Semester – V

19MBU511A **(4H – 2C)**

MANAGEMENT OF HUMAN MICROBIAL DISEASES - PRACTICAL

Instruction Hours / week: L: 0 T: 0 P: 4 **Marks:** Internal: **40**External: **60** Total: **100**

End Semester Exam: 9 Hours

COURSE OBJECTIVES

- To provide a strong base in the fundamentals of pathogens.
- To learn techniques and methods used in the cultivation and isolation of pathogens.
- To obtain with the knowledge about the habitat and characteristics of pathogens.

COURSE OUTCOME

Involves the identification, classification, and characterization of pathogenic species.

EXPERIMENTS

1. Diagnosis of respiratory tract disease.
2. Diagnosis of urinary tract disease.
3. Diagnosis of gastrointestinal tract disease.
4. Identification of dermatophytes

SUGGESTED READINGS

1. Ananthanarayan R. and Paniker C.K.J. (2009) Textbook of Microbiology. 8th edition, University Press Publication
2. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013) Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication.
3. Goering R., Dockrell H., Zuckerman M. and Wakelin D. (2007) Mims' Medical Microbiology. 4th edition. Elsevier.
4. Willey JM, Sherwood LM, and Woolverton CJ. (2013) Prescott, Harley and Klein's Microbiology. 9th edition. McGraw Hill Higher Education.
5. Madigan MT, Martinko JM, Dunlap PV and Clark DP. (2014). Brock Biology of Microorganisms. 14th edition. Pearson International Edition.

Experiment No: 1
DIAGNOSIS OF RESPIRATORY TRACT DISEASE

INTRODUCTION: The respiratory tract is far more prone to infection than other organ systems, as it frequently comes into contact with numerous pathogens present throughout the environment. Infections of the respiratory tract are caused by a wide range of viruses and bacteria that may vary seasonally and can impact the upper and lower respiratory tract, affecting different patient populations with varying degrees of severity.

AIM: To diagnose the received specimen and to isolate and to identify the organism suspected for respiratory tract disease.

SAMPLE: Sputum is the most usual specimen. Apart from it bronchial washings, biopsies, gastric aspirate, CSF, pleural fluids were also collected in certain cases.
Sputum is a mixture of bronchial secretion and inflammatory exudates coughed out and expedorated in some infections. During bacterial infection sputum will be purulent and contain yellow, green and opaque materials. Usually disposable wide mouthed plastic container of about 100 ml capacity is used to collect specimen.

SPECIMEN PROCESSING
Homogenization: The specimen is usually processed in 2 ways:
1. By means of adding sterile glass beads.
2. Equal volume of 1% buffered pancreatin was added and shaken well and was placed in water bath for an hour. The suspension was shaken well every 15 minutes and this helps in the uniform distribution of the specimen.

PROCEDURE
Microscopy
Gram staining: Make the smear and perform the gram staining to see the morphology.

Acid fast staining (or) Ziehl Nelson staining: The heat fixed smear of specimen was flooded with carbolfuchsin and heated. Then the smear was washed with water and decolorized with acid alcohol (20% H_2So_4 and then with 95% ethanol) for 2 minutes. The smear was then counterstained with Loeffelers methylene blue and then observed under oil immersion.

Culture method
Lowenstein Jenson's medium: Inoculate the specimen onto two bottles of LJ medium and incubated at 37°c and inspected for growth of typical colonies weekly once.

Blood agar: Prepare Blood agar plates and streak the given specimen on the surface of the medium and incubate at 37 ℃ for 24 h.

Nutrient agar: Prepare the nutrient agar plates and streak the given specimen on the surface and incubate at 37 °C for 24 h.

Macconkey agar: Prepare Macconkey agar plates and streak the given specimen on the surface and incubate at 37 °C for 24 h.

Mannitol salt agar: Prepare Mannitol salt agar plates and streak the given specimen on the surface and incubate at 37 °C for 24 h.

Biochemical characterisitics
Catalase test
Media-Nutrient agar
Culture – 24 h old culture
Reagent - H_2O_2 (3%)

Coagulase test
Tube coagulase: It is used to detect free coagulase. Add 0.4 ml of (1.5 dilution) plasma to 0.1 ml of overnight broth and incubate for 3-6 h and observe the tubes for coagulation.

Slide coagulase: It is used to detect bound coagulase. Place 2 drops of saline onto slide. Take a loopful of inoculum and introduce and following which add 2 drops of undiluted plasma. Then observe for coagulation.

Gelatin liquefaction test: Prepare Nutrient Gelatin media and inoculate with the given specimen. Incubate the plates at 37°C for 24 h.

