COURSE OBJECTIVES

Biology 251 – General Microbiology Laboratory Course Objectives

Laboratory Protocols

All protocols for lab are available online at the TMCC Biology 251 – General Microbiology Lab website.

See link for reference: https://www.tmcc.edu/microbiology-resource-center

Laboratory Safety Policies and Procedures

The Microbiology Lab Safety Rules handout will be disbursed the first day of lab each semester. The lab instructor will go over all lab safety rules the first day of lab each semester. Lab instructors will pass out, and students will sign, a lab safety signature sheet acknowledging the intent to follow all laboratory safety policies and procedures. This student signed lab safety signature sheet will be kept on file.

For more information see the website: https://www.tmcc.edu/microbiology-resource-center/getting-started/lab-safety

Laboratory Notebook Components

All students enrolled in Biology 251 will be given a lab composition book during the first lab of the semester. An overview of laboratory notebook components will be presented to students during the first day of lab each semester. Students are expected to download the lab protocols from the website, read them over, and fill out their lab book before coming to lab each time. Printed out lab protocols are not allowed in lab. Only student lab books are allowed in lab.

Lab Coat

A lab coat will be provided for each student enrolled in Biology 251 during the first day of lab. Students are required to wear their lab coat during lab.

Microscopy

Your instructor may expect you to know the following.

1. Know how to properly handle, transport, clean, and use the microscope.
2. Be able to identify and know the function of these parts of the microscope:
    - Ocular eyepieces
    - Objective lenses
    - Stage
    - Mechanical stage & controls
    - Arm
    - Base
    - Iris diaphragm
    - Condenser
    - Fine focus adjustment

- Coarse focus adjustment

3. Know the proper procedure for examining a specimen slide using the 100X objective lens.
4. Understand the relationship between the power of the objective to the magnification of the specimen examined.

Micromorphology

A brief overview of some clinically important bacteria, including common structures used for diagnostic and/or identification purposes

1. Be able to identify under the microscope and describe the appearance of each of the following types of micromorphology, at instructor's discretion:
    - Coccus
    - Diplococci
    - Streptococci
    - Staphylococci
    - Tetrad
    - Sarcinae
    - Bacillus
    - Diplobacilli
    - Streptobacilli
    - Coccobacillus
    - Vibrio
    - Spirillum
    - Spirochete
2. Be able to identify under the microscope and describe the appearance of each of the following bacterial cell structures, at instructor's discretion:
    - Monotrichous Flagella
    - Amphitrichous Flagella
    - Lophotrichous Flagella
    - Peritrichous Flagella
    - Capsules
    - Central Spores
    - Terminal Spores
3. Be able to identify under the microscope and describe the appearance of each of the following slide representatives of micromorphology, at instructor's discretion. Be able to make labeled drawings of each and list basic information about each.
    - Bacteria, yeast, and blood
    - Bacterial types
    - *Staphylococcus aureus*
    - *Micrococcus luteus*
    - *Neisseria gonorrhoeae*
    - *Streptococcus faecails*
    - Bacillus
    - *Bacillus anthracis*
    - *Clostridium tetani*
    - Mixed coliforms

- *Mycobacterium tuberculosis*
- *Pseudomonas aeruginosa*
- *Borrelia burgdorferi*
- *Treponema pallidum*
- Typical spirillum
- Vibrio cholera
- Bacterial capsules
- Bacterial flagella (peritrichous)
- Bacterial flagella (amphitrichous)
- Archaebacteria

Microbiological Survey

A brief overview of other important microscopic organisms, algae, fungi, protozoa, and helminthes

1. Be able to identify under the microscope and describe the appearance of each of the following slide representatives of micromorphology. Be able to make labeled drawings of each and list basic information about each. A subset of these may be chosen by the instructor.
    a. Bacteria
        - Anabaena
        - Gleocapsa
        - Oscillatoria

    b. Algae
        - Ceratium
        - Chlamydomonas
        - Chlorella
        - Diatoms
        - Peridinium
        - Spirogyra
        - Volvox

    c. Protozoans
        - Amoeba proteus
        - Crytosporidium parvum
        - Euglena
        - Foraminifera
        - Giardia lamblia
        - Leishmania donovani
        - Paramecium caudatum
        - Plasmodium falciparum
        - Plasmodium malariae
        - Trichomonas vaginalis
        - Trypanosoma cruzi
        - Tyrpanosoma gambiense

- Vorticella

d. Fungi

- Aspergillus
- *Candida albicans*
- Coprinus
- Penicillium
- Rhizopus
- *Saccharomyces cerevisiae*

e. Helminths

- Echinococcus ganulosa
- Enterobius vermicularis
- Fasciola hepatica
- Loa loa
- Necator americanus
- Planaria
- Paragonimus westermani
- Taenia
- Trichinella spiralis
- Trichiuris trichiura

Aseptic Technique

Demonstrate appropriate and safe microbiological procedures

1. Be able to demonstrate proper and appropriate aseptic technique in lab, including the following:
    - Decontaminating your lab bench
    - Safely organizing your work space
    - Properly adjust your Bunsen burner
    - Properly use a micro incinerator
    - Sterilize your inoculating tools
    - Aseptically transfer microorganisms using BSL 2 procedures
    - Handle biohazard spills
    - Dispose of biohazard materials
2. Define and describe the following terms related to aseptic technique:
    - Aseptic
    - BSL 1
    - BSL 2
3. Be able to distinguish between BSL 1 and BSL 2 procedures.

