuge at 1500 RPM for 1 minute. 10. Resuspend the cells and examine for agglutination macro and microscopically.

Observations and Result-

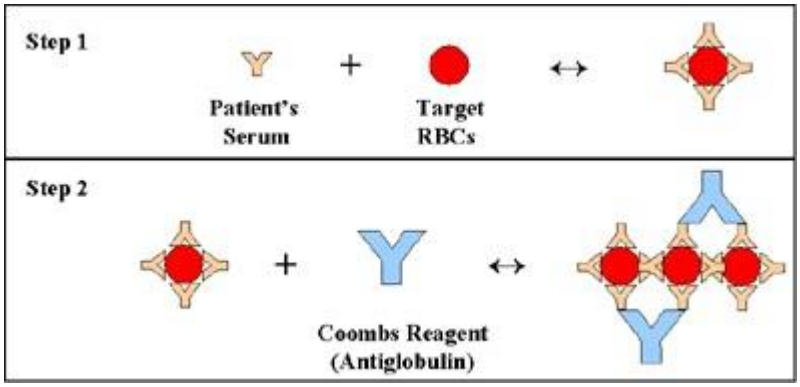


Fig. 29.1

Sr. No.	Tube	Observation	Conclusion
1	Positive control (p)	Agglutination No agglutination	Correctly performed test procedure Coomb's serum may not be proper. Repeat the test.
2	Negative control (N)	It should not show agglutination as saline does not contain anti-D or any other antibody.	
3	Test serum (T)	Agglutination (and if P tube results are correct) No agglutination	Patient's serum contains anti-D. Patient's serum do not contain Anti-D.

Clinical significance	1. AHG technique is useful in identifying weak immunologic reactions. 2. It is used to identify Rh variant (D^u). 3. Useful in compatibility testing. 4. Useful in detecting Rh antibodies or other antibodies in patients serum in case • When Rh negative woman married to Rh positive husband. • Rh negative person exposed to Rh antigen

	as in Transfusion of Rh positive blood, pregnancy when baby is Rh positive (if father is Rh positive),.
Skills to be achieved	Students can perform and interpret the result of indirect Coomb's test result correctly.
Skill evaluating criteria	1. Preparation of coomb's control- 2marks 2. Correct technique- 4marks 3. Incubation period – 1mark 4. Washing of incubated cells perfectly- 1mark 5. Correct interpretation- 2marks
FAQs	1. Principle of coomb's test 2. How to prepare coomb's control? 3. What is AHG? 4. Significance of Indirect coomb's test.
Assignment	Perform indirect coomb's test of 5 samples.
Reference	Chapter 17 and 18 Hand book of clinical pathology, Hematology and Blood Banking

Week no.	30
Practical No.	**30**
Title/ Aim	Direct Coomb's test
Objective	Students shall be able to do Indirect coombs test.
Principle	AHG test detects "sensitized red cells" where red cells are coated with antibody or globulin but do not agglutinate. Here red cells are sensitized within body (in vivo). When the sensitized red cells come in contact with AHG reagent they agglutinate.
Requirements	1. Test tubes 2. Pasteur pipettes 3. Centrifuge 4. Reagents • AHG serum 5. Specimen- • EDTA blood preferred. (can use oxalated or citrated blood can be used).
Environment	MLT laboratory
Procedure	Prepare 5% cell suspension of the red cells to be tested in isotonic saline. With the help of Pasteur pipette add one drop of the prepared cell suspension to a small tube. Wash with normal saline three times to remove all the traces of serum. Decant completely after last washing.

	Add two drops of AHG reagent. Mix it well and centrifuge at 1500 RPM for one minute. Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

Observations and **Result**

Observation	Conclusion
Agglutination	Presence of sensitized red cells.
No agglutination	Red cells are not Sensitized.

Table 30.1

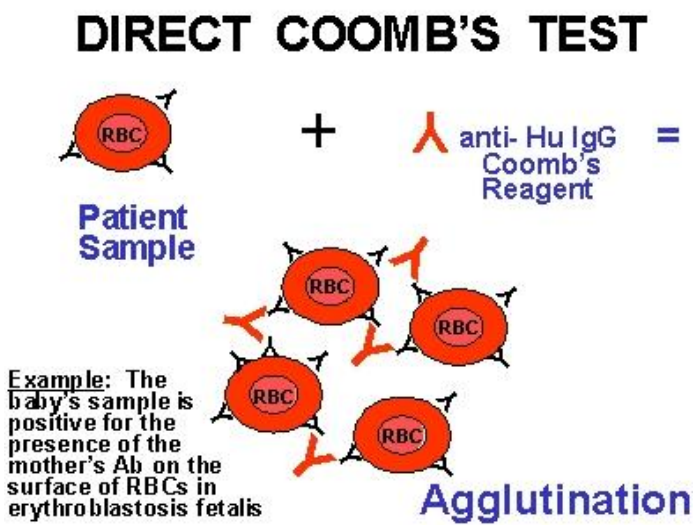


Fig. 30.1

Clinical significance	Useful in detecting Rh antibodies or other antibodies in patients serum in case 1. When there is Rh positive baby in the womb of a sensitized Rh negative mother; the antibodies produced in mother's serum cross the placenta and enter into fetal circulation. These antibodies coat baby's Rh positive

	cells. These coated cells are removed from the circulation causing haemolytic anemia (HDN). These coated cells are detected by Direct Coomb's test immediately after the birth of a baby. 2. Transfusion reactions. 3. Autoimmune haemolytic anemia.
Skills to be achieved	Students can perform and interpret the result of direct coomb's test result correctly.
Skill evaluating criteria	1. Preparation of 5% cell suspension- 2 marks 2. Correct technique- 4marks 3. Washing of cells perfectly- 2mark 4. Correct interpretation- 2marks
FAQs	1. What are sensitized red cells? 2. What is AHG? 3. Significance of direct coomb's test.
Assignment	Perform direct coomb's test of 5 samples.
Reference	Chapter 17 and 18 Hand book of clinical pathology, Hematology and Blood Banking