

Virginia Western Community College

MDL 237

Clinical Bacteriology

COURSE OUTLINE

Prerequisites:

N/A

Course Description:

Teaches handling, isolation, and identification of pathogenic bacteria. Emphasizes clinical techniques and associate bacteria with clinical symptoms.

Semester Credits: 4 Lecture Hours: 2 Lab/Recitation Hours: 3

Required Materials:

Textbooks: Textbook of Diagnostic Microbiology 5th edition by C.R. Mahon, et al.

ISBN: 978-0-323-08989-0

Laboratory Manual for Microbiology Fundamentals: A Clinical Approach, Second Edition by Steven Obenauf and Susan Finazzo, McGraw-Hill Education, ISBN: 978-1-259-29386-3

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
- 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 and molecular testing methods 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 *S. epidermidis*
- Learn proper isolation techniques for aerobic vs. anaerobic and facultative anaerobic bacteria
- Isolate and successfully identify pathogenic fungi and various non-bacterial pathogens such as Mycoplasma and Ureaplasma
- Understand the theory behind automated techniques of bacterial identification
- Recognize how pre-analytical, analytical, and post analytical errors can adversely affect results

Topics

I. Bacterial Cell Structure, Physiology, Metabolism, and Genetics

- A. Significance
- B. Overview of the Microbial World
- C. Classification/Taxonomy
 - Nomenclature
 - Classification by Phenotypic and Genotypic Characteristics
 - Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria
- D. Comparison of Prokaryotic and Eukaryotic Cell Structure
- E. Bacterial Morphology
 - Microscopic Shapes
 - Common Stains Used for Microscopic Visualization
- F. Microbial Growth and Nutrition
 - Environmental Factors Influencing Growth
 - Bacterial Growth
 - Fermentation and Respiration
 - Biochemical Pathways from Glucose to Pyruvic Acid
 - Anaerobic Utilization of Pyruvic Acid (Fermentation)
 - Aerobic Utilization of Pyruvate (Oxidation)
 - Carbohydrate Utilization and Lactose Fermentation
- G. Bacterial Genetics -Genetic Elements and Alterations
 - Mechanisms of Gene Transfer

II. The Laboratory Role in Infection Control

- A. General Concepts in Infection Prevention and Control Practice
 - Frequently Identified Microbes
 - Outbreak Investigation
 - Steps of an Outbreak Investigation
 - Investigation Support from the Laboratory
 - Environmental Culturing
 - Infection Prevention and Control Education
- B. Emerging and Reemerging Pathogens
 - Examples of Emerging and Reemerging Pathogens
 - Response Plans

III. Performance Improvement in the Microbiology Laboratory

- A. General Guidelines for Establishing Quality Control
 - Temperature
 - Thermometer Calibration
 - Media Quality Control
 - Reagent Quality Control
 - Antimicrobial Susceptibility Control

- Personnel Competency
- Use of Stock Cultures
- Quality Control Manual

B. Performance Improvement

- Vision and Mission Statements
- Indicators of Performance Improvement: Process vs. Outcome
- Establishing Performance Monitors
- Problem-Action Form
- Fixing the Process
- Benchmarking
- Commercially Purchased Monitors

C. Analytic Analysis of Tests

- Analytic Sensitivity and Specificity
- Accuracy

D. Clinical Analysis of Tests

- Clinical or Diagnostic Sensitivity

E. Operational Analysis of Tests

- Predictive Values of Tests
- Efficiency of Tests

F. Choosing a Laboratory Method

G. Test Validation

IV. Specimen Collection and Processing

A. Basic Principles of Specimen Collection

- Collection Procedures
- Patient-Collected Specimens
- Labeling and Requisitions
- Safety

B. Preservation, Storage and Transport of Specimens

- Specimen Storage
- Preservatives and Anticoagulants
- Holding of Transport Media
- Shipping Infectious Substances

C. Specimen Receipt and Processing

- Specimen Priority
- Rejection of Unacceptable Specimens
- Macroscopic and Microscopic Evaluation
- Primary Inoculation
- Specimen Preparation
- Isolation Techniques
- Incubation

D. Culture Workup

- Nonroutine Specimens

E. Communication of Laboratory Findings

V. Microscopic Examination of Materials from Infected Sites

A. Preparation of Samples

- Smears from Swabs
- Smears from Thick Liquids or Semisolids
- Smears from Thick, Granular or Mucoid Materials
- Smears from Thin Fluids
- Cytocentrifuge Preparations