Bacterial Isolation

Demonstrate the successful isolation of individual species from a mixed culture

1. Be able to successfully isolate a single bacterium species using the quadrant method of streaking for isolation.
2. Be able to demonstrate proper streaking for isolation using the quadrant method.

Macro-morphology & Small Volume Serial Dilutions

Demonstrate successful isolation of individual species from a mixed culture
Demonstrate successful technique of small volume serial dilution schemes
Identify major macro-morphology characteristics of isolates

1. Be able to identify on a plate culture and describe the appearance of each of the following types of macro-morphology:
    - Form – Spindle, Circular, Filamentous, Irregular, Rhizoid, Punctiform
    - Elevation – Flat, Raised, Convex, Pulvinate, Umbonate
    - Margin – Entire, Undulate, Filamentous, Lobate, Erose, Curled
    - Size – Pinpoint, Small, Medium, Large
    - Color – Non-pigmented, White, Creamy, Tan
    - Texture – Moist, Mucoid, Dry
    - Optical quality – Opaque, Translucent, Dull, Shiny
    - Hemolysis – Beta, Alpha, Alpha-prime, Gamma
2. Be able to successfully complete a small volume serial dilutions scheme.
3. Be able to calculate colony forming units per mL (CFU's/mL) and interpret what that means.

Bacterial Staining

Successfully complete the staining procedure appropriate for a given microorganism

1. Be able to demonstrate appropriate staining technique. Be able to identify under the microscope and describe the appearance of each using common micromorphology and bacterial structure terminology. A subset of these may be chosen by the instructor, but the Gram's stain is required.
    - Simple Stain
    - Negative Stain
    - Gram's Stain
    - Capsule Stain
    - Endospore Stain
    - Acid-Fast Stain

Unknown Identification

Learn the proper differential & selective media used to culture specific types of bacteria

1. Demonstrate proper inoculation techniques on specific biochemical tests. Be able to identify basic biochemical test media. A subset of these may be chosen by the instructor.
    - Motility Media
    - McFarland's Standard
    - Fluid Thyoglycolate Medium (FTM)
    - Catalase
    - Oxidase
    - Blood Agar
    - Mannitol Salt Agar (MSA)
    - Coagulase
    - Novobiocin Antibiotic Sensitivity
    - Hektoen Enteric Agar (HEA)

- MacConkey's Agar (Mac)
- Eosin Methylene Blue Agar (EMB)
- Simmon's Citrate
- Triple Sugar Iron Agar (TSI)
- Urea
- Salt Tolerance
- Bile Esculin Agar (BEA)
- Hippurate Hydrolysis
- Optochin Antibiotic Sensitivity
- Bacitracin Antibiotic Sensitivity
- SXT Antibiotic Sensitivity

2. Conduct biochemical testing appropriate to specific bacteria genera.
    - Streptococci, and Enteric organisms.
    - Be able to demonstrate the proper inoculation of differential and selective media.
3. Interpret biochemical test results properly to determine species identification.
    - Be able to recognize specific results for biochemical tests conducted.
    - Be able to determine the identification of specific bacteria species based on biochemical test results.

Antimicrobial Susceptibility Testing

Successfully complete the Kirby-Bauer disk diffusion method of antimicrobial susceptibility testing for a given microorganism

1. Be able to successfully complete the Kirby-Bauer disk diffusion method of antimicrobial susceptibility testing for a given microbe.
2. Be able to interpret zones of inhibition qualitatively as resistant, intermediate, or susceptible.
3. Be able to determine which antimicrobial agents are best for treatment when multiple zones of inhibition are present.

Additional Lab Activities

Your instructor may choose to do one, some, or all of the following lab activities.

Case Study:

Following traditional medical teaching techniques of using a case study to test and develop your knowledge of the subject matter and your ability to use that knowledge to problem solve, the case study lab is designed to emulate a clinical setting.

1. Given a mixed bacterial culture, be able to isolate individual bacterial species and identify them using the Gram stain and basic biochemical testing.
2. Given a patient assessment form and the identification of the bacterial species from your mixed culture: be able to determine the cause of disease, the proper treatment for the disease, and the prognosis of your patient.

Independent Research Project:

Investigate a topic of your choice, subject to your instructor's approval.

1. Research projects must be original, creative and NOT totally trivial.
    - Do NOT think of this as a glorified high school science fair project.
2. We are expecting you to conduct a college level experiment.
    - Your instructor is not interested in bacterial sampling of bathroom knobs, anti-microbial soap, etc.

3. You will ask one question of interest.
4. You cannot collect any samples from a hospital setting.
5. You will design your experiment as a comparison.
6. You must collect quantifiable data, preferably using serial dilutions.
    - If your protocol does not use serial dilutions you must still know how to do them and be able to calculate them for your final lab practical.
7. You will do replicate samples so that you can conduct a statistical analysis of you data to test your hypothesis.
    - This requires of minimum of 3 samples for your test.
8. You will work with your instructor and IA to design and come up with an appropriate experimental protocol. This is NOT EASY to do.
    - You will really have to think and rethink this whole process through.
    - You will undoubtedly revise your procedure several times before you have permission to proceed.
9. Your experimental design will be limited by the constraints of the materials we have available in the lab and the time available to complete your experiment.

Motility:
Learn the proper technique for making a hanging drop mount to visualize the motility of living bacteria.

1. Prepare hanging drop mounts using aseptic technique and visualize these living bacteria moving around under the microscope.

Undergraduate Research Projects:
Participation in various undergraduate research projects.