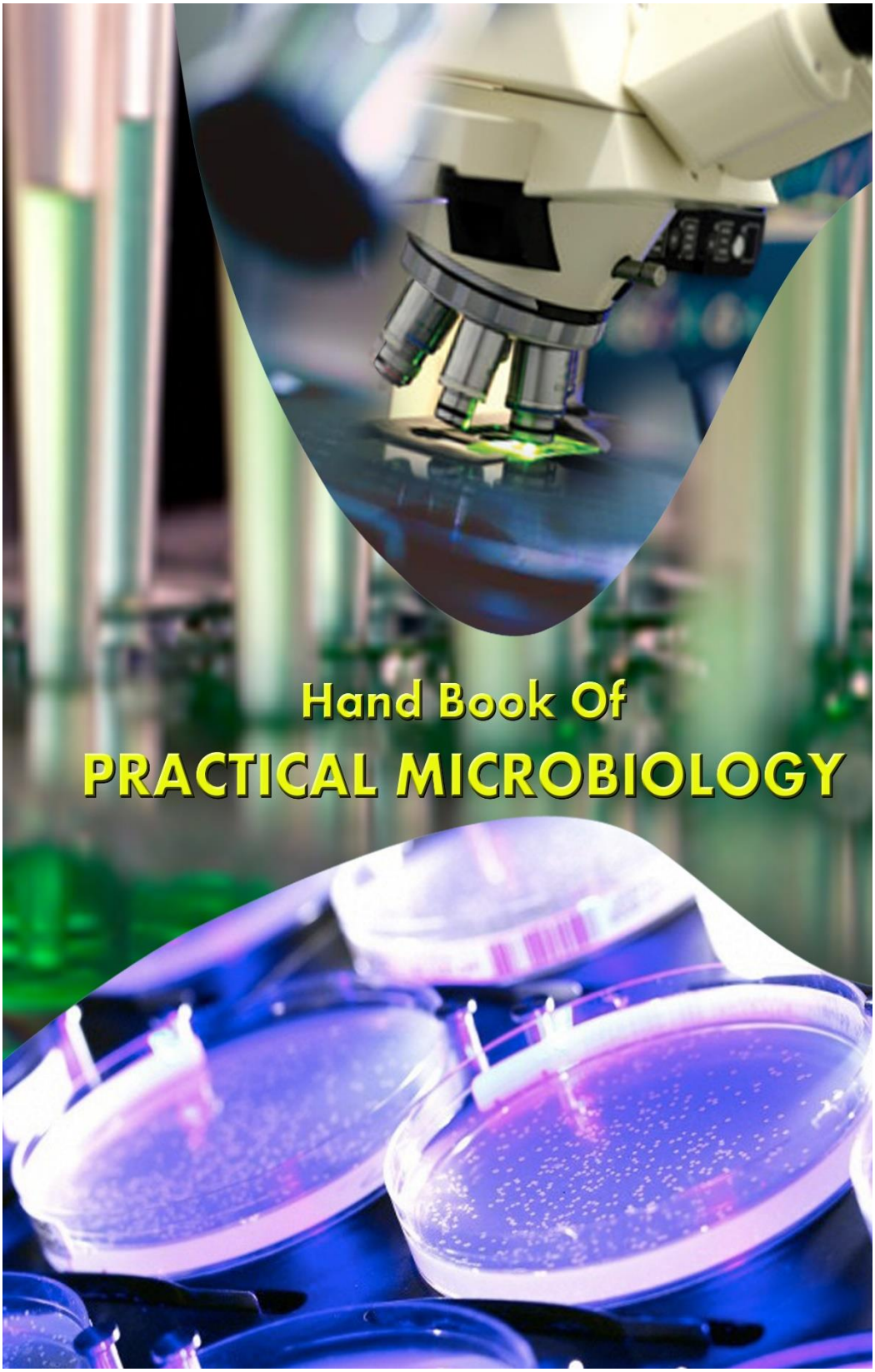
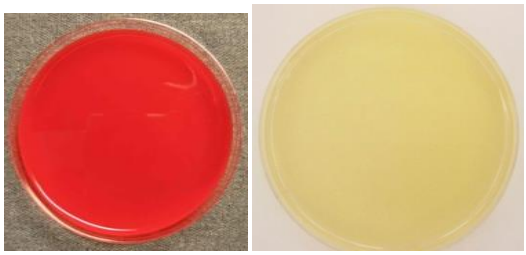

Hand Book Of
PRACTICAL MICROBIOLOGY

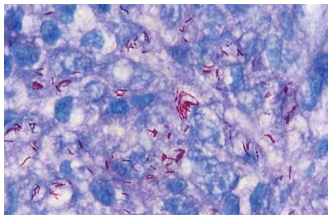
H.S.C. (VOCATIONAL)

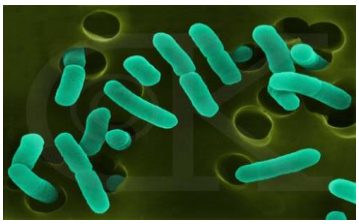
MEDICAL LABORATORY TECHNICIAN

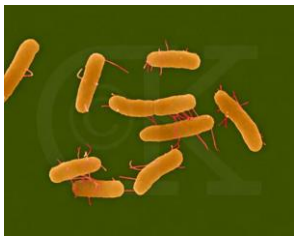
STD: XI(PAPER-3)



MICROBIOLOGY
PRACTICAL HANDBOOK







INDEX

INTRODUCTION TO MEDICAL MICROBIOLOGY

Medical microbiology is the study of microbes that infect humans, the disease they cause, and their diagnosis, prevention and treatment.

The diagnostic microbiology laboratory is engaged in the identification of infectious agents. These infections are broadly classified as viruses, bacteria, mycotic agents and parasites.

Identification of the infectious agent is the principle function of the diagnostic microbiology laboratory. The laboratory also provides information regarding the most effective antimicrobial agent and its dosage to be used for the specific patient.

Microbiology Laboratory Safety and Rules:

- Wear lab coat /apron in the laboratory.
- Do not invite friends into the laboratory.
- Wear gloves while working in laboratory.
- Wear Shoes in the laboratory.
- Open-toed sandals are not allowed.
- Keep pencils, fingers, etc. out of your mouth, ears, eyes, nose, etc
- Wear mouth mask where ever required.
- Do not bring food, drink, into the laboratory.
- Do not take any cultures out of the laboratory for any reason
- Wipe the bench tops down with disinfectant both before you begin your work and after you have completed your work.
- Keep nonessential books and clothing far away from your work area.
- Wash your hands with soap and water for 15 seconds before and after you work in the lab, or if you leave the laboratory for any reason.

- Mouth pipetting must not be done.
- Any plating/culturing/inoculating must be done in aseptic zone.
- Used glassware, media, etc must be sterilized before and after use.
- Be careful near Bunsen burners.
- Tie long hair back away from the face.
- Turn off burners when not in use.
- Beware of the chemicals and reagents used in biochemical tests.
- Report any accidents (spills, broken glass, cuts, or injuries) to your instructor immediately.
- Dispose of contaminated or used materials quickly and in the manner set forth by your instructor.
- Label all of your tubes and plates exactly as directed by your instructor.
- Place all cultures for incubation exactly where instructed or you risk their loss.
- Any cultures not handled accordingly will be discarded.
- Keep microscopes clean and the objectives free of oil.

Week No.	1st Week
Practical No.	1
Title/ Aim	Operation of instruments
Objectives	Student shall be able to operate different instruments used in microbiology laboratory
Requirements	Autoclave ,Incubator, water bath, pH meter, Hot air oven
Environment	MLT laboratory
Procedure	**pH METER** **Introduction**- The hydrogen ion conc. or pH is a measure of the acidity or alkalinity of a solution. At a given temperature,in an aqueous solution, the product of hydrogen ion concentration and hydroxyl ion concentration is constant. An acid solution has a pH value less than 7 and a basic solution has a pH value greater than 7. And a neutral solution has a pH value of 7. **Principle**- When a pair of electrodes is dipped in an aqueous solution, a potential is developed across a thin glass of bulb. The e.m.f. of complete cell formed by joining these two electrodes at a given temperature is E=E (ref) - E (glass) E (ref) = calomel electrode E (glass) =glass electrode

	Components- **Glass electrode**- it consists of a thin bulb blown on to a hard glass tube. It contains 0.1 mole per litre HCL connected to a platinum wire through a silver-silver chloride combination. **Calomel electrode**- it consists of a glass tube containing saturated KCL connected to a platinum wire through mercurous chloride paste. **Operation-** • Turn on and warm up the pH meter. • Immerse the electrodes in a beaker containing standard buffer pH 4. • If the pH reading is not 4, then adjust it to exact 4.0 by using calibration knob. • Wash the electrodes by distilled water and then wipe with soft tissue paper. • Use another standard buffer pH 7 to confirm the standardization. • Now immerse the electrodes in solution under test. • Note the pH readings. **Care and maintenance**- Glass electrodes must be clean and adequately watered. For cleaning the glass electrode never use

	any substance which has absolute alcohol and conc. HCL. For proper functioning, the calomel electrode must be kept filled with saturated KCL. When not in use keep dipped in distilled water. **HOT AIR OVEN** **Use**- Hot air oven is mainly used for Dry sterilization Preparation of anti coagulant bulbs Drying of glass ware **Principle** When electricity is passed through the heating coil, the electrical energy is converted to heat energy and the temperature is controlled by a thermostat. **Operation (details refer to expt. No:4)** Place the dry articles inside the hot air oven like powdered chemicals. Drain the washed glass ware first and then arrange inside the oven. Close the door firmly. Start the electricity. Control the temperature by using thermostat. Sterilize for required time. Put off the main switch.

	Allow to cool. Remove the articles. **Care and maintenance-** Put off the main switch when not in use. Clean the oven after each use. **Autoclave : refer to no:3**
Clinical significance	Knowing operation of various equipments helps to carry out procedures easily and its maintenance.Keeps instrument in working condition for a longer time.
Skills to be achieved	Handling of pH meter, autoclave and Hot air oven.(refer to expt. 3&4)
Skill evaluation criteria	Use of pH meter-----3marks Preparation of material for sterilization--- 4 marks Technique of autoclaving/ Hot air oven--- 3 marks
FAQs	1. What is principle of hot air oven/autoclave/pH meter? 2. How care of eachequipment is taken?
Assignment/Activity	Study operation of each equipment.
Reference	Theory topic -Handbook of Microbiology

Week No.	2ndweek
Practical No.	2

Title/ Aim	To disinfect inanimate objects.
Objectives	Student shall be able to disinfect inanimate objects.
Principle	Disinfection of the work area reduces the chances of contamination of media and cross-contamination of specimens, which help to prevent erroneous results.
Requirements	**5**% phenol solution**. (**Less effective in disinfecting viruses).
Environment	MLT laboratory
Procedure	1. Saturate a gauze pad or sponge with the disinfectant (5%phenol) and wipe off the entire work table including edges. 2. Keep a jar of disinfectant in a central position in which all the specimens can be discarded(e.g.throat swab, pus swab etc.) 3. Keep a bucket partially filled with 5%phenol and place under the bench.Discard Specimen bottles in the bucket.
Result	Thegiven Inanimate objects are disinfected appropriately.
Clinical significance	Inorder to avoid biological hazard,pathogenic microorganisms handled in the laboratory must be carefully destroyed. The harmless organisms present in the work area might contaminate the media therefore should be removed.
Skills to be achieved	Students will disinfect objects properly.

Skill evaluation criteria	Appropriate disinfection of inanimate objects. • At least10 different glassware---- - 5 marks. • Selection of disinfectant------ 2 marks. • Disinfected edges and corner--3 marks. Total: 10 marks.
FAQs	1. What is disinfection? 2. Name some commonly used disinfectants.
Assignment/Activity	• Prepare a list of different disinfectants used. • Disinfect the given specimen.
Reference	Theory topic 2-Handbook of Microbiology

Week No.	3nd week
Practical No.	**3**
Title/ Aim	To do sterilization of glassware, media etc using autoclave.
Objectives	Student shall be able to do sterilization of

	glassware, media etc using autoclave.
Principle	Water boils when its vapour pressure equals that of the surrounding atmosphere. Hence when pressure inside a closed vessel increases, the temperature at which water boils also increases. When steam comes into contact with a cooler surface it condenses to water and gives up its latent heat to that surface.
Requirements	1. Autoclave 2. Articles to be sterilized: Media / glassware / instruments etc. 3. Non – absorbent cotton, paper for wrapping and string.
Environment	MLT laboratory
Procedure	<u>Pre –preparations</u>:- • Test tubes, pipettes, flasks etc glassware must be first clean & dry. • Plug the glassware with non- absorbent cotton. • Liquid contents must not be more than ¾ full & then plugged loosely &arrow mark↑. • Moist media must be poured in conical flask, after plugging with cotton the necks of the flasks must be covered separately with paper and string. • All the articles to be sterilized must be wrapped with paper & tied with string.