INTERPRETATION OF RESULTS
Microscopy
Gram staining:

Acid fast staining (or) Ziehl Nelson staining:

Culture method
Lowenstein Jenson's medium:

Blood agar:

Nutrient agar:

Macconkey agar:

Mannitol salt agar:

Biochemical characteristics
Catalase test:

Coagulase test:

Tube coagulase:

Slide coagulase:

Gelatin liquefaction test:

Based on the observation of the microscopy, cultural and biochemical characteristics, the possible organism in the specimen may be interpreted as _____.

Experiment No. 2
DIAGNOSIS OF URINARY TRACT DISEASE

INTRODUCTION: Urinary tract infections occur more frequently in women than men due to shortness of female urethra. *E.coli* is the principle urinary tract pathogen. Other less common cause of UTI is *Staphylococci, Enterococci, Proteus, Klebsiella and other Coliforms and Pseudomonas aeroginosa*.

Most infections originate from the patients bowel flora (endogenous infection). Infections are usually caused by the entry of microorganisms from external sources. Microorganisms at the opening of the urethra, careless personal hygiene and sexual intercourse contribute to the incidence of UTI. Opportunistic organisms present in intestine can also cause the infection.

AIM: To diagnose the specimen and to isolate and to identify the organism suspected for urinary tract disease.

SPECIMEN COLLECTION: Specimen should be collected carefully without any urethral contamination.

Clean catch midstream urine: The first portion of the urine which is more likely to be contaminated must be avoided as the bacteria will have multiplied to high levels after overnight incubation in the bladder. Clean catch of midstream urine was collected in sterile wide mouth screw cap tubes.

PROCEDURE

Microscopic examination

Wet mount method: Place a drop of fresh uncentrifuged urine on slide and cover with cover glass and examine under light microscope.

Gram staining and Motility test: Place Gram stained smear of urine on slide and cover with cover glass and examine under light microscope. Carry out the Hanging drop technique using the specimen to check the motility.

Culture methods: Dilute the urine specimen and streak on nutrient agar, macconkey agar, mannitol salt agar, cystine–lactose–electrolyte-deficient agar (CLED) plates and incubate the agar plates at 37 °C for 24 h.

Biochemical characteristics

Indole test

Media- peptone broth
Reagent – 0.2 ml of Kovac's reagent
Incubation time & temperature – 37 °C for 24 h

Methyl red test
Media- MRVP broth
Reagent – methyl red
Incubation time & temperature – 37 ℃ for 48 h.

Voges-Proskauer test
Media- MRVP broth
Reagent – Barritt's reagent
Incubation time & temperature – 37 ℃ for 48 h.

Citrate test
Media- Simmon's citrate agar
Reagent – bromothymol blue
Incubation time & temperature – 37 ℃ for 48 h.

Carbohydrate fermentation test
Media- Peptone broth
Reagent - bromothymol blue
Sugars employed – 1 % (w/v) Glucose, Sucrose, Lactose, Mannitol
Incubation time & temperature – 37 ℃ for 24-48 h.

Urease test
Media-Nutrient agar
Reagent - Christensen's Urea Agar
Incubation time & temperature – 37 ℃ for 24 h.

Catalase test
Media-Nutrient agar
Reagent - H_2O_2 (3%)
Culture – 24 h old culture

INTERPRETATION OF RESULTS
Microscopic examination
Wet mount method:

Gram staining and Motility test:

Culture methods:

Biochemical characteristics

Indole test

Methyl red test

Voges-Proskauer test

Citrate test

Carbohydrate fermentation test

Urease test

Catalase test

Based on the observation of the microscopy, cultural and biochemical characteristics, the possible organism in the specimen may be interpreted as _____.

Experiment No. 3
DIAGNOSIS OF GASTROINTESTINAL TRACT DISEASE

INTRODUCTION: Gastrointestinal infections are viral, bacterial or parasitic infections that cause gastroenteritis, an inflammation of the gastrointestinal tract involving both the stomach and the small intestine. Symptoms include diarrhea, vomiting, and abdominal pain**.** Diarrhea is a condition where intestinal discharge is expelled in a liquid state which is known as the stool. Diarrhea is accompanied by discomfort, loss of water and electrolytes and it leads to severe dehydration. Pathogenic organisms such as *Vibrio cholera* causes to severe dehydration, *E. coli* causes travelers' diarrhea, some intestinal viruses and Rota viruses can also cause diarrhea.

AIM: To diagnose the specimen and to isolate and to identify the organism suspected for gastrointestinal tract disease.