B. Stains

C. Microscopes

D. Examination of Prepared Material

- Characterization of Background Materials
- Search for Microorganisms
- Evaluation of Choice of Antibiotic
- Initiation of Special Handling for Unsuspected or Special Pathogens

E. Grading or Classifying Materials

- Contaminating Materials
- Local Materials
- Purulence
- Mixed Materials

F. Reports of Direct Examinations

G. Examples of Sample Observations and Reports

- Quality Control in Direct Microscopic Interpretation

VI. Use of Colony Morphology for the Presumptive Identification of Microorganisms

A. Importance of Colonial Morphology as a Diagnostic Tool

B. Initial Observation and Interpretation of Cultures

C. Gross Colony Characteristics Used to Differentiate and Identify Microorganisms

- Hemolysis
- Size
- Form or Margin
- Elevation
- Density Color
- Consistency
- Pigment
- Odor

D. Colonies with Multiple characteristics

E. Growth of Organisms in Liquid Media

VII. Biochemical Identification of Gram Negative Bacteria

A. Carbohydrate Utilization

- Oxidation –Fermentation Tests
 - Triple Sugar Iron Agar
 - Ortho-Nitrophenyl- β -D-Galactopyranoside Test
- B. Glucose Metabolism and Its Metabolic Products
- Methyl Red Test
 - Voges-Proskauer Test
- C. Amino Acid Utilization
- Decarboxylase and Dihydrolase Tests
 - Deaminase Test
- D. Miscellaneous Tests
- Citrate Utilization
 - DNase
 - Gelatin Liquefaction
 - Indole Production
 - Malonate Utilization
 - Motility
 - Nitrate and Nitrate reduction
 - Oxidase
 - Urease
 - Lysine Iron Agar Slant
 - Motility-Indole-Ornithine Agar
 - Sulfide-Indole-Motility Agar
 - D. Manual Multitest Systems
 - Principles of Identification
 - Analytical Profile Index
- E. Rapid and Automated Identification Systems
- Rapid Biochemical Tests Performed on Isolated Colonies
 - Identification Systems Relying on Carbohydrate Utilization or Chromogenic Substrates
 - Automated Identification Systems
 - Evaluation of Identification Systems

VIII. Applications of Molecular Diagnostics

- A. Nucleic Acid Hybridization Techniques
- Hybridization Reaction Variables
 - Hybridization Formats
 - Applications of Nucleic Acid Hybridization Techniques
- B. Nucleic Acid Amplification Procedures
- Polymerase Chain Reaction
 - Other Nucleic Acid Amplification Reactions
- C. Strain Typing and Identification
- Nonamplified Typing Methods
 - Amplified Typing Methods

D. Future of Molecular Diagnostics Testing in the Clinical Microbiology Laboratory

- Sequencing
- Pyrosequencing
- DNA Microarrays and Nanoarrays
- Proteomics
- MALDI-TOF Mass Spectrometry

E. Nanomedicine

IX. Antimicrobial Agent Mechanisms of Action and Resistance

A. Antibiotic Targets and Mechanisms of Action

- Inhibition of Bacterial Cell Wall Biosynthesis
- Inhibition of Folate Synthesis
- Interference with DNA Replication
- Interference with DNA Transcription
- Interference with mRNA Translation
- Origins of Antibiotic Resistance
- Acquired Mechanisms of Resistance

B. Dissemination

C. Nanotechnology to Deliver Therapeutic Agents

X. Antimicrobial Susceptibility Testing

A. Reasons and Indications for Performing Antimicrobial Susceptibility Tests

- Factors to Consider When Determining Whether Testing is Warranted

B. Selecting Antimicrobial Agents for Testing and Reporting

- Selection of Test Batteries
- Reporting of Susceptibility Test Results

C. Traditional Antimicrobial Susceptibility Testing

- Inoculum Preparation and Use of McFarland Standards
- Dilution Susceptibility Testing Methods
- Disk Diffusion Testing
- Modified Methods for Testing Slow-Growing or Fastidious Bacteria
- Additional Organism and Antimicrobial Agent Testing Concerns

D. Automated Antimicrobial Susceptibility Test Methods

- Principles of Technologies Used
- Currently Available Automated Systems
- Nonautomated Antimicrobial Susceptibility Test Methods: Etest