	Important: - Now place the articles in dressing drum with the perforations open of the dressing drum to allow steam to enter. Sterilization procedure:- • Fill water in the autoclave through the water – inlet. Open the valve. Water must be filled till the **mark** indicated. • Close the lid & tighten the screws. • Open air outlet valve. • Plug- in and ON the switch. • Till steam comes out from air outlet valve(saturated steam) • Then close the air – outlet valve. • Now, pressure indicator will move from zero and wait till it comes to required pressure (e.g. 15 lbs). After attaining this pressure count the time (e.g. 20mins). • Switch OFF the electric supply. Let the pressure drop to zero by opening the outlet valve. • Slowly open the autoclave & remove the dressing drum with articles. Use clean thick cloth duster forhandling heated articles & opening the lid.
Observations	Proper maintenance of pressure, temperature and time during sterilization process.
Result	Sterilization of following done

	✚ Culture media – 10 lbs pressure for 10mins. ✚ Glassware, specimen collection bottle – 15 lbs pressure for 20mins ✚ Infected material -15 lbs pressure for 20mins.
Clinical significance	• Avoid biological hazard. • Sterilization using autoclave is highly effective that kills microorganisms without destroying media.
Skills to be achieved	Students will operate autoclave and sterilize any given material.
Skill evaluating criteria	1. Proper handling of autoclave. • Correct water level ------1 mark • Requiredpressure -----1 mark • Requiredtime ----1 mark • Safe removal of glassware----2 marks. 2. Proper preparation of material for autoclaving. • Correct wrapping of glassware---2 marks • Plugging of glassware ----2 marks • Labeling ----1 mark. ▪ Total: 10 marks.
FAQs	1. What is sterilization? 2. What are the different methods of sterilization? 3. What is principle of working of autoclave?
Assignment/Activity	Sterilize the given material using autoclave.
Reference	Theory topic 2-Handbook of Microbiology

Week No.	4th week
Practical No.	4
Title/ Aim	To sterilize glassware etc using hot air oven.
Objectives	Students shall be able to sterilize glassware etc using dry heat (hot air oven).
Principle	When electricity is passed through the heating coil, the electrical energy converted to heat energy and the temperature is controlled by a thermostat.
Requirements	1. Hot Air Oven 2. Articles to be sterilized: glassware / instruments etc.

	3. Non – absorbent cotton, paper for wrapping and string.
Environment	MLT laboratory
Procedure	1. Place the dry articles inside the hot air oven like powdered chemicals. 2. Drain the washed glassware first and then arrange inside the oven. 3. Close the door firmly. Start the electricity. 4. Control the temperature by using thermostat. 5. Sterilize for required time. The proper time and temperature for Dry-Heat sterilization is - 160 °C (320 °F) for 2 hours or - 170 °C (340 °F) for 1 hour. 6. Put off the main switch. 7. Allow to cool. Remove the articles.
Observations	Time and temperature maintenance during the process.
Result	Given articles are sterilized.
Clinical significance	• Avoid biological hazard. • Sterilization using Hot air oven is highly effective that kills microorganisms without destroying media.
Skills to be achieved	Students will operate Hot air oven correctly and sterilize any given material..
Skill evaluating criteria	1. Proper handling of Hot air oven. • Correct temperature-------2 marks • Dry glassware------1 mark • Selection of material---2 marks

	2. Proper preparation of material for sterilization by using Hot air oven. • Correct wrapping of glassware---2 marks • Plugging of glassware ----2 marks • Labeling ----1 mark. Total : 10 marks.
FAQs	1. What is sterilization? 2. What are the different methods of sterilization? 3. Explain principle of sterilization by dry heat.
Assignment/Activity	Sterilize the given material using hot air oven.
Reference	Ref. Theory Topic 2&3- Handbook of Microbiology

Week No.	5th week
Practical No.	**5**
Title/ Aim	To prepare Nutrient Agar.
Objectives	Student shall be able to prepare Nutrient Agar.
Principle	Nutrient agar is used to cultivate those bacteria, which are not nutritionally fastidious.
Requirements	1. Peptone – 5 Grams 2. Beef extract – 3 Grams 3. Sodium Chloride – 8 Grams 4. Agar – 15 Grams 5. Distilled Water – 1000 ml 6. pH paper
Environment	MLT laboratory
Procedure	1. Place the ingredients except Agar in 1 liter conical flask. 2. Add about 800 ml of distilled water. 3. Dissolve the ingredients either by using the

	stirrer or if necessary heat. 4. Adjust pH to 7.3 using 1 N NaOH . 5. Add Agar and boil for 1-2 min. 6. Make volume to 1000 ml by adding distilled water. 7. Sterilize by Autoclaving at 121^{o}C for 15 min.
Observations	 **Figure 4.1**
Result	Given media is prepared as per the procedure.
Clinical significance	• Used for culturing microorganisms in laboratory to detect the causative organism from given specimen. • Reporting AST.
Skills to be achieved	Students can prepare nutrient agar by using ingredients in required proportion.
Skill evaluating criteria	1. Exact weighing of the ingredients---4 marks. 2. Media preparation technique---4 marks 3. Storage---2 marks. Total : 10 marks

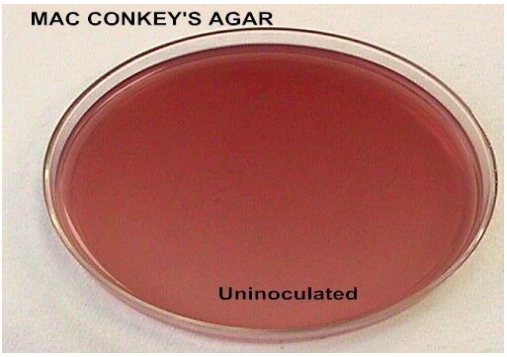
FAQs	• What is the composition of nutrient agar? • What are the different types of media?
Assignment/Activity	Prepare 5 nutrient agar plates.
Reference	Ref. Theory Topic 7- Handbook of Microbiology

Week No.	6th week
Practical No.	**6**
Title/ Aim	To prepare Blood Agar.
Objectives	Student shall be able to prepare Blood Agar.
Principle	Enriched and differential solid medium and detecting hemolytic bacteria
Requirements	1. Nutrient Agar- 500 ml 2. Sterile defibrinated blood-25 ml
Environment	MLT laboratory
Procedure	1. Transfer sterilized Nutrient Agar to 50°C water bath. 2. Add aseptically sterile defibrinated sheep or horse blood. 3. Mix gently. Adjust pH to 7.3.
Observations	 **Figure 5.1**
Result	Given media is prepared as per the procedure.
Clinical	• Used for culturing wide range of pathogens

significance	in the laboratory to detect the causative organism from given specimen. • Differentiate bacteria on the basis of type of hemolysis.
Skills to be achieved	Students can prepare blood agar by using ingredients in required proportion.
Skill evaluating criteria	1. Exact weighing of the ingredients---4 marks. 2. Media preparation technique---4 marks 3. Storage---2 marks. Total : 10 mark
FAQs	1. What is the composition of blood agar? 2. How will you differentiate between alpha, beta and gamma hemolysis?
Assignment/Activity	Prepare 5 blood agar plates
Reference	Ref. Theory Topic 7- Handbook of Microbiology

Week No.	7^{th} week
Practical No.	**7**

Title/ Aim	To prepare MacConkey's Agar.
Objectives	Student shall be able to prepare MacConkey's Agar.
Principle	Bile salt inhibits gram positive and gram negative cocci. Lactose fermenters produce red or pink colonies and Non lactose fermenters produce colorless colonies on the medium.
Requirements	1. Peptone - 20 grams 2. Lactose-10 grams 3. Bile salt - 5 grams 4. Sodium chloride - 5 grams 5. Neutral red - 0.075 grams 6. Agar-12 grams 7. Distilled water-1000 ml 8. pH paper/pH meter
Environment	MLT laboratory
Procedure	1. Place the ingredients except Agar in 1 liter conical flask. 2. Add about 800 ml of distilled water. 3. Dissolve the ingredients either by using the stirrer or if necessary heat. 4. Adjust pH to 7. 5. Add Agar and heat with frequent agitation and boil for one minute to completely dissolve the medium. 6. Make volume to 1000 ml by adding distilled water.

	7. Sterilize by Autoclaving at 121°C for 15 min.
Observations	 **Figure 7.1**
Result	Given media is prepared as per the procedure.
Clinical significance	Detection of coli forms organisms and pathogenic species of enteric bacilli.
Skills to be achieved	Students can prepare Mac Conkey's agar by using ingredients in required proportion.
Skill evaluating criteria	1. Exact weighing of the ingredients---4 marks. 2. Media preparation technique---4 marks 3. Storage---2 marks. Total : 10 marks
FAQs	1. What is the composition of Mac conkey's agar? 2. How will you differentiate between lactose fermenters and Non –lactose fermenters?
Assignment/Activity	Prepare 5 Mac Conkey agar plates.
Reference	Ref. Theory Topic 7- Handbook of Microbiology

Week No.	8th week
Practical No.	**8**

Title/ Aim	To do motility of bacteria.
Objectives	The student shall be able to do motility of bacteria.
Principle	Under reduced illumination motility is observed at the edge of the drop.
Requirements	1. Cavity slide 2. Cover slip 3. Mineral oil / Liquid paraffin / Vaseline 4. Culture 5. Microscope
Environment	MLT laboratory
Procedure	1. Take a cover slip on a clean smooth table and put mineral oil / liquid paraffin / Vaseline at the four corners of the cover slip. 2. Put a single loopful of culture in the center of the cover slip. 3. Invert cavity slide (concave side down) over the cover slip and press gently to form a seal. 4. Turn the slide and observe the drop hanging from the cover slip. 5. Observe under low power of microscope and focus the edge of the drop. 6. Now turn to high power & focus with moving the slide. 7. Carefully observe motility of bacteria and

	report.

Observations

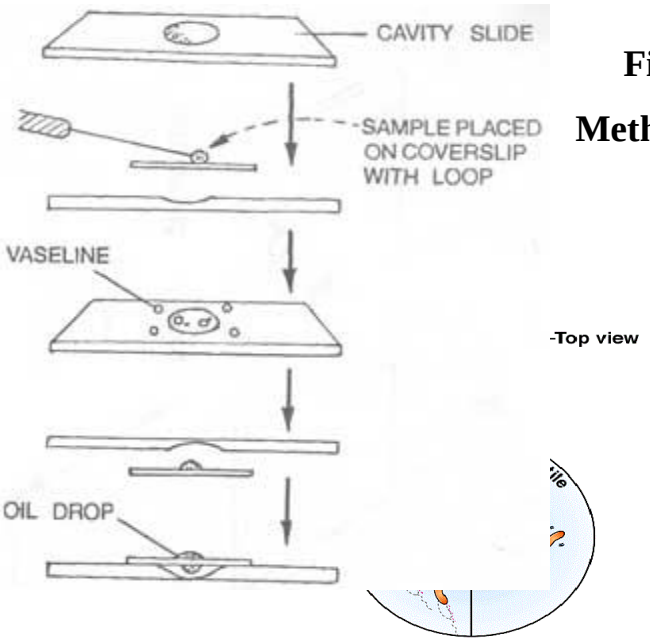


Figure 7.1 Hanging Drop Method

Figure 7.2

Morphology	Actively motile	Sluggishly motile	Nonmotile

Table no.8.1

Result	Bacteria are sluggishly motile / actively motile / non-motile.
Clinical significance	• Hanging drop technique is used to differentiate between motile and non-motile bacteria. • Motile bacteria possess flagella except spirochetes.

Skills to be achieved	Students can observe motility of bacteria by hanging drop method. Students can differentiate between motile and non motile bacteria.
Skill evaluating criteria	• Technique of preparation of hanging drop---4 marks. • Focusing of the edge of drop under low and high power---4 marks • Observing the movement of microorganisms--2 marks Total : 10 marks
FAQs	1. How will you detect that the bacteria are motile or non-motile?
Assignment/Activity	Perform hanging drop examination of 5 different organisms.
Reference	Theorytopic 9- Handbook of Microbiology

Week No.	9th week
Practical No.	**9**
Title/ Aim	Collection of Urine Specimen for bacteriological examination [Patient must not be on antibiotics]
Objectives	Student must be able to instruct patient to collect

	urine specimen for successful isolation of microorganisms.
Principle	To obtain midstream sample of the patient taking care that contaminants are avoided andspecimen must deliver to the laboratory promptly,as bacteria continue to grow in urine.
Requirements	1. Container: Wide-mouthed, screw capped, dry, sterile leak proof bottle provided by the Laboratory. 2. Mid-Stream (clean catch) Sample.
Environment	MLT laboratory
Procedure	1. First morning discharge of urine is desirable. 2. Ask the patient to wash the genital organ with clean water (do not use soap) before passing the urine. 3. Ask the patient to collect midstream urine sample about (20ml) after discarding initial portion of urine. 4. After collecting required quantity replace the cap securely. 5. In case of infants a bag is fixed with an adhesive mouth to the genitalia and left for 2-3 hrs. 6. In unavoidable instance catheterization can be done. 7. Clearly label the specimen.

