Virginia Western Community College
MDL 237 Clinical Bacteriology

Prerequisites
Bio 101 or equivalent

Course Description
This course will introduce the student to the basic concepts of clinical microbiology with an emphasis on bacterial identification of clinically significant pathogenic bacteria and fungi. Topics covered include biochemical identification and molecular biology identification techniques of pathogenic bacteria. Proper specimen collection and plating of bacteria from human body sites will be emphasized also. Susceptibility testing and antibiotic resistance will also be discussed.

Semester Credits: 4 **Lecture Hours: 2** **Lab/Clinical/Internship Hours: 3**

Required Materials

Textbook:

1. Textbook of Diagnostic Microbiology 6th edition by C.R. Mahon, et al., ISBN: 978-0-323-08989-0 (Lecture)
2. Microbiology Fundamentals 2nd edition by S. Obenauf and S. Finazzo, ISBN: 978-1-259-29386-3 (Lab)

Supplementary Materials:

Course Outcomes

At the completion of this course, the student should be able to:

- Perform a Gram stain of samples containing bacteria to aid in a presumptive identification
- Perform other staining techniques (negative, acid-fast, endospore) of samples containing bacteria to aid in a presumptive identification
- Plate biological samples containing bacteria onto the proper media and be able to identify the purpose of various selective media
- Identify bacteria by their colony characteristics and growth on selective media
- Identify bacteria by using biochemical testing methods
- Describe and discuss molecular testing methods for bacterium identification such as PCR and DNA fingerprinting
- Understand the mechanism of action of common antibiotics and the basis of antibiotic resistance
- Describe and differentiate the characteristics of the various species of pathogenic bacteria including the staphylococci, streptococci, and enterococci, and be able to differentiate one species from another such as Staphylococcus aureus and epidermidis
- Learn proper isolation techniques for aerobic vs. anaerobic and facultative anaerobic bacteria

- Describe and discuss isolation and identification of pathogenic fungi and various non-bacterial pathogens such as *Mycoplasma* and *Ureaplasma*
- Understand the theory behind automated techniques of bacterial identification

Topical Description

MDL 237 Clinical Bacteriology Schedule

Unit	Topic	Chapter(s)	Objectives
I	Bacterial Cell Structure, Physiology, Metabolism, and Genetics	1	1. Identify and define the methods used by epidemiologists to subdivide bacterial species. 2. Differentiate the cell walls of gram-positive from gram-negative bacteria. Explain the gram stain reaction of each type of cell wall type. 3. Explain the use of the following stains: Acid-fast stains, acridine orange, methylene-blue, calcofluor white, lactophenol cotton blue, and India ink. 4. Compare the nutritional and environmental requirements for bacterial growth and define the categories of media used for culturing bacteria. 5. Define the atmospheric of obligate aerobes, microaerophiles, facultative anaerobes, obligate anaerobes, and capnophilic bacteria. 6. Describe the stages in the growth of bacterial cells. 7. Differentiate between fermentation and oxidation (respiration). 8. Summarize the three biochemical pathways that bacteria use to convert glucose to pyruvate.
II	The Laboratory Role in Infection Control and Performance Improvement in the Microbiology Laboratory – See Lab 1	3 and 5	1. Describe surveillance and outbreak and steps followed for each. 2. Identify when and how environmental culturing is appropriate in infection prevention and control programs. 3. Describe the roles of the microbiology laboratory in preparing for potential bioterrorism activities. 4. Define quality control as it applies in the clinical microbiology laboratory. 5. Describe proper documentation and institution of appropriate corrective action.
III	Specimen Collection and Processing	6	1. Explain the goal of specimen preservation, storage and transport to the laboratory. 2. Distinguish the appropriate conditions for storage of specific specimen types. 3. Explain the prioritization guidelines used during processing to prevent degradation of the specimen. 4. Summarize biochemical reactions and growth patterns of general purpose, selective, differential, enrichment and transport media. 5. Apply Gram stain reactions to facilitate the presumptive identification of microorganisms. 6. Determine the appropriate isolation technique to be use when inoculating solid and liquid media. 7. Outline the appropriate temperature and atmospheric conditions for incubation of routine specimens and to recover fastidious bacteria.
IV	Microscopic Examination of Materials from Infected Sites – See Lab 3	7	1. Given a list of stains commonly used in the diagnostic laboratory, identify the appropriate stain type for determining whether a microbe is a bacillus, fungus, mycobacterium or viral inclusion. 2. Evaluate the following morphology with common species: Gram negative bacilli, small, pleomorphic Gram-positive cocci, groups Yeast and pseudohyphae Hyphae, septate and branched Enlarged cell with intranuclear and cytoplasmic inclusions 3. Apply quality control and quality improvement activities in the laboratory to the results of the direct microscopic examination and association with the culture

V	Use of Colony Morphology for the Presumptive Identification of Microorganisms	8	1. Describe how growth on blood, chocolate , and MacConkey agars is used in the preliminary identification of isolates. 2. Differentiate α-hemolysis from β-hemolysis on a blood agar plate. 3. Identify colony characteristics shown on blood, chocolate and MacConkey agars and correlate to microscopic findings.
VI	Biochemical Identification of Gram-Negative Bacteria	9	1. Discuss the utilization of lactose by bacteria 2. Compare oxidation and fermentation 3. Explain the different reactions that may be observed on the triple sugar iron agar 4. Describe the reactions involved and the products of metabolisms tested in each of the following: Ortho-nitrophenyl-B-D-galactopyranoside test Methyl red and Voges-Proskauer test Decarboxylase, dihydrolase and deaminase tests Citrate utilization DNase test Gelatin liquefaction test Indole test Malonate utilization Motility test Nitrate/nitrite reduction tests Oxidase test Urease test
VII	Applications of Molecular Diagnostics and Antimicrobial Agent Mechanisms of Action and Resistance	11 and 12	1. Discuss what nucleic acid probes are and how they are used in molecular diagnostics techniques. 2. Explain the concept of nucleic amplification reactions and how these techniques may be used in the clinical microbiology laboratory. 3. Compare the various strain typing methodologies and discuss the advantages and disadvantages. 4. Describe the mechanism of action of the different classes of antimicrobials 5. Distinguish between intrinsic and acquired mechanisms of antibiotic resistance.
VIII	Antimicrobial Susceptibility Testing – See Lab 6	13	1. Explain the rationale behind the performance of antimicrobial susceptibility tests. 2. Describe the method for selection of specific drugs in testing and reports 3. Explain minimal inhibitory concentration, disk diffusion and interpretive criteria 4. Define the variables that must be controlled when antimicrobial susceptibility tests are performed. 5. Explain use of the D-zone test. 6. Explain extended spectrum B-lactamases and how organisms that produce these enzymes are detected in the clinical laboratory 7. Explain the meanings of non-susceptible, susceptible, intermediate and resistant as applied to antimicrobial susceptibility test results
IX	Staphylococci	14	1. Describe the general characteristics of the genus Staphylococcus. 2. Describe the natural habitat, virulence factors and clinical infections associated Staphylococcal species. 3. Construct a chart of key tests to differentiate among clinically relevant Staphylococcal species. 4. Distinguish gram-positive cocci that are clinically relevant from those that are considered normal flora. 5. Explain why methicillin resistance among Staphylococcus aureus isolates is a serious clinical problem. 6. Summarize current practices for detecting resistance to oxacillin, clindamycin and vancomycin.
X	*Streptococcus, Enterococcus*, and Other Catalase-Negative, Gram-Positive Cocci	15	1. Compare the general characteristics and isolation of streptococci and similar organisms. 2. Explain the Lancefield classifications of streptococci 3. Describe the natural habitat, virulence factors and clinical infections associated Streptococcal and Enterococcal species. 4. Evaluate the screening of pregnant women for group B streptococci.

			5. Discuss the major serologic tests used to detect antibodies that are produced after recent streptococcal infections.
XI	Aerobic Gram-Positive Bacilli	16	1. Discuss the general characteristics of the aerobic Gram-positive bacilli. 2. Describe the natural habitat, virulence factors and clinical infections associated with the following*: Listeria monocytogenes, Streptococcus agalactiae, Erysiplothrix rhusiopathiae, Arcanobacterium haemolyticum, Gardnerella vaginalis*, Nocardia, and Streptomyces. 3. Summarize the clinical infections associated with Bacillus anthracis and Bacillus cereus. 4. Compare the relationship among the three exotoxin proteins of *B. anthracis.*
XII	Enterobacteriaceae	19	1. Identify the general characteristics of organisms that belong to the family Enterobacteriaceae 2. Compare the virulence factors of the *Escherichia coli* strains pathogenic for the GI tract and those involved in extraintestinal disease. 3. Describe the pathogenesis of the clinically relevant members of the family Enterobacteriaceae. 4. Given an organism's characteristic growth on nonselective and selective differential media, presumptively identify the isolate to the genus level.
XIII	Neisseria Species and *Moraxella catarrhalis*	17	1. Describe the general characteristics of the genus Neisseria 2. Identify the major pathogens within the genus Neisseria 3. Describe nonselective and selective media for the isolation of Neisseria pathogenic species 4. Discuss the tests used in identification of *N. gonorrhoeae* and *N. meningitidis* cultures 5. Identify high-risk groups for acquiring *N. meningitidis* infection and specimens for recovery 6. Summarize the clinical significance and laboratory features of *Moraxella catarrhalis*
XIV	Haemophilus and Other Fastidious Gram-Negative Bacilli	18	1. Distinguish Haemophilus and other Fastidious Gram-negative bacilli by colony and microscopic morphology, habitat, nutritional requirements, and key identifying characteristics. 2. Compare the appearance of the Haemophilus spp. when performing tests for determination of X and V factors requirement. 3. Explain the clinical significance of these organisms when isolated in the clinical laboratory.
XV	*Vibrio, Aeromonas, Plesiomonas,* and *Campylobacter* Species	20	1. Discuss the habitat, colony morphology and microscopic characteristics of the organisms 2. Identify the media of choice for isolation of each organism 3. Compare and contrast each group of organisms with respect to macroscopic and microscopic morphology, biochemical reactions, media, epidemiology, and clinical infection. 4. Identify risk factors associated with acquiring infections by these organisms
XVI	Nonfermenting and Miscellaneous Gram-Negative Bacilli	21	1. Describe the general characteristics of non-fermentative, gram-negative rods. 2. Identify and discuss the most clinically significant non-fermentative gram-negative bacilli 3. Describe the typical biochemical reactions and characteristic of the most commonly encountered non-fermentative organisms
XVII	Anaerobes of Clinical Importance	22	1. Describe anaerobic bacteria, including their sensitivity to oxygen, why they are sensitive, and where they might be found in the environment and human body. 2. Differentiate the various types of anaerobes with regard to atmospheric conditions: Obligate, facultative, aerotolerant anaerobes. 3. Given the signs and manifestations of an anaerobic infection, identify the most probable causative agents: botulism, Tetanus, gas gangrene, actinomycosis.

XVIII	*Mycobacterium tuberculosis* and Nontuberculous Mycobacteria	26	1. Compare the general characteristics of mycobacteria to those of other groups of bacteria 2. Discuss safety precautions to be followed while working in a mycobacteriology laboratory 3. Compare the different culture media used for isolation of mycobacteria 4. Discuss the clinical disease cause by Mycobacterium tuberculosis
XIX	Medically Significant Fungi	27	1. Describe the general characteristics and structures of fungi. 2. Compare asexual and sexual reproduction of fungi 3. Identify the four divisions of fungi. 4. Characterize the following different types of mycoses, defining the tissues they affect: Superficial, cutaneous, systemic and opportunistic. 5. Summarize staining, culture and biochemical methods for the identification of clinically significant of yeast.

MDL 237 Lab Topics

Unit	Topic	Objectives
I	Laboratory Safety	1. Differentiate the functions and purposes of a disinfectant and antiseptic. 2. Summarize the general modes of microbial action. 3. Discuss the way each physical agent controls the growth of microorganisms. 4. List the mechanism of action for each type of chemical agent commonly used in antiseptics and disinfectants. 5. Discuss the appropriate use of antiseptics by health care personal: Handwash, surgical scrub, and patient skin preparation. 6. Describe the hazards encountered in the microbiology lab. 7. Differentiate Standard Precautions from Transmission Based precautions. 8. Compare and contrast the three types of biosafety cabinets. 9. Explain the four categories of biosafety levels.
II	Introduction to Clinical Microbiology:	1. Identify the parts of a microscope and explain their function 2. Define terms relating to microscope functionality such as resolution contrast, magnification, parfocal lens, and field of view. 3. Demonstrate how to prepare a wet mount and how to focus a microscope 4. Locate and focus a specimen using oil immersion techniques 5. Explain the goal of specimen preservation, storage and transport to the laboratory. 6. Select the appropriate conditions for storage of specific specimen types. 7. Explain the prioritization guidelines used during processing to prevent degradation of the specimen. 8. Demonstrate the ability to aseptically transfer bacteria between various media. 9. Describe and explain the steps involved in the aseptic transfer of an inoculum 10. Describe the importance of aseptic technique in laboratory and clinical settings. 11. Explain the purpose of the isolation streak plate procedure 12. Demonstrate how to perform an isolation streak plate procedure 13. Examine a plate and determine if isolated colonies were obtained and whether it was a success.
III	Survey of the Microbial World:	1. List the major groups of microbial phototrophs (algae and cyanobacteria) 2. Recall the environmental and clinical significance of these organisms 3. Describe the major characteristics, structures, and by-products of microbial phototrophs. 4. Describe the four major groupings of protozoa based on their means of movement 5. Locate and identify three examples of free-living protozoa: Amoeba, Euglena, and Paramecium 6. List the major groups of phyla of fungi 7. Differentiate between yeasts and molds, macroscopically and microscopically 8. Recognize the vegetative and reproductive structures of molds

		9. Distinguish between buds, hyphae, and pseudohyphae
IV	Staining Techniques	1. Successfully prepare a bacterial smear 2. Identify the cellular morphology and arrangement of a bacterial culture 3. Explain the purpose of heat-fixing and staining procedures 4. Describe the general chemical basis for microbiological staining 5. Properly prepare a negatively stained slide 6. Explain the basis for and advantages of negative staining 7. Successfully make a capsule smear 8. Identify a bacterial capsule on a slide 9. Explain the clinical significance of bacterial capsules 10. Properly prepare a Gram stain 11. Interpret the Gram reaction observed on a slide to facilitate the presumptive identification of microorganisms 12. Explain the chemical basis for the staining technique 13. Describe the differences between the cell wall structure of gram-negative and gram-positive cells 14. List the common genera of acid-fast bacteria and the clinically significant diseases they cause 15. Correctly perform and interpret an acid-fast stain 16. Explain the basis for the steps in the staining technique 17. List the common genera of endospore-forming bacteria 18. Explain the significance of endospore formation to bacteria 19. Correctly perform an endospore stain 20. Explain the basis for the steps in the staining technique 21. Postulate on the possible impact of endospore-forming bacteria on human health
V	Media: Enrichment, Differential, and Selective	1. Construct a chart summarizing biochemical reactions and growth patterns of general purpose, selective, differential, enrichment and transport media. 2. Understand he function of blood agar 3. Describe the types if hemolysins and their functions 4. Interpret the types of hemolysis associated with growth on a blood agar plate 5. Understand the selective and differential functions of mannitol salt agar 6. Interpret growth on a mannitol salt agar plate and determine the bacterium it represents 7. Describe the use on mannitol salt agar in identification of *Staphylococcus aureus* 8. Understand the selective and differential functions of Eosin Methylene Blue (EMB) agar 9. Explain how MacConkey agar is both selective and differential 10. Interpret the various colors of growth on an EMB agar plate and/or a MacConkey agar plate and determine the category of bacteria they represent 11. Explain how and when MacConkey agar would be used in a clinical setting 12. Determine which type of medium (defined or undefined) best supports the growth of bacteria 13. Compare the growth of fastidious and non-fastidious organisms 14. Define and exemplify fastidious organisms, complex medium, and defined medium
VI	Growth	1. Perform a standard plate count 2. Identify countable plates 3. Calculate dilution factors and be able to calculate cells per milliliter given colony count and dilution factor 4. Understand the effect of different concentrations of salt on bacterial growth 5. Be able to interpret growth data and use them to categorize the salt preferences of bacteria being tested 6. Describe the various ways bacteria carry out metabolism in the absence of oxygen

		7. Interpret growth patterns in brain-heart infusion (BHI) agar deeps and determine what category of aerotolerance they represent 8. Understand the function of catalase in cells that produce the enzyme 9. Perform and interpret the results of a catalase test and know their value in differentiating bacteria 10. Explain the chemical basis for the oxidase test 11. Perform and interpret reactions in the oxidase test
VII	Control of Microbial Growth	1. Demonstrate how to perform antimicrobial susceptibility testing using the Kirby-Bauer method 2. Understand the origin of a zone of inhibition and how it should be measured 3. Interpret the results of the Kirby-Bauer method and determine if an antibiotic is effective against a particular bacterium 4. Describe the differences between normal biota and transient organisms 5. Understand the nature of detergents and their role in removing microbes from skin 6. Interpret the results of hand washing procedures involving soap, scrubbing, or antiseptics
VIII	Hydrolytic Enzymes	1. Explain the appearance of a positive and negative gelatin hydrolysis test result 2. Explain the basis for the gelatin hydrolysis test 3. Explain the appearance of a positive and negative starch hydrolysis test result 4. Explain the basis for the starch hydrolysis test 5. Identify the substrate, enzyme, product, and reagent used in the starch hydrolysis test
IX	Biochemical Testing	1. Describe the appearance of tubes with the following reactions in Phenol Red Broth: acid, acid plus gas, and alkaline 2. Explain the function of the Durham tube, peptone, pH indicator, and carbohydrate source in a medium 3. Explain why incubation time is critical in this experiment utilizing Phenol Red Broth 4. Explain the appearance of a positive and a negative urease test result 5. Explain the basis for the urease test
X	Identifying Microbes	1. Understand the basic function of multi-test systems 2. Demonstrate how to inoculate and read EnteroPluri-Test or a similar bacterium identification system 3. Calculate an ID code from the results and use it to identify a bacterium 4. Complete appropriate testing to isolate, identify and perform susceptibility testing for an unknown organism(s) sample