E. Interpretation of In Vitro Antimicrobial Susceptibility Test Results

F. Methods of Detecting Antimicrobial-Inactivating Enzymes

- β -Lactamase Tests

G. Quality Control of Antimicrobial Susceptibility Tests

H. Selecting an Antimicrobial Susceptibility Test Method

I. Susceptibility Testing Challenges

- Rapid Susceptibility Determination

J. Special Antimicrobial Susceptibility Tests

- Minimum Bactericidal Concentration Test
- Controlling Test Variables
- Time-Kill Assays
- Synergy Tests
- Serum Bactericidal Test
- Molecular Probes for Identifying Determinants of Antimicrobial Resistance
- Measurement of Antimicrobial Agents in Serum and Body Fluids

XI. The Staphylococci

A. General Characteristics

B. Clinically Significant Species

- *Staphylococcus epidermidis*
- *Staphylococcus saprophyticus*
- *Staphylococcus lugdunensis*
- Other Coagulase Negative Staphylococcus

C. Laboratory Diagnosis

- Specimen Collection and Handling
- Microscopic Examination
- Isolation and Identification
- Rapid Methods of Identification

D. Antimicrobial Susceptibility

- Methicillin-Resistant Staphylococci
- Vancomycin-Resistant Staphylococci
- Macrolide Resistance

XII. *Streptococcus*, *Enterococcus*, and Other Catalase-Negative Gram-Positive Cocci

A. General Characteristics

- Cell Wall Structure
- Hemolysis

B. Clinically significant Streptococci and Streptococcus-like Organisms

- *Streptococcus pyogenes*
- *Streptococcus agalactiae*
- Groups C and G Streptococci
- *Streptococcus pneumoniae*
- *Viridans Streptococci*
- Streptococcus-like Organisms

C. Laboratory Diagnosis

- Classification Schemes

D. Noncultural Identification

E. Susceptibility Testing

XIII. Aerobic Gram-Positive Bacilli

A. Non-Spore-Forming, Nonbranching Catalase Positive Bacilli

- *Corynebacterium*
 - *Rothia*
 - Related Genera and Undesignated CDC *Corynebacterium* Groups
- B. Non-Spore-Forming, Nonbranching Catalase Negative Bacilli
- *Erysipelothrix rhusiopathiae*
 - *Arcanobacterium*
 - *Gardinerella vaginalis*
- C. Non-Spore-Forming, Branching Aerobic Actinomycetes
- *Nocardia*
 - Other Actinomycetes
- D. Spore-Forming, Nonbranching Catalase-Positive Bacilli
- *Bacillus anthracis*
 - *Bacillus cereus*
 - Other *Bacillus* species

XIV. Neisseria Species and *Moraxella catarrhalis*

- A. Pathogenic *Neisseria* Species
- Virulence Factors
 - *Neisseria gonorrhoeae*
 - *Neisseria meningitides*
- B. Commensal *Neisseria* Species
- *Neisseria cinerea*
 - *Neisseria flavescens*
 - *Neisseria lactamica*
 - *Neisseria mucosa*
 - *Neisseria polysaccharea*
 - *Neisseria sicca*
 - *Neisseria subflava*
 - *Neisseria elongata*
 - *Neisseria weaver*

XV. *Haemophilus* and Other Fastidious Gram-Negative Bacilli

- A. *Haemophilus*
- General Characteristics
 - *Haemophilus influenza*
 - Infections Associated with Other *Haemophilus* Species
 - Laboratory Diagnosis
- B. HACEK Group
- *Aggregatibacter aphrophilus*
 - *Aggregatibacter actinomycetemcomitans*
 - *Cardiobacterium hominus*
 - *Eikenella corrodens*
 - *Kingella*

*C. Capnocytophaga**D. Pasteurella**E. Brucella**F. Franciella**G. Legionella*

- Clinical Significance
- Virulence Factors
- Infections Caused by *Legionella*
- Epidemiology
- Laboratory Diagnosis
- Specimen Collection and Handling
- Microscopic Examination
- Isolation and Identification
- Serologic Testing

H. Bordetella

- Clinically Significant Species
 - *Bordetella pertussis*
 - *Bordetella parapertussis*
 - Miscellaneous species
- Laboratory Diagnosis
 - *Specimen Collection and Handling*
 - *Nucleic Acid Detection*
 - *Microscopic Examination*
 - *Isolation and Identification*
 - *Serologic Testing*
- Antimicrobial Susceptibility