Observations	  **Fig 8.1 Containers for urine**
Result	Specimen is obtained taking all the precautions.
Clinical significance	Urine specimen is submitted for the diagnosis of urinary tract infection.
Skills to be achieved	Students will acquire proper knowledge and significance of sterile containers used for urine collection, its labeling and handling.
Skill evaluating criteria	1. Patient instructions---2 marks. 2. Knowledge of containers---2 marks. 3. Selection of containers------2 marks. 4. Specimen labeling---- 1mark. 5. Correct quantity-------1 mark. 6. Specimen handling---2 marks. Total : 10 marks.
FAQs	1. How will you obtain /collect the specimen from patient? 2. What instructions you will give the patient before sample collection?
Assignment/Activity	Collect specimen from 10 different patients.
Reference	Theory Topic 21- Handbook of Microbiology

Week No.	10th week
Practical No.	**10**
Title/ Aim	To collect blood for bacteriological examination.
Objectives	Student shall be able to collect blood for bacteriological examination.
Principle	Blood is collected by vein puncture taking

	allthe precautions during the acute phase of the disease and before any antibiotic administration.
Requirements	1. Disposable sterile Syringe & needle (10 ml) 2. Spirit / 70 % alcohol 3. 2% iodine Solution 4. Tourniquet 5. Sterile swabs 6. Biphasic media
Environment	MLT laboratory
Procedure	1. Disinfect the skin with spirit at the vein puncture site. 2. Then apply 2% iodine solution wait for minute. 3. Draw blood by venipuncture. 4. Clean with sprit / 70% alcohol 5. Add 5 ml of blood immediately into each collecting bottles before clotting takes place by inserting the needle through the rubber line of the bottle cap.
Result	Blood specimen is collected taking all the precautions for bacteriological examination.
Clinical significance	Blood is probably the most important specimen submitted to the bacteriological laboratory for culture for the diagnosis of septicemia,bacteraemia,PUO, etc.
Observations- The method of collecting blood is illustrated by image as follows-	

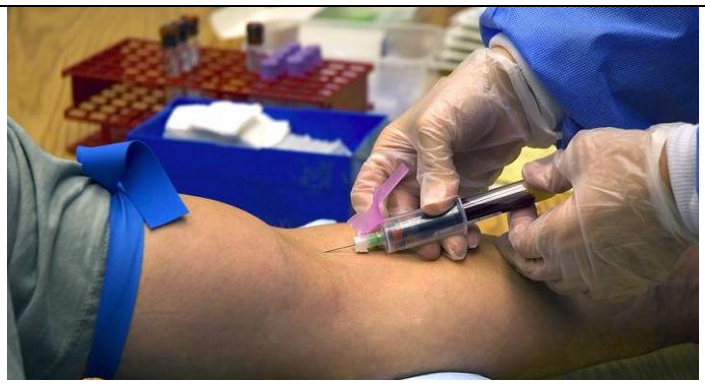


Figure 10.1

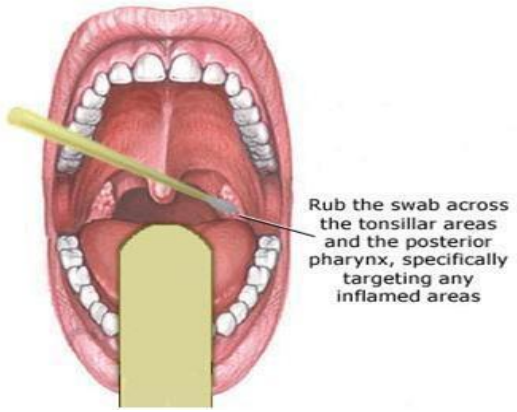
Skills to be achieved	• Collection of blood by vein punctures under aseptic precautions.
Skill evaluating criteria	1. Patient instructions---2 marks. 2. Knowledge of containers---2 marks. 3. Selection of containers------2 marks. 4. Specimen labeling---- 1mark. 5. Correct quantity-------1 mark. 6. Specimen handling---2 marks Total 10 marks.
FAQs	1. How will you collect blood from patient? 2. What instructions you will give the patient before sample collection?
Assignment/Activity	Collect specimen from 5 different patients.
Reference	Theory Topic 21- Handbook of Microbiology
Week No.	11th week
Practical No.	11
Title/ Aim	To know the technique of collecting stool sample for bacteriological study.
Objectives	Students must know the technique of collecting stool sample for bacteriological study.

Requirements	1. Containers- Screw capped, clean, dry, wide mouth, 250 ml bottle or bed pan or sterile swab in case of rectal swab. 2. Cary – Blair medium in case of delay / transport of Specimen.
Environment	MLT laboratory
Procedure	1. In case of freshly discharged faces give the container and culture within 2hrs. 2. In case of bed-pan specimen transfer small portion ofspecimen to a clean dry container. 3. For rectal swabs- Take the swab after ejection of stool. Insert the swab well, swab the rectal wall. Take 2 swabs. 4. Transportation: Transfera portion of Specimen in to Cary –Blair transport medium. 5. Refrigerate the Specimen if prolonged delay. 6. Label the Specimen correctly.
Observations  **Figure 11.1Containers for Stool Collection.**	
Result	Specimen is obtained taking all the precautions
Clinical significance	To see for the presence of an enteric

	pathogen causing acute intestinal infection(diarrhoea).
Skills to be achieved	Proper technique of collection of specimen. Knowledge of containers.
Skill evaluating criteria	1. Patient instructions---2 marks. 2. Knowledge of containers---2 marks. 3. Selection of containers------2 marks. 4. Specimen labeling---- 1mark. 5. Correct quantity-------1 mark. 6. Specimen handling---2 marks. Total 10 marks
FAQs	1. How will you obtain /collect the specimen from patient? 2. What instructions you will give the patient before sample collection?
Assignment/Activity	Collect specimen from 10 different patients
Reference	(Ref. Theory Topic 21)- Handbook of Microbiology

Week No.	12th week
Practical No.	12
Title/ Aim	Collection of Throat swab and Vaginal Swab
Objectives	Students must be aware of appropriate Specimen collection for identification of causative organism.
Principle	Obtain specimen without any contaminants
Requirements	Sterile cotton swabs within test tube.
Environment	MLT laboratory
Procedure	Throat Swab • Tilt the head of the patient slightly backwards. • Ask him to open his mouth wide. • Press the tongue with the tongue depressor using left hand.

	• With right hand pass the swab well over both the tonsils and area of inflammation and return to container. Take 2 Swabs
	
	Vaginal Swab: It is taken by a physician /trained by nurse or in some cases by an experienced technician.
	• Collect the vaginal discharge with sterile cotton swab.
	  • Put it into**Amies Transport Medium**under sterile condition.
	  • Make smears for gram staining.
	  • The Specimen should not be refrigerated.

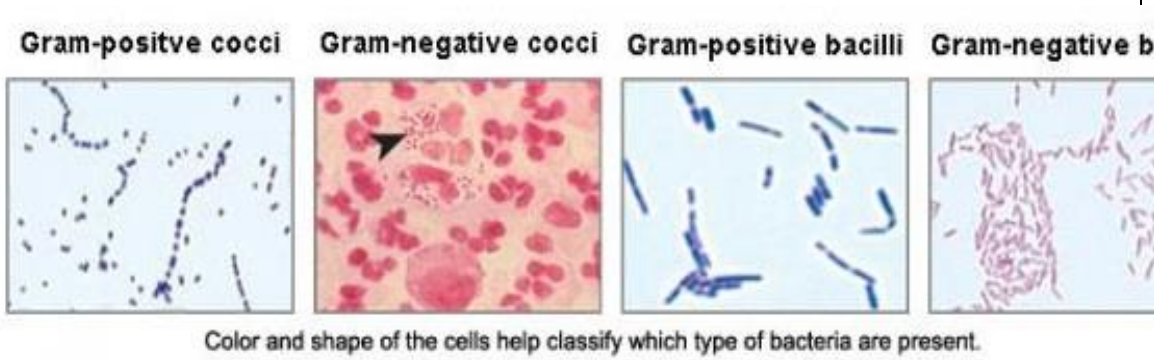
Observations   **Figure 11.1 Collection of Throat Swab**	
Result	All the specimen collection technique must contain only those organisms from which it is collected avoiding contamination. It should be appropriate for further

	identification of causative organism
Clinical significance	**Throat swab:**Diagnosis of streptococcal sore throat which might lead to serious problems, such as rheumatic fever,scarlet fever and acute glomerulonephritis. **Nasopharyngeal swab**: Diagnose whooping cough and diphtheria. **Vaginal swab:**Diagnosis of Gonorrhoea
Skills to be achieved	Collection of throat swab and vaginal swab
Skill evaluating criteria	1. Patient instructions---2 marks. 2. Knowledge of containers---2 marks. 3. Selection of containers------2 marks. 4. Specimen labeling---- 1mark. 5. Correct quantity-------1 mark. 6. Specimen handling---2 marks. Total :10marks.
FAQs	• How will you obtain /collect the specimen from patient? • What instructions you will give the patient before sample collection?
Assignment/Activity	Take 10 specimens of throat swab
Reference	(Ref. Theory Topic 21)- Handbook of Microbiology

Week No.	12th week
Practical No.	12
Title/ Aim	To do Gram staining of given specimen.
Objectives	Student shall be able to identify gram positive and gram negative organisms after staining with gram staining technique.
Principle	Bacteria pick up purple color when stained with crystal violet. Iodine fixes the stain to the bacterial cell**.** Acetone- alcohol decolorizes gram negative bacteria whereas gram positive bacteria retain the purple color counterstaining with saffranine or basic fuschin stain. Gram negative bacteria appear pink.
Requirements	• Crystal violet stain • Gram's Iodine • Decolourizer (alcohol-acetone) • Saffranine stain

	• Bunsen burner and Nichrome wire loop • Specimen
Environment	MLT laboratory
Procedure	1. Make a thin smear of the specimen. Allow it to dry. 2. Pass the slide two to three times through flame until it feels comfortably warm on the back of the hand (heat fixing) 3. Place the slide on staining rack. Add crystal violet stain for one minute. Wash gently with water 4. Add Gram's Iodine for one minute. Drain the Iodine. Do not wash. 5. Pour Acetone-alcohol on smear till no more blue color comes out from smear (decolourization) 6. Wash with water. 7. Stain with saffranine for 10 seconds (counterstaining) 8. Wash with water. Dry. 9. First observe under low power objective (10x). then under high power (45x) and finally under oil immersion lens using a drop of Cedar wood oil

Observations



Color and shape of the cells help classify which type of bacteria are present.

Figure 5.1.2 Gram Staining	
Result	**Gram positive** bacteria appear **purpl**e and **Gram negative** bacteria appear **pink** in color.
Clinical significance	• Differentiating into gm positive and gm negative is helpful in determining the subsequent biochemical tests and media for their culture. • A preliminary report can be submitted which might help the physician to initiate therapy well before culture results are available
Skills to be achieved	Gram staining skill of given specimen/culture.
Skill evaluating criteria	1. Heat fixation of smears---2 marks. 2. Working under aseptic conditions-- 2marks. 3. Selection of stains----1 mark. 4. Staining technique--- 5 marks. Total 10 marks.
FAQs	1. How you will do gram staining of given specimen? 2. Report the findings of gram staining.
Assignment/Activity	Do gram staining of urine sample,throat swab,nasal swab, sputum specimen(2 samples each)
Reference	(Ref. Theory Topic 5)- Handbook of Microbiology

Week No.	13th week
Practical No.	13
Title/ Aim	To do Albert's staining
Objectives	Student shall be able to identify metachromatic granules of Corynebacterium diphtheriae after staining with Albert's stain.
Principle	Malachite green stains the metachromaticgranules bluish black and the bacterial body green.
Requirements	• Albert's solution A • Albert's solution B • Bunsen burner • Nichrome wire loop • Specimen
Environment	MLT laboratory
Procedure	• Make a thin smear of specimen. Allow to dry.

	• Pass the slide two to three times through flame until it feels comfortably warm on the back of the hand (heat fixing) • Place the slide on staining rack. Flood with Albert's solution A for five minutes. • Drain the solution. Do not wash with water. • Flood with solution B and keep for one to two minutes. • Wash gently under running tap water. • Drain. Dry. And examine under oil immersion objective (100x).

Observations

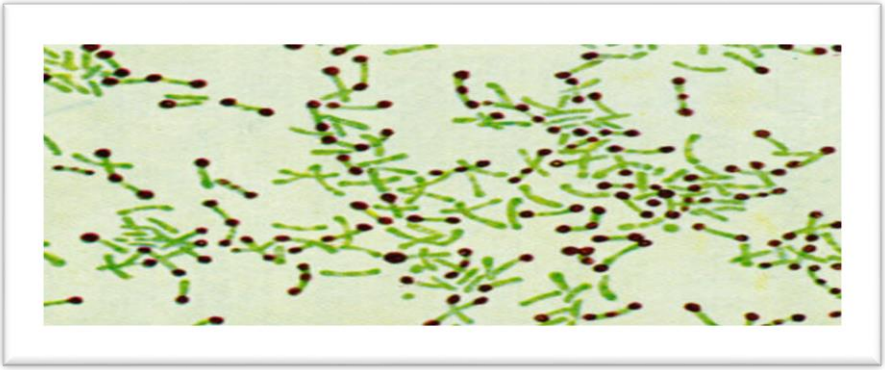


Figure 13.1 ALBERT STAIN -C. Diphtheriae

Result	Metachromatic granules appear bluish black, Bacilli green or bluish green.
Clinical significance	To detect Corynebacterium diphtheria
Skills to be achieved	Skill to do albert staining and keenly observe the metachromatic granules and identify the granules.
Sk+ill evaluating criteria	1. Heat fixation of smears---2 marks.

	2. Working under aseptic conditions---2 marks. 3. Selection of stains----1 mark. 4. Staining technique--- 5 marks. Total 10 marks.
FAQs	1. How you will do Albert's staining ? 2. Report the findings of Albert's staining.
Assignment/Activity	Do Albert's staining of 3-5 specimen.
Reference	(Ref. Theory Topic 5)

Week No.	15th week
Practical No.	15
Title/ Aim	To do Z.N. staining (Ziehl-Neelsen Staining)
Objectives	Students shall be able to identify Acid Fast organisms after staining with Z.N. stain
Principle	Mycobacteria are extremely difficult to stain by ordinary methods because of the lipid containing cell walls called mycolic acid.Heat is applied in hot stain method for detection of Mycobacterium tuberculosis and cold stain method is used for detection of Mycobacterium leprae. Acid Fast bacilli resist de-staining with strong decolorizing agent such as alcohol and strong acids. Acid fast negative are counterstained with methylene blue stain.
Requirements	• Carbolfuchsin stain • 20% Sulphuric acid (for M. Tuberculosis) or 5% Sulphuric acid (for M.leprae)

	• Methylene blue stain • Bunsen burner • Nichrome wire loop • Specimen
Environment	MLT laboratory
Procedure	1. Make a thin smear of the specimen. Allow it to dry. 2. Pass the slide two to three times through flame until it feels comfortably warm on the back of the hand (heat fixing). 3. Place the slide on staining rack. Add carbolfuchsin stain. 4. Heat gently with flame until steam rises. Avoid boiling and continue heating for about five minutes. Do not allow stain to dry. 5. Wash the slide with water. 6. Cover the slide with 20% Sulphuric acid for one minute. (decolourization) 7. Wash the slide with water. 8. Cover the slide with methylene blue for one minute (counter staining) 9. Wash with tap water. Allow the water to drain off completely. Dry. 10. Observe the slide first under low power objective (10x) and then under oil immersion objective (100x).