SPECIMEN: Stool sample

COLLECTION AND TRANSPORT: The specimen was collected in a clean, dry, disinfectant free wide mouthed container without urine contamination. When fecal sample is not available rectal swab is preferable. It can transport using sterile Cary Blair medium.

PROCEDURE

Microscopic examination

Gram staining & Motility test: Make the smear and perform the gram staining to see the morphology. Carry out the Hanging drop technique using the specimen to check the motility.

Culture methods: Inoculate the specimen on various media like EMB agar, Macconkey agar, thiosulphate citrate bile salt media, xylose lysine deoxycholate agar, SS agar and incubate for 16-24 h at 37 °C.

Biochemical tests

Indole test

Media- peptone broth
Reagent – 0.2 ml of Kovac's reagent
Incubation time & temperature – 37 °C for 24 h

Methyl red test

Media- MRVP broth
Reagent – methyl red
Incubation time & temperature – 37 °C for 48 h.

Voges-Proskauer test

Media- MRVP broth
Reagent – Barritt's reagent

Incubation time & temperature – 37 ℃ for 48 h.

Citrate test
Media- Simmon's citrate agar
Reagent – bromothymol blue
Incubation time & temperature – 37 ℃ for 48 h.
Catalase test
Media-Nutrient agar
Reagent - H_2O_2 (3 %)
Culture – 24 h old culture

INTERPRETATION OF RESULTS

Microscopic examination

Gram staining & Motility test:

Culture methods

EMB agar:

MacConkey agar:

Thiosulphate citrate bile salt media:

Xylose lysine deoxycholate agar:

SS agar:

Biochemical tests
Indole test

Methyl red test

Voges-Proskauer test

Citrate test

Catalase test

Based on the observation of the microscopy, cultural and biochemical characteristics, the possible organism in the specimen may be interpreted as _____.

Experiment No. 4
IDENTIFICATION OF DERMATOPHYTES

INTRODUCTION: Dermatophytes are a unique group of closely related filamentous fungi that invade keratinized cutaneous structures, including the stratum corneum, nails, and hair, of humans and animals, resulting in an infection referred to as dermatophytosis, ringworm, or tinea. Diagnosis of dermatomycoses is based on the detection of septate hyphae by direct microscopic examination of clinical samples. Direct microscopic examination is rapid and inexpensive but does not provide genus or species identification. *Arthroderma* now contains 21 species, *Ctenomyces* one species, *Epidermophyton* one species, *Lophophyton* one species, *Microsporum* three species, *Nannizzia* nine species and *Trichophyton* 16 species. In addition, two new genera have been introduced: *Guarromyces* containing one species and *Paraphyton* three species. Although the number of genera has increased, the species that are relevant to routine diagnostics now belong to smaller groups, which should enhance their identification.

AIM: To identify the dermatophytes

SPECIMEN: Skin, hair and nail samples

COLLECTION AND TRANSPORT: Skin, hair and nail samples were collected from clinically suspected cases of dermatophytosis under aseptic conditions using 70 % alcohol into a sterile black paper.

PROCEDURE

KOH wet mount: The specimens collected were subjected to KOH wet mount preparation. 10 % KOH used for skin and hair samples and kept for 10-15 min and 40 % KOH used for nail samples, kept overnight. A small amount of sample is taken and added to the drop of KOH placed on a glass slide and covered with a glass slip and observed under microscope.

Culture: The specimen was inoculated onto Sabouraud's dextrose agar with 0.05 % Chloramphenicol and 0.5 % Cycloheximide and incubated at 28 °C for up to four weeks, and was observed periodically for growth and (reverse) pigmentation.

Tease mount by lactophenol cotton blue: A small portion of a colony was picked and suspended in two drops of lactophenol cotton blue placed on a clean slide. The mycelial mat was teased apart with dissecting needles, covered with cover-slip and observed under microscope for presence of aseptate slender hyphae, macro and microconidia and their arrangement.

Dermatophyte Test Media (DTM): All isolated dermatophytes were inoculated onto DTM and incubated at 28 °C for 7 days and observed for color change.

Urease test: All isolated dermatophytes were inoculated onto Christensen's Urea Agar and incubated at 28 °C for 7 days and observed for colour change. This test is to differentiate between *T. mentagrophytes* and *T. rubrum*.

INTERPRETATION OF RESULTS

KOH wet mount:

Culture:

Tease mount by lactophenol cotton blue:

Dermatophyte Test Media (DTM):

Urease test:

Based on the observation of the microscopy, cultural and biochemical characteristics, the possible organism in the specimen may be interpreted as _____.