XVI. Enterobacteriaceae

A. General Characteristics

- Microscopic and Colony Morphology
- Classification
- Virulence and Antigenic Factors
- Clinical Significance

B. Opportunistic Members of the Family Enterobacteriaceae and Associated Infections

- *Escherichia coli*
- *Klebsiella* and *Raoutella*
- *Enterobacter*, *Cronobacter*, and *Pantoea*
- *Serratia*
- *Proteus*
- *Morganella*
- *Providencia*
- *Edwardsiella*
- *Erwinia* and *Pectobacterium*

- *Citrobacter*

C. Primary Intestinal Pathogens of the Family Enterobacteriaceae

- *Salmonella*
- *Shigella*
- *Yersinia*

D. Other Genera of the Family Enterobacteriaceae

- *Budivicia*
- *Buttiauxella*
- *Cedecea*
- *Ewingella*
- *Kluyvera*
- *Leclercia*
- *Leminorella*
- *Moellerella*
- *Obesumbacterium*
- *Photorhabdus*
- *Rahnella*
- *Tatumella*
- *Trabulsiella*
- *Yokenella*

E. Laboratory Diagnosis of Enterobacteriaceae

- Specimen Collection and Transport
- Isolation and Identification
- Screening Stool Cultures for Pathogens
- Serologic Grouping

XVII. *Vibrio*, *Aeromonas*, *Plesiomonas*, and *Campylobacter* Species

A. *Vibrio*

- General Characteristics
- *Vibrio cholera*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Vibrio alginolyticus*
- Laboratory Diagnosis

B. *Aeromonas*

- General Characteristics
- Clinical Manifestations
- Laboratory Diagnosis
- Antimicrobial Susceptibility

C. *Plesiomonas*

- Epidemiology
- Clinical Manifestations

- General characteristics
- Laboratory Diagnosis
- Antimicrobial Susceptibility

D. *Campylobacter* and *Campylobacter*-Like Species

- Epidemiology
- Clinical Manifestations
- Laboratory Diagnosis
- Antimicrobial Susceptibility

XVIII. Nonfermenting and Miscellaneous Gram-Negative Bacilli

A. General Characteristics of Nonfermenters

- Clinical Infections
- Biochemical Characteristics and Identification
- *Pseudomonas* Fluorescent Group
- *Acinetobacter*
- *Stenotrophomonas maltophilia*
- *Burkholderia*
- *Moraxella*, *Oligella*, and *Psychrobacter*

B. Less Commonly Encountered Nonfermentative, Gram-Negative Bacilli

- *Alcaligenes* and *Achromobacter*
- *Brevundimonas*
- CDC Groups EO-3, EO-4, and *Paracoccus*
- *Chromobacterium*
- *Comamonas* and *Delftia*
- *Flavobacteriaceae*
- *Methylobacterium* and *Roseomonas*
- *Ralstonia* and *Cupriavidus*
- *Shewanella*
- *Sphingomonas*

XIX. Anaerobes of Clinical Importance

A. Important Concepts in Anaerobic Bacteriology

- Anaerobes Defined
- Why Some Organisms Are Anaerobes
- Where Anaerobes Are Found
- Anaerobes at Specific Anatomic Sites
- Factors That Predispose Patients to Anaerobic Infections
- Indications of Anaerobe Involvement in Human Disease

B. Frequently Encountered Anaerobes and Their Associated Diseases

- Gram-Positive Spore-Forming Anaerobic Bacilli
- Gram-Positive Non-Spore Forming Bacteria
- Anaerobic Gram-Negative Bacilli

- C. Specimen Selection, Collection, Transport, and Processing
 - Specimen Quality
 - Processing Clinical Samples for Recovery of Anaerobic Pathogens
- D. Procedures for Identifying Anaerobic Isolates
 - Preliminary Procedures
 - Indications of the Presence of Anaerobes in Cultures
 - Presumptive Identification of Clinically Significant Anaerobes
 - Definitive Identification of Anaerobic Isolates
 - Identification of Clostridium Species
 - Identification of Anaerobic Non-Spore-Forming, Gram-Positive Bacilli
 - Identification of Anaerobic Gram-Negative Bacilli
 - Identification of Anaerobic Cocci
- E. Antimicrobial Susceptibility Testing
 - Problems in Susceptibility Testing of Anaerobic Isolates
- F. Treatment of Anaerobe-Associated Diseases
 - Surgical Therapy
 - Hyperbaric Oxygen
 - Antimicrobial Therapy
 - Antitoxins