Observations

- Acid Fast organisms are bright red bacilli on blue background.
- Other organisms and cells appear blue.

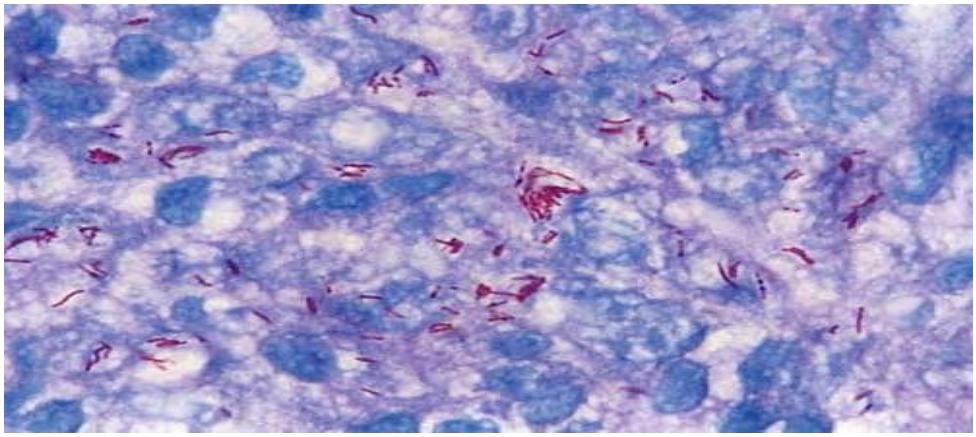


Figure 14.1 Z.N. Staining

Result

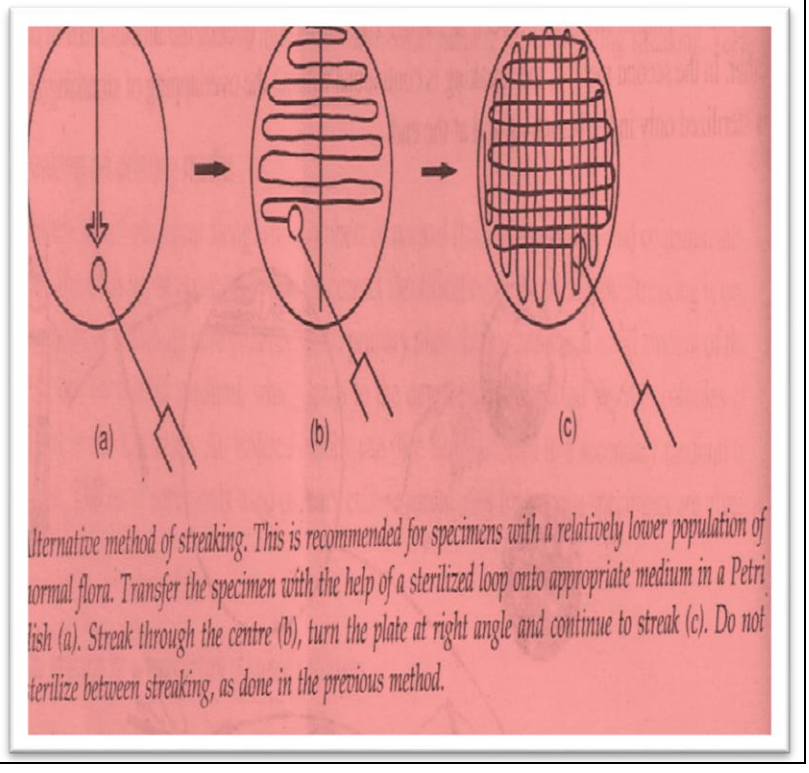
No. of acid fast bacteria in 10 fields	Report
None	Absent
1 to 2	Positive, +
2 to 10	Positive, ++
10 to 100	Positive, +++
Above 100	Positive, ++++

Clinical significance	To detect Mycobacterium tuberculosis
Skills achieved	Skillfully do acid fast staining without any error.
Skill evaluation criteria	1. Heat fixation of smear. Heat fixation of smears---2 marks. 2. Working under aseptic conditions---2 marks. 3. Selection of stains----1 mark.

	4. Staining technique--- 5 marks. Total 10 marks.
FAQs	How you will do acid fast staining of given specimen?
Assignment/Activity	Do Acid fast staining of 5 Sputum specimen.
Reference	Theory Topic 5)- Handbook of Microbiology
Week No.	16th week
Practical No.	16
Title/ Aim	To do Streak culture method**.**
Objectives	Learning technique of streak culture to enable the students to isolate microorganisms (bacteria).
Principle	Streaking is done on primary plate to provide information on the number of types of bacteria in the sample To obtain pure cultures for the secondary plate.
Requirements	• Petri plates with media. • Specimen / Culture. • Inoculating loop. • 2 Burners / Safety hood (Aseptic zone). • Marker pen.
Environment	MLT laboratory
Procedure	Apply this method only for specimens with a low number of organisms. **Continuous method** **Procedure:-** 1. Swab the table, burners with a disinfectant. Ignite two burners. All the plating / streaking technique must be done in- between 2 burners is aseptic zone / safety hood.

	2. At the back of the plate mark a straight line exactly in the center (diameter). 3. Open the plate with your left hand, thumb& index finger on the lid & other three fingers at the base of the plate. 4. Place a loopful of the specimen / inoculums on the marked central line of the plate touching the media and spread in a single line across the diameter of the plate. 5. Streak back & forth (zigzag) in lines perpendicular to the initial streak, covering the entire plate. 6. Now, turn the plate 90° and streak back and forth in lines parallel to the initial streak, again covering the entire plate

Observations



lternative method of streaking. This is recommended for specimens with a relatively lower population of normal flora. Transfer the specimen with the help of a sterilized loop onto appropriate medium in a Petri lish (a). Streak through the centre (b), turn the plate at right angle and continue to streak (c). Do not terilize between streaking, as done in the previous method.

Figure 16.1 Streak culture - Continuous Method	
Result	Well isolated colonies obtained.
Clinical significance	Identification of infectious agent.
Skills achieved	1. Use of inoculating loop----3 marks 2. Technique of aseptic transfer---3 marks 3. Actual streaking------3 marks. 4. Obtaining isolated colonies---1 mark. Total :10 marks.
FAQs	How you will do streaking on primary culture plate?
Assignment/Activity	Do streak culture---continuous method of few specimen.
Reference	(Ref. Theory Topic 8)- Handbook of Microbiology

Week No.	17^{th} week
Practical No.	17
Title/ Aim	Identification of isolated bacteria by biochemical test-TSI (triple sugar iron) test
Objectives	The student shall be able to do TSI test.
Principle	• It indicates whether a bacterium ferments glucose only, or lactose and sucrose also, with or without gas formation, besides indicating H2S production as well. • The medium is distributed in tubes, with a butt and slant. After inoculation if slant remains red and the butt becomes yellow,all the sugars—glucose,lactose and sucrose –are fermented. Bubbles in butt indicate gas production and blackening shows H2S formation.
Requirements	• TSI slant, • Incubator, • culture of organism • inoculating loop and straight needle
Environment	MLT laboratory
Procedure	Streak the TSI slant with a loop and stab with a straight needle. Incubate at 37^0C.for 18 to 24 hrs.
Observation • **Alkaline slant (red) and acid butt (yellow) with or without gas**	

production (breaks in the agar butt):

Only glucose fermentation has occurred. The organisms preferentially degrade glucose first. Since this substrate is present in minimal concentration, the small amount of the acid produced on the slant surface is oxidized rapidly. The peptones in the medium are also used in the production of alkali. At the butt, the acid reaction is maintained because of the reduced oxygen tension and slower growth of the organisms.

- **Acid slant (yellow) and acid butt (yellow) with or without gas production:**

Lactose or sucrose fermentation has occurred. Since these substances are present in higher concentrations, they serve as substrates for continued fermentative activities with maintenance of an acid reaction in both the slant and the butt.

- **Alkaline slant (red) and alkaline butt (red) or no change (orange-red) butt:**

No carbohydrate fermentation has occurred. Instead; peptones are catabolized under anaerobic and /or aerobic conditions resulting in alkaline pH due to production of ammonia. If only aerobic degradation of peptones occurs, the alkaline reaction is evidenced only on the slant surface. If there is aerobic and anaerobic utilization of peptone, the alkaline reaction is present on the slant and the butt.

- **Hydrogen sulfide (H_2S) production:**

Some bacteria utilize thiosulfate anion as a terminal electron acceptor, reducing it to sulfide. If this occurs, the newly-formed hydrogen sulfide (H2S) reacts with ferrous sulfate in the medium to form ferrous sulfide, which is visible as a black precipitate. The blackening of the medium is almost always observed in the butt (bottom) of the medium.

- **Carbon dioxide (CO_2) production:**

It is recognized simply as bubbles of gas between the agar and the wall of the tube or within the agar itself. The carbon dioxide production is sufficient to split the agar into two or more sections. To obtain accurate results, it is absolutely essential to observe the cultures within 18-24 hours following incubation. This will ensure that the carbohydrate substrates have not been depleted and that degradation of peptones yielding alkaline end products has not taken place.

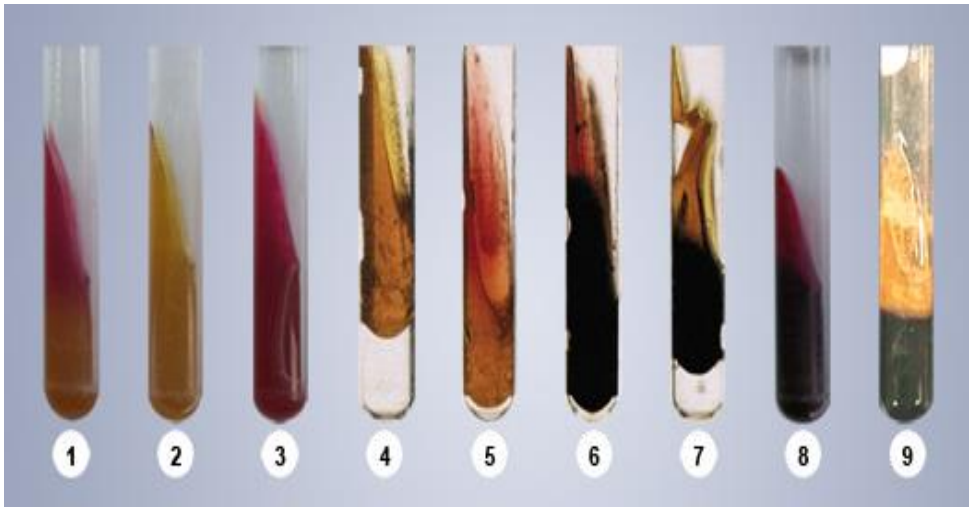


Figure 17.1 TSI test

Result

Sr. No.	Result(Slant/Butt)	Symbol	Interpretation
1	Red/Yellow	K/A	Glucose fermentation only, peptone catabolized.
2	Yellow/Yellow	A/A	Glucose and lactose and/or sucrose fermentation.
3	Red/Red	K/K	No fermentation, Peptone catabolized.

4	Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
5	Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
6	Red/Yellow with bubbles and black precipitate	K/A,G,H_2S	Glucose fermentation only, Gas produced, H_2S produced.
7	Yellow/Yellow with bubbles and black precipitate	A/A,G,H_2S	Glucose and lactose and/or sucrose fermentation, Gas produced, H_2S produced.
8	Red/Yellow with black precipitate	K/A,H_2S	Glucose fermentation only, H_2S produced.
9	Yellow/Yellow with black precipitate	A/A,H_2S	Glucose and lactose and/or sucrose fermentation, H_2S produced.

Clinical significance	The TSI medium facilitates preliminary identification of Gram negative bacilli.
Skills to be achieved	Skillfully perform the TSI test.
Skill evaluation criteria	1. Inoculation---1mark. 2. Aseptic condition----1 mark 3. Stabbing--- 2 marks 4. Observing correctly: • Slant colour change----2 marks. • Butt colour change---2 marks. • Gas production---2 marks. Total: 10 marks.
FAQs	What is the principle of TSI medium?
Assignment/Activity	Inoculate 4-5 TSI medium tubes.

Reference	(Ref. Theory Topic 9)- Handbook of Microbiology
Week No.	18th
Practical No.	18
Title/ Aim	The student shall be able to do IMViC test-indole test
Objectives	The student shall be able to do Indole test
Principle	Certain bacteria breakdown amino acid trptophan to give indole.indole reacts with kovac's reagent to give red colour.
Requirements	• Charts,ppt,you –tube. • Peptone water • Culture. • Kovac's reagent
Environment	MLT laboratory
Procedure	1. Subculture on media containing trptophan(eg peptone water. 2. Incubate at 37 deg. cent. For 24 hrs. 3. Add few drops of kovac's reagent 4. Examine for development of red ring.
Observations Positive test is identified by a development of red ring.	

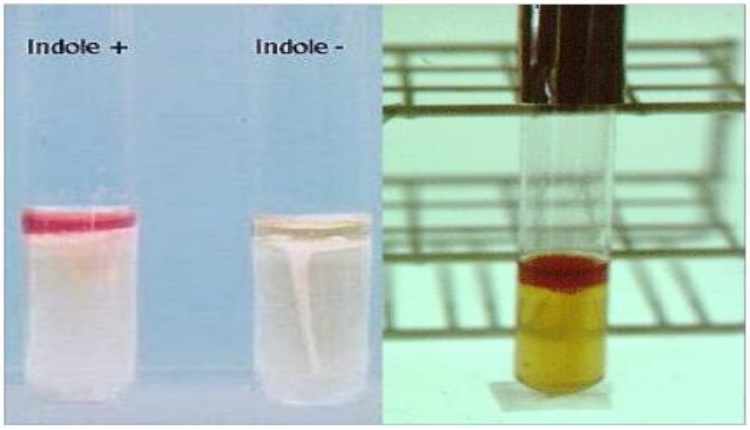


fig:18.1

Positive organisms: E.coli

Negative organism:Klebsiella

Result	Development of red ring:positive No development of red ring:negative.
Clinical significance	• Using the IMViC(indole) test will determine the biochemical properties and enzymatic reactions of enterobacteriaeceae. • The IMViC (indole)will determine whether they are pathogenic, occasional pathogenic or normal intestinal flora.
Skills to be achieved	• Skillfully perform IMViC (indole)test under aseptic conditions with no contamination
Skill evaluating criteria	1. Inoculation into medium-----4 marks 2. Aseptic conditions-----4 marks 3. Differentiating between +ve and -ve test---2 marks. Total: 10 marks.
FAQs	How you will do Indole test?
Assignment/Activity	Study Indole formation of 3-4 bacteria.
Reference	(Ref. Theory Topic 9)- Handbook of Microbiology

Week No.	19th week
Practical No.	19
Title/ Aim	To do IMViC-methyl red (MR) test
Objectives	The student shall be able to do methyl red (MR) test
Principle	This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they overcome the buffering action of the system. Methyl Red is a pH indicator, which remains red in color at a pH of 4.4 or less.
Requirements	• Charts,ppt,you-tube • glucose phosphate broth • methyl red reagent • culture • inoculating loop • incubator
Environment	MLT laboratory
Procedure	The bacterium to be tested in inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37oC for 48 hours. The pH of the medium is tested by the addition of 5 drops of MR reagent. Development of red color is taken as positive. MR negative organism produceyellow colour.
Observations	

Development of red color is taken as positive. MR negative organism produceyellow colour

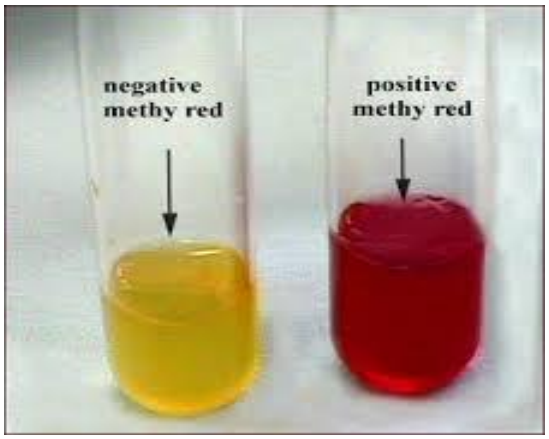


Fig:19.1

Eschericihia coli: Positive; Klebsiella pneumoniae: Negative

Result	Development of red color is taken as positive. MR negative organism produceyellow colour
Clinical significance	Using the IMViC (MR) test will determine the biochemical properties and enzymatic reactions of enterobacteriaeceae. The IMViC (MR)will determine whether they are pathogenic, occasional pathogenic or normal intestinal flora.
Skillsto be achieved	Skillfully perform IMViC(MR) test under aseptic conditions with no contamination
Skill evaluating criteria	1. Inoculation into medium-----4 marks 2. Aseptic conditions-----4 marks 3. Differentiating between +ve and -ve test---2 marks. Total: 10 marks.
FAQs	How you will do Methyl Red (MR) test?
Assignment/Activity	Perform Methyl Red test of 3-4 bacteria.
Reference	(Ref. Theory Topic 9)- Handbook of Microbiology

Week No.	20th week

Practical No.	20
Title/ Aim	To doIMViC-Voges proskauer (VP) Test
Objectives	The student shall be able to doVoges proskauer (VP)test
Principle	VP test detects butylene glycol producers. Acetyl-methyl carbinol (acetoin) is an intermediate in the production of butylene glycol.If acetoin is present, it isoxidized in the presence of air and KOH todiacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha-naphthol to produce red color.
Requirements	• glucose phosphate broth • culture to be inoculated • inoculating loop • alpha-naphthol • 40% KOH
Environment	MLT laboratory
Procedure	1. Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48 hours. 2. 0.6 ml of alpha-naphthol is added to the test broth and shaken. 3. 0.2 ml of 40% KOH is added to the broth and shaken. 4. The tube is allowed to stand for 15 minutes.

	5. Appearance of red color is taken as a positive test. 6. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.

Observations

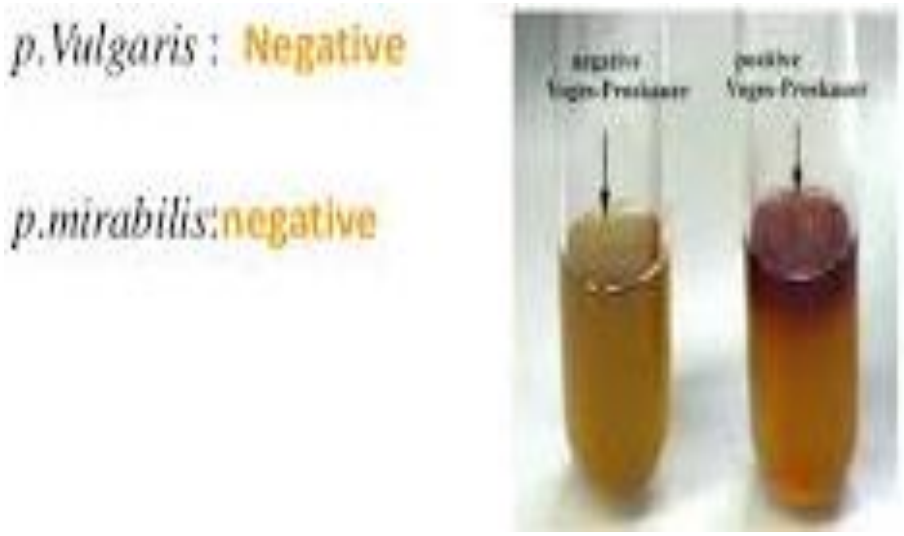


fig 20.1

Escherichia coli: Negative; Klebsiella pneumoniae: Positive

Result	Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents.
Clinical significance	• Using the IMViC (VP)test will determine the biochemical properties and enzymatic reactions of enterobacteriaeceae. • The IMViC(VP)will determine whether they are pathogenic, occasional pathogenic

	or normal intestinal flora.
Skills to be achieved	• Skillfully perform VP test under aseptic conditions with no contamination
Skill evaluating criteria	• 1.Inoculation into medium-----4 marks • 2.Aseptic conditions-----4 marks • Differentiating between +ve &–vetest----------2marks Total: 10 marks.
FAQs	How you will do Voges Proskauer (VP) test?
Assignment/Activity	Do Voges Proskauer (VP) test of 3-4 bacteria.
Reference	(Ref. Theory Topic 9)- Handbook of Microbiology

Week No.	21th week
Practical No.	21
Title/ Aim	To doIMViC-citrate utilization test
Objectives	The student shall be able to do citrate utilization test.
Principle	This test detects the ability of an organism to

	utilize citrate as the sole source of carbonand energy. Bacteria are inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue.Utilization of citrate involves the enzyme citritase, which breaks down citrate and results in alkaline pH. This results in change of medium's color from green to blue.
Requirements	Charts,ppt, you tube • Simmon's citrate agar • Culture to be tested • Inoculating loop
Environment	MLT laboratory
Procedure	1. Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37oC. 2. If the organism has the ability to utilize citrate, the medium changes its color from green to blue.
Observations	

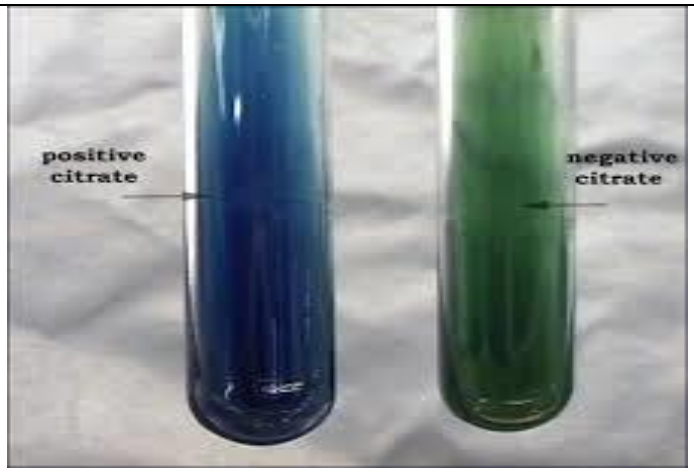


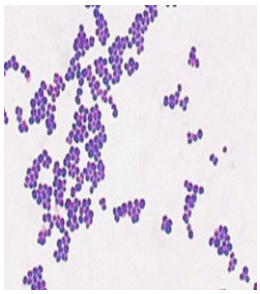
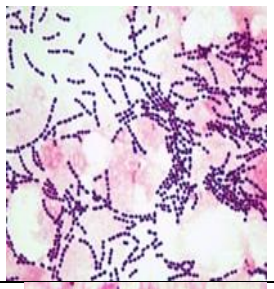
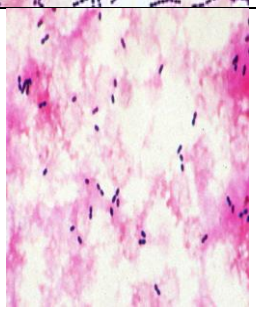
Fig 21.1
Klebsiella pneumoniae: Positive Escherichia coli: Negative

Result	If the organism has the ability to utilize citrate, the medium changes its color from green to blue.
Clinical significance	• Using the IMViC (citrate test) will determine the biochemical properties and enzymatic reactions of enterobacteriaeceae. • The IMViC (citrate test)will determine whether they are pathogenic, occasional pathogenic or normal intestinal flora.
Skills to be achieved	Skillfully perform citrate test under aseptic conditions with no contamination
Skill evaluating criteria	1.Inoculation into medium-----4 marks 2.Aseptic conditions-----4 marks 3. Differentiating between +ve &–vetest-----2marks
FAQs	How you will do citrate utilization test?
Assignment/Activity	Study citrate utilization of 3-4 bacteria.
Reference	Theory Topic 9- Handbook of Microbiology

Week No.	22st week
Practical No.	22
Title/ Aim	To identify the bacterium from permanent mounts

Objectives	To give knowledge to the student how to identify microorganisms on the basis of their gram character and morphology.
Principle	Identification of bacterium.
Requirements	• Permanent mounts of bacteria • Microscope.
Environment	MLT laboratory
Procedure	Focus the given slide under oil immersion objective and report

Observations

Gram charac t-er	shape	Arrangement	Name of bacteria	Observation
Gram positi ve	Cocci	Grape-like clusters	Staphylococc aureus	
Gram positi ve	Cocci	Chains	Streptococcus pyogenes	
Gram positi ve	Cocci	Lanceolate diplococcic	Pneumococci Streptococcus pneumoniae	 Fig:21.3

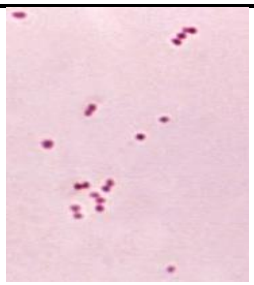
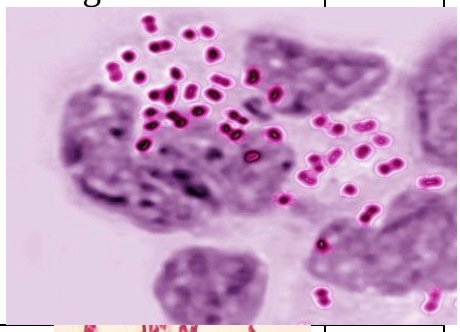
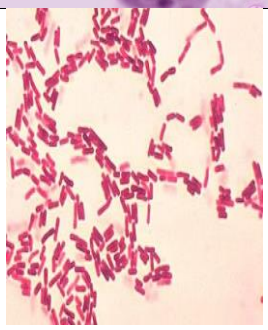
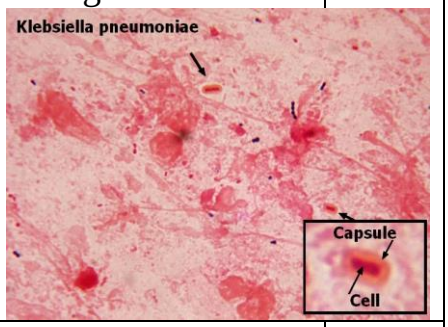
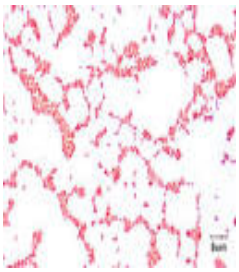
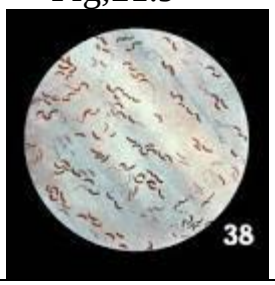
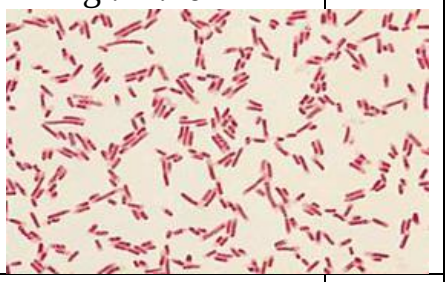
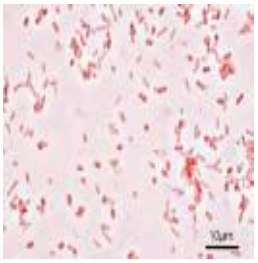
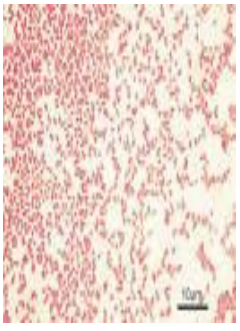
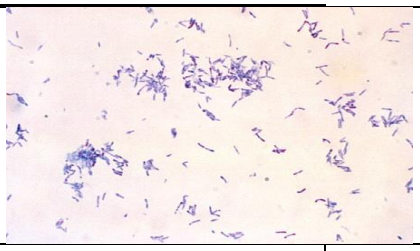
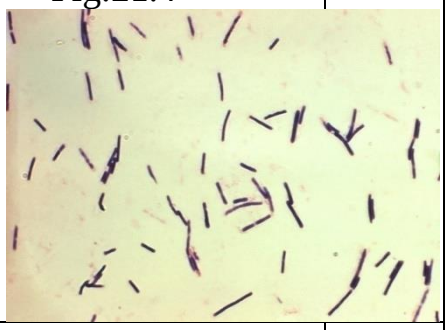
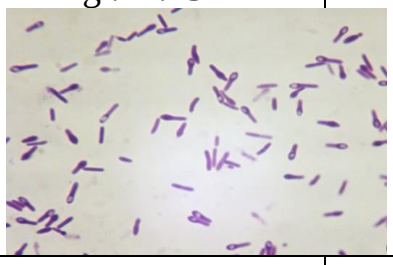
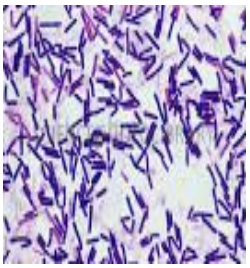
Gram negative	cocci	Diplococcic	Neisseria meningitides	 Fig :21.4	
Gram negative	Cocci	Diplococci kidney bean shaped.	Neisseria Gonorrhoeae	Fig21.5 	
Gram negative	bacilli	Single	Escherichia Coli	 Fig:21.6	
Gram negative	bacilli	Single	Klebsiella pneumoniae	Fig:21.7 	
Gram negative	bacilli	Single	Proteus		

				Fig :21.8	
Gram negative	bacilli	Comma shaped	Vibrio Cholerae	Fig;21.9 	
Gram negative	bacilli	Single	Shigella	Fig :21.10 	
Gram negative	bacilli	Single	Salmonella	 Fig:21.11	
Gram negative	bacilli	Single	Pseudomonas	 Fig :21.12	
Gram positive	bacilli	Chinese letter arrangement	Corynebacteri-um	Fig:21.13	

				
Gram positive	bacilli	Single with oval and subterminal spores	Clostridium welchii	Fig:21.4 
Gram positive	bacilli	Single with round and terminal spores	Clostridium tetani	Fig :21.15 
Gram positive	bacilli	Single with oval and subterminal	Clostridium botulinum	 Fig:21.16

Result	Same as in table
Skills to be achieved	Knowledge of the identifying characteristics of given organism
Skill evaluating criteria	Each organism: a. Gram character----2 marks b. Shape-----2 marks c. Arrangement---2 marks d. Name----2 marks

	e. Diagram----2 marks. Total: 10 marks.
FAQs	State the gram character and morphology of given organism?
Assignment/Activity	Draw diagrams of given organisms focused under the microscope stating their gram character and morphology.
Reference	**Refer: theory topic:12-** Handbook of Microbiology

Week No.	23nd week
Practical No.	23
Title/ Aim	Bacteriological examination. Bacteriological examination of Air,Water,Milk.
Objectives	Student shall be able to perform bacteriological examination of Air, water,Milk.
Requirements	1. Water sample,

	2. Milk sample 3. sterile water, 4. nutrient agar, 5. MacConkey agar 6. Incubator
Environment	MLT laboratory
Procedure	**Air Examination** • Place agar plate (containing selective or non-selective agar, depending on organism of interest) on a flat surface in the test location, and remove the lid. • Leave the agar exposed for the agreed period of time (this may vary depending on the likely level of contamination in the test environment. But time periods of at least 30 minutes, and up to 4 hours, are usually recommended). Monitor the exposure time with a timer. • Replace the lid; place the plates in an incubator overnight. • Count the number of colonies. • Perform biochemical tests to determine the organism. **Water examination** • The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000, etc.) in sterile water and cultivating

	these on nutrient agar in a dish that is sealed and incubated. • MacConkey agar is used to count Gram-negative bacteria such as *E. coli*. • One set of plates is incubated at 22°C for 24 hours and a second set at 37°C for 24 hours. • The composition of the nutrient usually includes reagents that resist the growth of non-target organisms and make the target organism easily identified, often by a color change in the medium • At the end of the incubation period the colonies are counted by eye, a procedure that does not require a microscope as the colonies are typically a few mm **Milk examination** • Serial dilution of milk samples(1:10, 1:100, 1:1000, etc.) in sterile water is carried out to obtain the different dilutions. • These milk dilutions are further transferred into sterile nutrient agar Petri plates and distributed uniformly. • Nutrient agar plates are incubated for 24 h at 37deg.C. • Bacterial colonies are observed and counted after incubation and the count is multiplied by dilution factor.

Observations	Colonies on nutrient agar and macConkey agar.
Result	The given air,water,milk sample contains bacteria.
Clinical significance	It is a method of analyzing air,water,milk to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are
Skills to be achieved	Air,Water,Milk analysis technique.
Skill evaluation criteria	1. Dilution technique---4 marks 2. Plating technique----4 marks 3. Observation -----2 marks. Total: 10 marks.
FAQs	How you will do bacteriological examination of given air, water,milk sample?
Assignment/Activity	Analyse 4 to 5 air,water,milk sample for their bacterial content.
Reference	-

Week No.	24th week
Practical No.	24
Title/ Aim	To do Antibiotic Sensitivity Test**(AST)**
Objectives	Student shall be able to perform antibiotic sensitivity test.
Principle	Whatman no. 2 filter paper circular disks (6 mm) impregnated with known concentration of antibiotics are placed on an Agar plate which is inoculated with a culture of the bacteria under test. The plate is incubated at 37°C for 24 hrs. During incubation, the antibiotics diffuse through the Agar. Susceptibility effectiveness is proportional to the diameter of the inhibition zone around the disc. Organisms which row up

	to the edge of the disc are resistant.
Requirements	• Nutrient broth • Muller Hinton Agar plates • Sterilized cotton swabs • Commercially available antibiotic discs
Environment	MLT laboratory
Procedure	1. Subculture bacteria from the isolated colonies in 1 ml of nutrient broth for 2-4 hrs.at 37^{o}C. This subculture is used as inoculums for the antibiotic disc diffusion plate. 2. Divide the plate into sections according to the number of antibiotics. 3. Inoculate properly by using a sterile swab so as to obtain uniform distribution of the inoculums. 4. Place commercially available antibiotics discs on the inoculated plates using sterile forceps. 5. Incubate the plates overnight at 37^{o}C 6. Measure the diameter of the zone of inhibition of growth in mm. 7. Report the result as follows:
Observations	

Figure 23.1

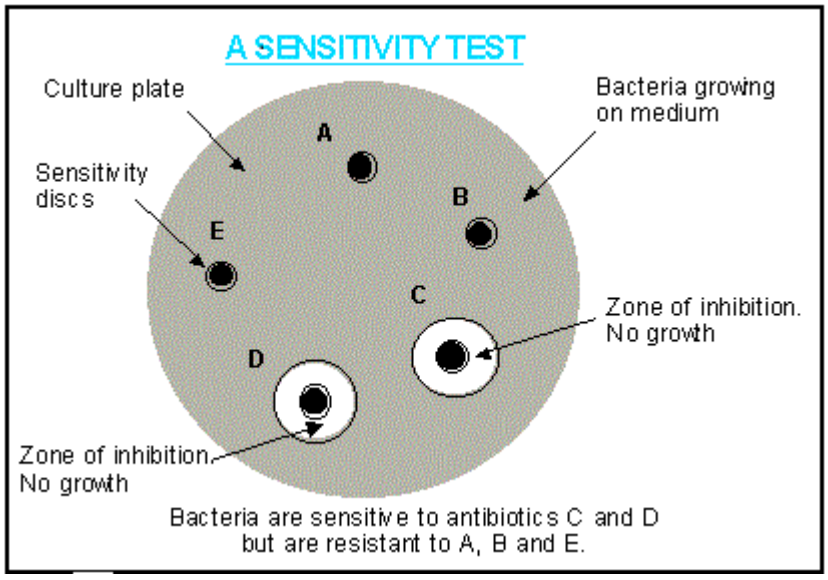


Figure 43.2 Antibiotic Sensitivity Test

Result

Observations	Reports
Zone less than 4 mm	Resistant
Zone 4-12 mm	Intermediate
Zone more than 12 mm	Sensitive

Clinical significance	Anti biotic sensitivity test is mainly useful if usually effective antibiotics fail to produce the desired effect. In the treatment and control of

	infectious diseases which are caused by pathogens that are drug resistant, sensitivity testing is helpful in selecting effective antimicrobial drugs. These tests measure antimicrobial activity against bacteria under laboratory conditions and not in the patient.
Skills to be achieved	Skillfully perform AST
Skill evaluation criteria	1. Plating technique---2 marks 2. Aseptic condition----2 marks 3. Selection of disc---1 mark.. 4. Placing the disc on the agar plate----2 marks 5. Zone measurement------2 marks. 6. Sensitivity reporting----1 mark Total: 10 marks.
FAQ	How you will perform AST for given organism?
Assignment/Activity	Do AST of 3-4 organisms.
Reference	**(Ref. Theory topic17)-** Handbook of Microbiology

Week No.	25th week
Practical No.	25
Title/ Aim	Fungal examination by wet mount.
Objectives	Students shall be able to stain the fungal preparation by lacto phenol cotton blue
Principle	Lactic acid preserves the fungal structure and clears the tissue, phenol acts as a disinfectant.
Requirements	• Lacto phenol cotton blue • Fungal specimen
Environment	MLT laboratory
Procedure	1. Place a drop of lacto phenol cotton blue on a clean slide. 2. Place fungal specimen on the drop and tease gently. 3. Place a coverslip on the mount and press down gently. 4. Examine under low power first and then under high power
Observations	
Observations	

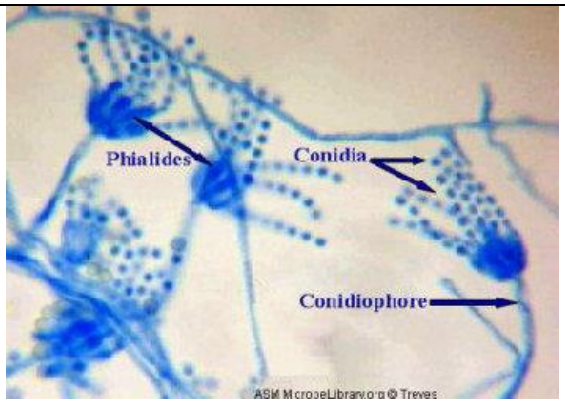
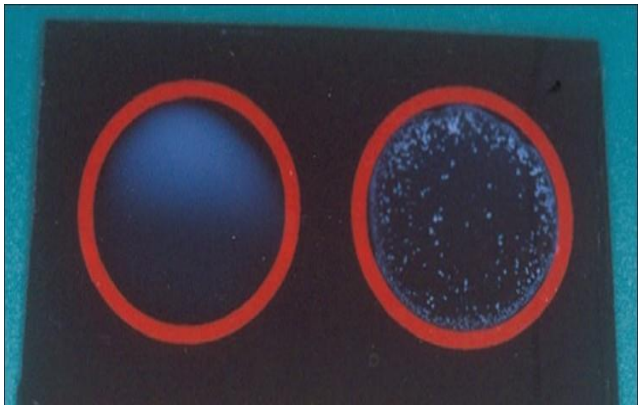


Figure 25.1 Aspergillus, Stained with Lacto Phenol Cotton Blue

Result	Fungal material appears pale to dark blue.
Clinical significance	Fungal infection.
Skills to be achieved	Skillfully prepare wet mount of fungal specimen and observe under the microscope.
Skill evaluation criteria	1. Preparing wet mount with no air bubbles----4 marks. 2. Proper teasing-----2 marks. 3. Focusing----2 marks. 4. Identification----2 marks. Total:10 marks.
FAQs	How you will prepare wet mount of given fungal specimen
Assignment/Activity	prepare wet mount of 4-5 fungal specimen
Reference	-

Week No.	26th week
Practical No.	26 -
Title/ Aim	**Serology** **RAtest (**Rheumatoid Arthritis test)
Objectives	The student shall be able to perform R.A. test accurately.
Principle	Small latex particles are coated with specially treated IgG. When serum containing Rheumatoid Factor is mixed with the IgG-coated latex particles, the rheumatoid factors gets bond to the IgG and cause agglutination.
Requirements	1. Serum sample. 2. Kit contains:- a. RF Antigen b. Positive control c. Negative control d. Glass slide with reaction circles e. Rubber teats.
Environment	MLT laboratory
Procedure	**<u>Qualitative methods</u>**

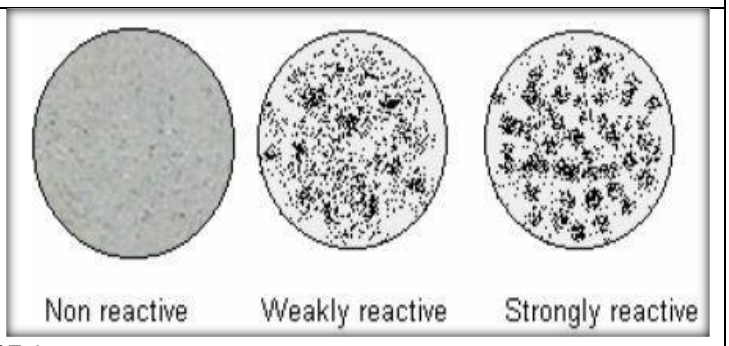
	1. Pipette one drop of serum on to the glass slide using the disposable pipette provided with the kit. 2. Add one drop of RHELAX RF latex reagents to the drops of serum on the slide. Do not let the dropper tip touch the liquid on slide. 3. Using a mixing sticks mix the serum and the RHELAX RF factor reagent uniformly over entire circles. 4. Immediately start a stop watch rock the slide gently back and forth, observing for agglutination macroscopically at 2mins. **<u>Semi Quantitative methods</u>:** 1. Using isotonic solution prepare serials dilutions of the serum sample positive in the quantitative method 1:2, 1:4, 1:8, 1:32, 1:64, and so on. 2. Pipette each dilution of serum on to the separate reaction circles. 3. Add one drop of RHELAX RF latex reagents to each drop of a diluted serum sample on the slide. Do not let the dropper tip touch the liquid on the slide. 4. Using a mixing sticks, mix the sample and the latex reagents uniformly over the entire circles.

	5. Immediately start a stopwatch. Rock the slide gently back and forth, observing for agglutination macroscopically at 2mins.
Observations	 **Figure 26.1RA test**
Result	**<u>Interpretation of Test Result</u>:** **<u>Qualitative method</u>:** • Agglutination is a positive test result and indicates the pleasance of rheumatoid factors in the test specimen. • No agglutination is a negative tests result and indicates the absence rheumatoid factors in the test specimen.
Clinical significance	Helps in the detection of Rheumatoid arthritis.
Skills to be achieved	Skillfully perform RA test
Skill evaluation criteria	1. Dilution technique--4 marks 2. Addition of correct reagents in proper sequence---3 marks. 3. Addition of correct samples to correct circle---2 marks. 4. Observation ---1 mark. Total : 10 marks.

FAQs	1. How you will do qualitative RAtest? 2. How you will do quantitative RA test?
Assignment/Activity	Perform RA test of 3-4 samples.
Reference	Theory chapter20- Handbook of Microbiology

Week No.	27th week
Practical No.	27
Title/ Aim	To do **VDRL** test
Objectives	Student shall be able to perform VDRL test in the laboratory.
Principle	Complement present in serum interferes with the Flocculation reaction. It is inactivated by keeping the serum at 56ºC. Antibodies of Syphilis react with the VDRL antigen (particles of lipid coated with cardiolipin) and forms floccules.
Requirements	1. Fasting serum specimen 2. VDRL plate 3. Commercially available VDRL kit containing • VDRL Carbon Antigen Suspension, • Positive Control Serum, • Negative Control Serum, • Test Cards, • Mixing Sticks. • Buffered saline • Normal Saline Note: Prepare a working VDRL antigen fresh before the test. Follow manufacturer's instructions.
Environment	MLT laboratory

Procedure	Method 1:slide test **Procedure-** • Keep 0.5 ml of serum in a water bath at 56^{o}C for inactivation for 30 minutes. • Cool to room temperature. • Add about 0.05 ml inactivated serum in the cavity of VDRL plate. • Add one drop of working VDRL antigen to the cavity containing serum. • Rotate the plate on the rotor immediately for four minutes or by hand ten times in five seconds. • Examine visually in bright light and confirm by observing under low power objective. Process one negative and one positive control serum in same manner. **<u>Method 2:Tube test</u>** **Procedure-** • Label test tubes 1 to 9. • Prepare serial dilution of inactivated serum. o Pipette o.1 ml of serum in tube no.1. o Add 0.1 ml of normal saline and mix (dilution 1:2) o Transfer 0.1 ml of dilution 1:2 to tube no.2 add 0.1 ml of saline and mix (dilution 1:4) o Similarly prepare other dilutions (i.e. 1:8,

	1:16, 1:32, 1:64, 1:128, 1:256) • Add 0.5 ml of each dilution to the numbered cavities of VDRL slide. • Add one drop of freshly prepared VDRL antigen to the diluted serum on the VDRL slide. • Rotate the plate for 4 min. • Observe the floccules
Observations	 fig:27.1

Result

- Report as follows:

Test Report	**Observation**
VDRL test Non-Reactive	No clumps.
VDRL test Weakly Reactive	Small clumps with free particles.
VDRL test Reactive	Medium and large clumps on a clear background.
Tube method ➢ **Report as follows:**	

Tube no.	1	2	3	4	5	6	7	8
Dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Reaction	R	R	R	R	R	WR	NR	NR

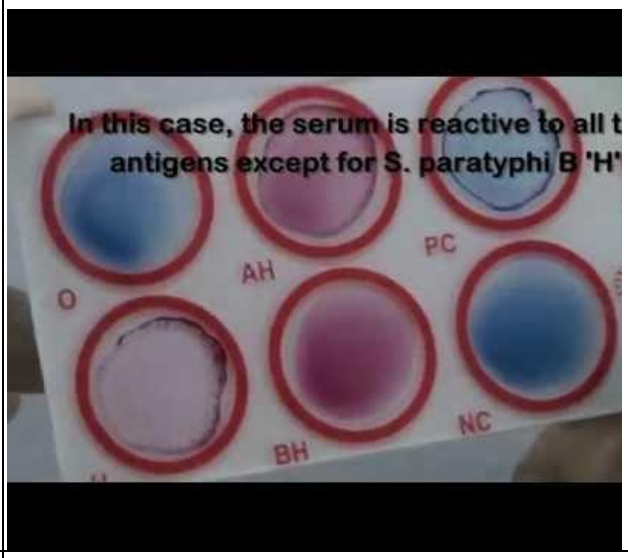
KEY**: R=Reactive, WR= weakly reactive, NR= nonreactive**

Note**: In case of weakly reactive serum, use dilution up to 1:32.**

Clinical significance	VDRL test includes determination of serological response to Treponema infection Syphilis
Skills to be achieved	Skillfully perform VDRL test
Skill evaluation criteria	1. Dilution technique--4 marks 2. Addition of correct reagents in proper sequence---3 marks. 3. Addition of correct samples to correct circle---2 marks. 4. Observation ---1 mark. Total: 10 marks.
FAQs	How you will do VDRL test Qualitative and Quantitative?
Assignment/Activity	Do VDRL test of 10 samples
Reference	Theory chapter20- Handbook of Microbiology

Week No.	28th week
Practical No.	28
Title/ Aim	To do Widal test- slide method

Objectives	The student must be able to perform Widal test accurately and independently.
Principle	Antibodies found in patients serum in response to exposure to Salmonella organisms agglutinate a bacterial suspension of Salmonella (non infective), which carries homologous antigens.
Requirements	1. Specimen : Serum (fresh) 2. Commercial kits containing four specific antigen suspensions:- • Salmonella typhi 'O' • Salmonella typhi 'H' • Salmonella paratyphi 'AH' • Salmonella paratyphi 'BH' 3. Slides with ceramic rings. 4. Droppers 5. Applicator sticks.
Environment	MLT laboratory
Procedure	Two agglutination methods are available. Slide method **Slide method** (for screening purposes) 1. Mark the circles on the glass plate as O, H, AH, BH. 2. Add one drop of serum to each of the circles. 3. Add corresponding antigen one drop to the marked circles. 4. Mix antigen and serum of each circle using

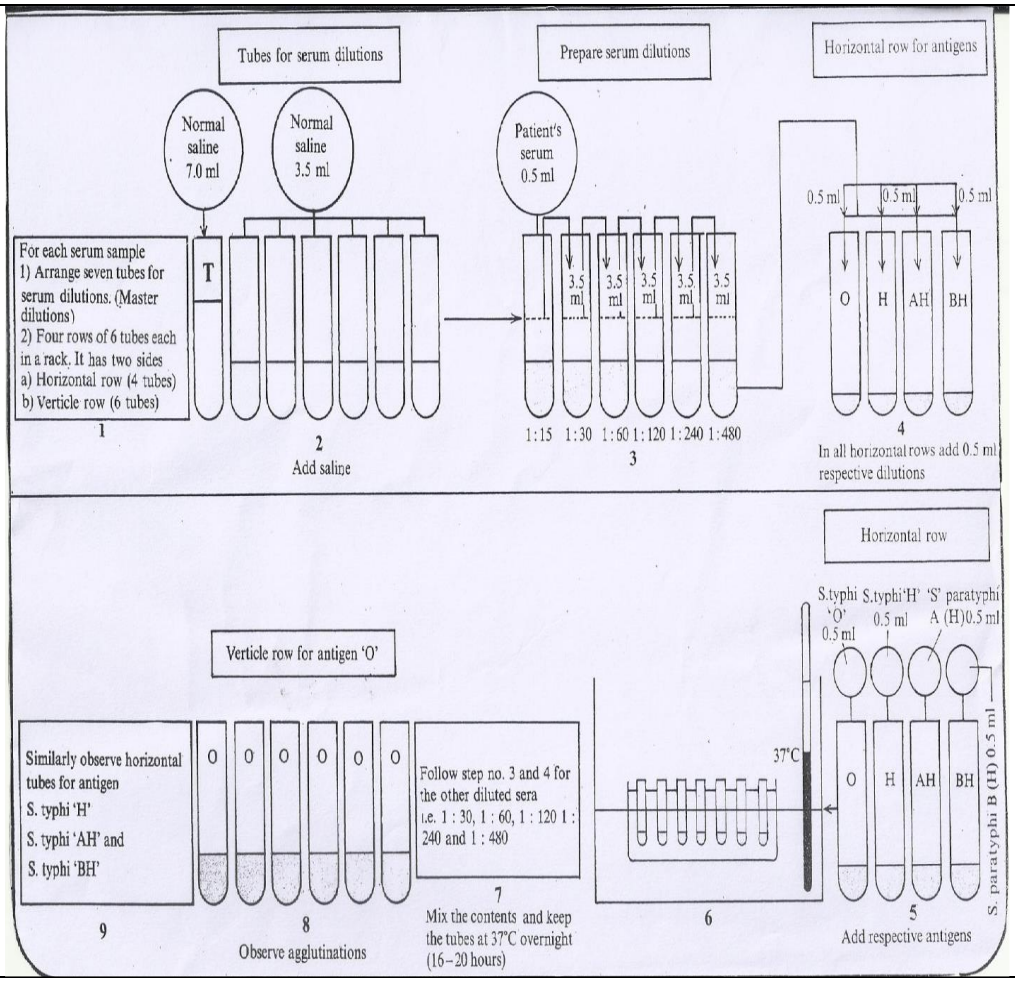
	separate applicator sticks. 5. Slowly rock and tilt the glass plate for 3mins and observe for agglutination. **Note:** A slight change in procedure may exist depending on the kit manufactures instructions.
Observations	**figure 28.1 Widal test - slide method** 
Result	Agglutination seen / not seen in each circle.
Clinical significance	Diagnosis of typhoid and paratyphoid
Skills achieved	Performing Widal test by slide method accurately
Skill evaluation criteria	1. Dilution technique--4 marks 2. Addition of correct reagents in proper sequence---3 marks. 3. Addition of correct samples to correct circle---2 marks. 4. Observation ---1 mark. Total : 10 marks.
FAQs	1. Do Widal test by slide method of given specimen and report

	2. Name the antigens which you will use for the test?
Assignment/Activity	Do Widal test by slide method of given 4 -5 specimen.
Reference	Theory chapter 20- Handbook of Microbiology.

Week No.	29th week
Practical No.	29
Title/ Aim	To do Widal test by tube method.
Objectives	The student must be able to perform Widal test accurately and independently.
Principle	Antibodies found in patients serum in response to exposure to Salmonella

	organisms agglutinate a bacterial suspension of Salmonella (non infective), which carries homologous antigens.
Requirements	1. Specimen: Serum (fresh) 2. Commercial kits containing • 5 test tubes • 36 Widal tubes 3. Widal rack 4. Serological pipettes 0.1ml and 1.0 ml. 5. Serological water bath / incubator 6. Normal saline (NS)
Environment	MLT laboratory
Procedure	**For one specimen** **Procedure:** 1. Take 5 test tubes in a rack & add 7 ml normal saline to 1st tube. 2. Add 3.5 ml NS to the remaining test tubes. 3. Now add 0.5 ml of serum to 1st tube only mix. 4. Now add 3.5 ml of mixture from 1st tube to 2nd tube mix. 5. Continue the transfer similarly till into last tube. 6. Now arrange 4 rows of 6 widal tubes each in widal rack. 7. Label four rows as ‘O’, ‘H’, ‘AH’, & ‘BH’.

	8. Now transfer 0.5ml from the master dilution tubes to each of the corresponding vertical four &widal tubes. 9. Add 0.5ml of NS to the 6th widal tube of each row this will be your control of 4 antigens. 10. Add 0.5ml of antigen e.g. 'O' to each of the 6 widal tubes in the first row. 11. Add similarly other antigen to the corresponding 6 widal tubes in the row. 12. Mix well each tube and keep at 37°C for 20-24 hrs. 13. Observe for agglutination & report the titre The dilutions of the tubes are 1:30, 1:60, 1:120, 1:240, & 1:480
Observations	



Result

Report:

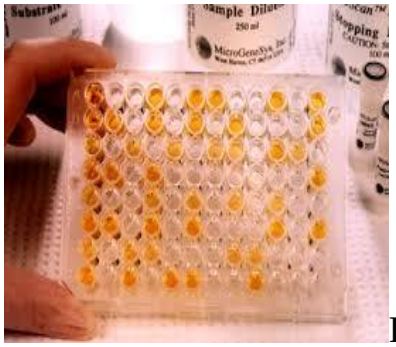
Dilution	Antigen			
	'O'	'H'	'AH'	'BH'
1:30				
1:60				
1:120				
1:240				
1:480				

Positive result should be correlated with clinical findings and precious

history immunization.	
Clinical significance	Diagnosis of typhoid and paratyphoid
Skills achieved	Performing Widal test by tube method accurately
Skill evaluation criteria	1. Dilution technique--4 marks 2. Addition of correct reagents in proper sequence---3 marks. 3. Addition of correct samples to correct circle---2 marks. 4. Observation ---1 mark. Total: 10 marks.
FAQs	Do Widal test by slide method of given specimen and report. Name the antigens which you will use for the test?
Assignment/Activity	Do Widal test by slide method of given 4 -5 specimen
Reference	Theory chapter 20- Handbook of Microbiology

Week No.	30th week
Practical No.	30

Title/ Aim	ELISA Test (Enzyme-linked Immunosorbent Assay**)**
Objectives	The student shall be able to do ELISA test for detection of HBsAg / HIV antibody
Principle	HIV antigen is fixed on the surface of a test well. Patients serum (if anti HIV is present) will attach to the HIV antigen, after washing, an enzyme conjugate reagent is added and re-incubated. The enzyme conjugate antihuman IgG attaches to the HIV antigen-antibody complex. After washing, a substrate – chromogen reagent is added. This is acted on the enzyme and a colour is produced. A stop reagent stops the reaction. The color is read spectrophometrically
Requirements	1. Patients serum 2. Spectrophotometer 3. HIV – ELISA Kit containing • Control serum • Diluents buffer • Enzyme conjugates, etc. • Substrate – chromogen • Stop solution • Wash solution
Environment	MLT laboratory
Procedure	**Sample example of a procedure** **Procedure**:

	1. Add indicated amount of diluent of the micro plate well. 2. Add serum sample and controls to the separate wells (amount as specified in kit procedure) 3. Shake and incubate at 37°C for 1 hr. 4. Wash with wash solution. 5. Add enzyme conjugate, and incubate at 37°C for say 30mins. 6. Wash with wash solution. 7. Add substrate – chromogen reagent. 8. Incubate at 37°C for say 20mins. 9. Stop reagents is added to stop the reaction. 10. Color produced in test and control wells is measured spectrophotometrically.
Observations	 Fig:30.1 **ELISA Test**
Result	The given specimen is positive/ negative for ELISA test.
Clinical significance	ELISA test for detection of HIV antibody.
Skills to be achieved	Performing HIVtest by ELISA method accurately.

Skill evaluation criteria	1. Stepwise addition of correct reagentsspecimen to the respective test wells---6 marks. 2. Observing colour produced---2marks. 3. Difference between negative and positive-2marks.
FAQs	1. Do HIVtest by ELISA method of given specimen and report 2. Name the reagentsstepwise which you will use for the test?
Assignment/Activity	Do HIV test by ELISAmethod of given 4 -5 specimen.
Reference	Theory chapter 20- Handbook of Microbiology

Week No.	31st week
Practical No.	31
Title/ Aim	TORCH ELISA Test
Objectives	The student shall be able to know about the TORCH ELISA test
Principle	Same as ELISA method provided in the kit

Requirements	Sample to be tested, reagents supplied in the kit
Environment	MLT laboratory/hospital visit.
Procedure	**1. Toxoplasma gondii (toxoplasmosis)** A parasite that can be acquired from ingesting cysts from the feces of infected cats, drinking unpasteurized milk, or eating undercookedcontaminated meat. Infection early in pregnancy can cause miscarriage.Later in pregnancy it can cause eye infections, and mental retardation. The detection of IgG/ IgM specific antibodies to T.gondii is particularlyhelpful for diagnosis of acute or primary infections. The determination isdone by using diagnostic kit of ELISA. **2. Rubella (German Measles)** Infection early in pregnancy can cause birth defects such as heart disease, growthRetardation and eye defect. The absence of Rubella specific IgG antibodies in sera of long term duration after primary infections inpresence of virus specific IgM antibodies is indicative of risk of defects in newborn infants. This test provides the clinician a help for monitoring of the immunological response upon vaccination. The test is carried out using

	diagnostic kit of ELISA for Rubella virus. **3. Cytomegalo virus (CMV)** This virus is transmitted through body secretions (including breast milk) as well as sexual contact. Infection can cause death, hearing loss and mental retardation. The detection of virus specific IgM antibodies is of great value in the diagnosis of acute/primary virus infections in the absence of typical clinical symptoms. Asymptomatic infections usually happen for Cytomegalo virus in healthy individuals during pregnancy. The test is carried out using diagnostic kit of ELISA for Cytomegalo virus. **4. Herpes simplex virus (HSV)** It is a common infection that is spread by oral and genital contact. The detection of virus specific IgM antibodies is of great value in the diagnosis of acute/primary virus infections in the absence of typical clinical symptoms. Asymptomatic infections usually happen for Herpes II in healthy individuals during pregnancy. The test provides the clinician a help for monitoring of risk population and for the follow-up of primary infections. The test is carried out using diagnostic kit of ELISA for Herpes II.

Observations	 **Figure 31.1** **TORCH Test Kit**
Result	The given specimen is positive/negative
Clinical significance	ELISA test performed for TORCH panel are mainly to detect the presence or absence of specific antibodies in serum for following organisms: A. Toxoplasma gondii (TO) B. Rubella (R) C. Cytomegalo virus (C) D. Herpes II (H) TORCH infections are very common in adult population. Pregnant woman are exposed to these infections. Fatal infection usually occurs by the trans placental root after invasion of the mother's bloodstream
Skills to be achieved	Performing TORCH test by ELISA method accurately
Skill evaluation criteria	1. Knowledge of different tests----4 marks. 2. Use of test-----4 marks. 3. Observation of tests----2 marks.

	Total:10 marks.
FAQs	How you will do TORCH test by ELISA method of given specimen?
Assignment/Activity	Prepare a PPT of procedure manual of different manufacturer's kits available in market.
Reference	Theory chapter 20- Handbook of Microbiology

Week No.	32nd week
Practical No.	32
Title/ Aim	**Immunological Pregnancy Test**
Objectives	The student shall be able to do pregnancy test accurately.
Principle	Monoclonal antibodies to HCG have been produced. These have been attached to enzymes which can later interact with a dye molecule and produce a color change.

Requirements	**Pregnancy test kit** **1.** Pregnancy test kit contains three regions – reaction region, test region and control region • Reaction region: contains monoclonal anti-HCG antibodies linked to enzyme. • Test region: contains polyclonal anti-HCG antibodies, which will bind to HCG molecules bound to monoclonal anti-HCG antibodies. Also contains dye molecules which will be activated if monoclonal antibodies bind to polyclonal anti-HCG antibodies. • Control region: contains anti-mouse antibodies and dye molecules which will be activated if monoclonal antibodies bind here.
Environment	MLT laboratory
Procedure	**1.** When pregnant woman's urine travels up the pregnancy test, HCG will bind to monoclonal antibodies in reaction region. 2. Movement of the urine will move the monoclonal antibodies up to the test region 3. Monoclonal antibodies with bound HCG will bind to antibodies in test region and activate dye molecules, producing a color change

	4. Any unbound monoclonal antibodies will continue to travel to control region and will bind to anti-mouse antibodies, activating dye molecules and producing a color change.

Observations

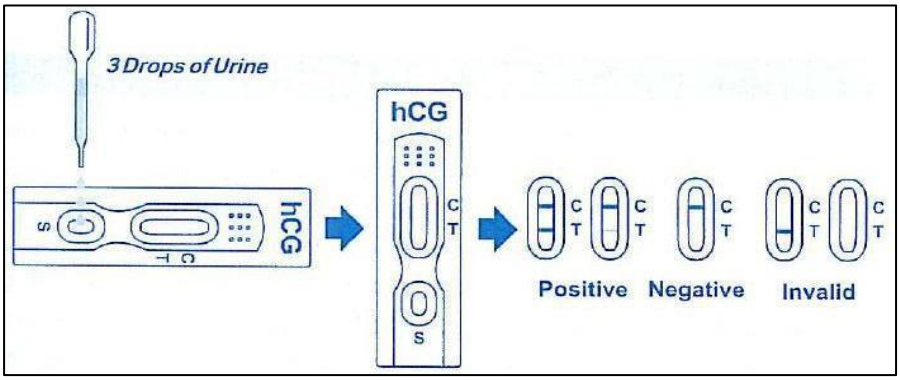


Figure32.1 Pregnancy test results

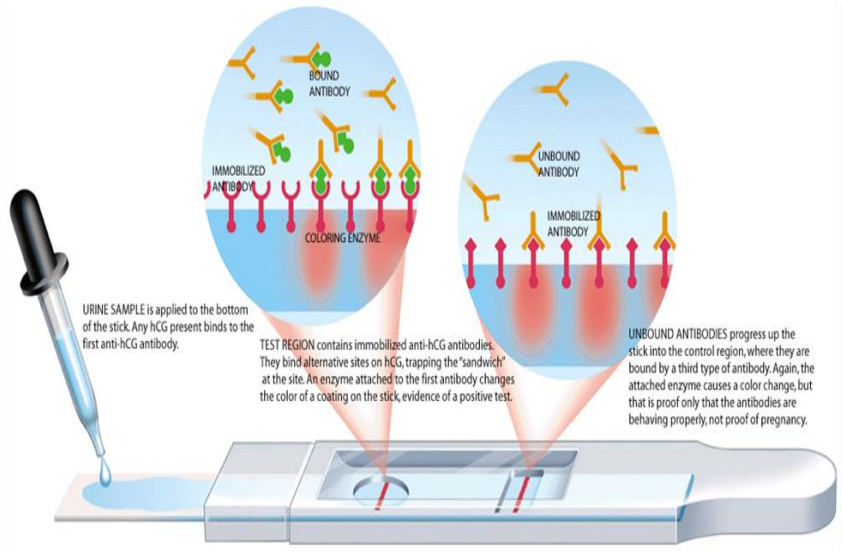


Figure 32.2 Pregnancy Tests – Mechanism

Result	The given urine specimen is positive/negative for pregnancy test.
Clinical significance	A pregnant woman has the hormone human chorionic gonadotrophin (HCG) in her urine

	which is tested and accordingly pregnancy report submitted.
Skills to be achieved	Performing pregnancy test of urine sample accurately.
Skill evaluation criteria	1.Patient instructions---2 marks 2.Addition of correct reagents---4 marks 3.Sample addition----2 marks 4. Correct reporting----- 2 marks. Total: 10 marks.
FAQs	Do pregnancy test of urine sample and report
Assignment/Activity	Do pregnancy test of urine sample (4 -5 specimen) and report
Reference	Theory chapter 20- Handbook of Microbiology

Week No.	33rdWeek
Practical No.	33
Title/ Aim	**C-reactive protein test**
Objectives	Student shall be able to perform C-reactive protein test in the laboratory.
Principle	Latex particles coated with anti- CRP, agglutinate in the presence of CRP.
Requirements	• Latex CRP kit • Normal saline • Serological pipettes 0.1ml and 1.0 ml.
Environment	MLT laboratory
Procedure	**1.** Dilute patient serum 1:5 with normal saline. **2.** Place 1 drop of dil. Serum on the test slide.

<table>
<tr><td></td><td>3. Place 1 drop of positive and negative control also in the respective zone of the test slide.
4. Add one drop of reagent to each zone.
5. Mix well and observe for agglutinations.</td></tr>
<tr><td>Result</td><td>Marked agglutinations generally indicate presence of CRP concentration above 6 mg/l
<table>
<tr><th>Serum dilution</th><th>CRP mg/l</th></tr>
<tr><td>1:10</td><td>12</td></tr>
<tr><td>1:20</td><td>24</td></tr>
<tr><td>1:30</td><td>48</td></tr>
<tr><td>1:40</td><td>96</td></tr>
</table></td></tr>
<tr><td>Clinical significance</td><td>The C-reactive protein is normal alpha globulin and it is elevated in patients who have an inflammatory condition of infectious or non-infectious origin. The results are simple index of the disease activity and treatment status.</td></tr>
<tr><td>Skills to be achieved</td><td>Performing C-reactive protein test in laboratory.</td></tr>
<tr><td>Skill evaluation criteria</td><td>• Proper dilution----4 marks.
• Use of test-----4 marks.
• Observation of tests----2 marks.
Total:10 marks.</td></tr>
<tr><td>FAQs</td><td>1. Perform C-reactive protein test of given specimen and report
2. Name the reagents stepwise which you will use for the test?</td></tr>
<tr><td>Assignment/Activity</td><td>Perform C-reactive protein testof given 4 -5 specimen.</td></tr>
<tr><td>Reference</td><td>Theory topic -Handbook of Microbiology</td></tr>
</table>