XX. The Spirochetes

- A. Leptospire
 - General Characteristics
 - Virulence Factors and Pathogenicity
 - Infections Caused by Leptospire
 - Epidemiology
 - Laboratory Diagnosis
 - Antimicrobial Susceptibility
 - Clinically Significant Species
 - *Borrelia recurrentis* and Similar *Borreliae*
 - *Borrelia burgdorferi*
- B. Treponemes
 - General Characteristics
 - Clinically Significant Species
 - *Treponema pallidum* Subsp. *pallidum*
 - Other Treponemal Diseases

XXI. Mycoplasma and Ureaplasma

- A. General Characteristics
- B. Clinical Infections
 - *Mycoplasma pneumoniae*
 - *Mycoplasma hominus* and *Ureaplasma* Species

- C. Laboratory Diagnosis
 - Specimen Collection and Transport
 - Direct Examination
 - Culture
- D. Antimicrobial Susceptibility
- E. Interpretation of Laboratory Results

XXII. *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria

- A. Clinical Significance of the *Mycobacterium tuberculosis* Complex
 - *Mycobacterium tuberculosis*
 - *Mycobacterium bovis*
 - Rapidly Growing Species
- B. *Mycobacterium leprae*
- C. Isolation and Identification of the Mycobacteria
 - Laboratory Safety Considerations
 - Specimen Collection
 - Digestion and Decontamination of Specimens
 - Staining for Acid-Fast Bacilli
 - Culture Media and Isolation Methods
 - Laboratory Identification
- D. Susceptibility Testing of *Mycobacterium tuberculosis*
- E. Immunodiagnosis of *Mycobacterium tuberculosis* Infection
 - Skin Testing
 - Serology

XXIII. Medically Significant Fungi

- A. General Characteristics
 - Yeast vs. Molds
 - Hyaline vs. Phaeoid
 - Dimorphism and Polymorphism
 - Reproduction
- B. Taxonomy
 - Mucorales
 - Ascomycota
 - Basidiomycota
 - Fungi Imperfecti
- C. Mycoses
 - Superficial mycosis
 - Cutaneous mycosis
 - Subcutaneous mycosis
 - Systemic Mycosis
- D. Clinically Significant Species
 - Agents of Superficial Mycosis

- Agents of Cutaneous Mycosis
 - Agents of Subcutaneous Mycosis
 - Agents of Systemic Mycosis
 - Agents of Opportunistic Mycosis
 - Agents of Yeast Infections
 - Pneumocystis Infection
- E. Laboratory Diagnosis of Fungi
- Specimen Collection, Handling, and Transport
 - Direct Microscopic Examination of Specimens
 - Isolation Methods
 - Identification of Fungi
- F. Immunodiagnosis of Fungal Infections
- G. Antifungal Susceptibility
- Antifungal Agents
 - Antifungal Susceptibility Testing

Laboratory Topics

1. Laboratory Safety
 - Safety requirements for VWCC Microbiology Lab
 - Clinical Lab Safety
2. Basic Techniques I
 - Use of the Microscope
 - Survey of the Microbial World
 - Protozoa
 - The Fungi
 - Motility: Hanging Drop Method
3. Basic Techniques II
 - Aseptic Technique
 - Isolation Streak Plate
4. Staining Techniques I
 - Introduction to Staining
 - Negative Stain
 - Capsule Stain
 - Gram Stain
5. Staining Techniques II
 - Acid-fast Stain
 - Endospore Stain

6. Media: Enriched, Differential and Selective

- Blood Agar
- Mannitol Salt Agar
- EMB Agar
- MacConkey Agar

7. Growth

- Standard Plate Count
- Osmotic Growth
- Hydrolytic Enzymes
 - Catalase, Oxidase, Coagulase, Gelatin Hydrolase

8. Biochemical Testing

- Phenol red Broth
- Triple-Sugar Iron Agar
- IMViC (Indole, Methyl Red, Vogler-Proskauer, Citrate) Reactions
- Urease Test

9. Control of Microbial Growth

- Antimicrobial Susceptibility Testing
- Hand Washing

10. Diagnostic Testing

- Rapid Strep Test

11. Identifying Microbes

- Enteropluri- Test (or equivalent)
- Isolation Streak Plate

12. Identification of Unknown Bacteria

Notes to Instructors: