SUGGESTED LABORATORY PROCEDURES AND SAFETY REGULATIONS

Suggested Procedures

1. PREPARE AHEAD OF TIME: Read over each new laboratory exercise before coming to class so you are familiar with the materials and procedures to be used.

2. WAIT FOR INSTRUCTIONS: The instructor will begin each laboratory period with a short introductory session providing specific instructions. Wait for these instructions before beginning protocols and ask questions if there are parts of the exercise you do not understand. Make sure you know what you are expected to do **before** you do it.

3. KEEP ACCURATE RECORDS: Record observations of all laboratory data when first collected and determine results as indicated. Make sketches or drawings where applicable. Record any variations in procedures or materials used, times required, etc.

4. COMPLETE ALL EXERCISES: It is your responsibility to complete all laboratory assignments, some of which will require data collection over several lab sessions. Do not expect the instructor or other students to collect data for you.

5. TEST YOUR UNDERSTANDING: Answer the questions at the end of each exercise to test your understanding of the laboratory material covered. Recognize that the same or similar questions often appear on laboratory exams.

6. TAKE TIME TO REVIEW: After completing each exercise, reread that portion of the laboratory syllabus to make sure you thoroughly understood the procedures being carried out, and/or the basic principles being demonstrated.

7. BE ALERT: Pay attention to instructional notices posted in the laboratory, they are there for your benefit.

Some Safety Regulations

1. WORK ON A CLEAN SURFACE: Clean the surface of your laboratory table with the laboratory disinfectant provided at both the beginning and the end of each lab session. Note - It is your responsibility to keep your disinfectant container filled and ready for use. A supply of additional disinfectant is available in the large plastic container at the back of the laboratory.

2. MINIMIZE CLUTTER: To avoid problems with stains, toxic chemicals, or burners, keep your tabletop free of non-essential materials at all times during the laboratory. Place your books and folders on the floor or in the table shelf provided.

3. BE CAREFUL WITH FIRE: Take note of the locations of Bunsen burners in this laboratory and recognize that they are usually lit when laboratories are in session. Be aware of the potential hazard presented by lose hair, fuzzy sweaters, papers and other flammable materials. Never leave your lighted burner unattended, and avoid placing it in a position likely to bring it into contact with other students.

4. WEAR PROTECTIVE CLOTHING: Since students will often work with potential pathogens and a variety of materials that may cause permanent stains, it is strongly recommended that a lab coat or other protective covering be worn. In the event of contaminating spills, this clothing can be removed and disinfected. Provisions will be made to keep protective clothing at school until the end of the semester. Since broken glass is an occasional hazard, bare feet are prohibited.

5. PROTECT YOUR VISION: Wear safety glasses whenever you are heating liquids in tubes or working with materials that might splatter and cause eye injury. Know the location of the emergency eyewash station and how to use the eyewash correctly.

6. CLEAN UP SPILLS: Clean up accidental microbial spills using an abundant quantity of laboratory disinfectant. Avoid handling broken glass or other materials that might cause injury (sharps). Place broken glass in the glass boxes and slides, tubes or pipettes in the containers provided within the discard area. **Do not place sharps in the waste paper receptacles**.

7. DISPOSE OF SOLID AND LIQUID WASTES PROPERLY: Place all used culture tubes in the plastic baskets or racks provided (**keep them vertical please**), discard Petri plates in the metal bins indicated and any contaminated flasks/beakers on the shelves available in the discard area. Remove all tape labels from glass containers (tubes, flasks, etc.). **Do not place culture tubes, flasks or beakers in the metal bins, never dispose of culture containers in trash receptacles, and do not pour contaminated liquids down sink drains**.

8. LEARN AND USE ASEPTIC TECHNIQUE: **Remember** that you will be working with a variety of potentially pathogenic microorganisms in this laboratory, and that you should practice aseptic techniques whenever dealing with living cultures. Recognize also that laboratory air contains microorganisms that can contaminate sterile materials.

9. AVOID CONTACT WITH PATHOGENS: Avoid unnecessary exposure to potential pathogens by following a few simple rules; **do not consume food or drinks while working in the microbiology laboratory**, avoid nail-chewing or other hand-to-mouth activities, and never pipette by mouth.

10. AVOID INFECTION: Report any accidents or injuries (cuts, burns, etc.) to your instructor as quickly as possible. First aid materials are available, and all precautions should be taken to avoid possible infection. If you have sustained and injury resulting in broken skin prior to entering the laboratory, make sure such areas are appropriately covered. Gloves are not used routinely in this laboratory, but can be made available under some circumstances. Ask your instructor if you believe you require gloves.

11. BE INFORMED: Know the locations and appropriate use of eyewash stations, glass containers, fire extinguishers, fire blanket, first aid kit, MSDS folders and clean up materials for spills/broken glass. Know the proper exits to use and procedures to follow in the event of an emergency evaculation.

12. USE COMMON SENSE: This facility, the laboratory equipment and supplies are available for your educational use. Generally be careful and think about what you are doing. If you need help, please contact the instructor.

Sanitation, Storage and Discard Procedures

The use of these procedures aids in the maintenance of uncontaminated cultures (especially important once you begin working with unknowns), and prevents the spread of viable, possibly pathogenic microorganisms. They are designed to protect everyone working within this laboratory from unnecessary exposure, and should be taken seriously.

1. Before beginning your work with any live microbial culture, **clean the countertop** with the micro-disinfectant provided. Spread the disinfectant in a thin film over the counter surface with a paper towel, allow it to stand for a moment and then **wipe it off**. At the end of the laboratory period, after all materials have been put away or properly discarded, repeat the counter cleaning procedure, and **wash your hands** thoroughly.

2. The **incubation and storage** of live microorganisms should be as follows:

   In the incubators – All cultures requiring 37^{o} C incubation must be placed in the appropriate section of the incubator as assigned. Culture tubes must be placed in a plastic tube rack or basket, and stored in an upright position. Plates must be labeled on the bottom surface (agar side) and placed in an inverted position (agar-side-up). Plates requiring extended incubation (48-72 hours) should be sealed in zip-lock bags to prevent desiccation and labeled accordingly.

   In storage boxes – Most materials placed in the incubator will be removed after 24 hours and transferred to the appropriate storage areas located at the back of the laboratory. Instructors will transfer these materials to the side counter for student access during the following laboratory period. **Note** – Placing cultures in the correct section of the incubator will significantly impact data collection, as materials incubated in the wrong location will be transferred to the wrong storage area and made available during the wrong laboratory session.

   In drawers – Each student will be assigned a section of a lab drawer for the storage of culture materials. Please do not store lab coats, shoes, notebooks or other materials in these drawers. All culture tubes must be maintained in an upright position. Plates must be labeled on the bottom surface (agar side) and placed in an inverted position (agar-side-up). If dust mites are detected, place newly prepared plates in a zip-lock bag, and SEAL IT SHUT.

   DO NOT STORE LIVE MICROBIAL CULTURES IN REFRIGERATORS CONTAINING STERILE MEDIA! If you do, and they are discovered, they will be removed and returned to a more appropriate location.

   DO NOT TAKE YOUR MICROBIAL CULTURES HOME FOR ANY REASON!

   Although bacteria are abundant throughout the environment, their concentrations on agar plates are much higher than in most other locations. If you find the brightly colored colonies scattered across an agar surface attractive, take a photograph, but leave the cultures in the laboratory.

3. The **discard procedures** used in this laboratory require that all used culture media and contaminated materials be placed on the appropriate shelves in the discard area at the front of the laboratory as follows:

   Disposable Materials – Used Petri plates must be placed in the metal containers provided, and should be handled carefully to avoid breaking or opening. It is not necessary to remove tape labels since plastic plates cannot be reused after being autoclaved. Contaminated pipettes, cotton swabs, toothpicks, and other disposable items must be placed in the metal containers along with plastic Petri plates.

   Recycled Materials - Plastic bags, microfuge tubes, pipette tips and other plastic materials can be recycled, and will be collected for this purpose. Students are expected to conscientiouly support recycling efforts.

   Glass tubes – Contaminated culture tubes must be placed **in an upright position** in the 50-tube baskets provided. All tubes containing live cultures should be capped, and students are expected to make an effort not to spill any tube-contents. Remember that plastic snap-caps do not seal, and tubes will leak if placed on their sides. Remove all tape labels before discarding culture tubes (writing on the glass is O.K.). Tape found with a student's name on it will be noted, and the student may lose points.

   Glass slides (except depression slides) – Place glass slides contaminated with bacteria or fungi in the metal container indicated. Slides (and cover-slips) used for wet mounts of algae or free-living protozoa can be rinsed with tap water, placed on a flat paper towel to dry, and used again during another lab session. If immersion oil is used, dispose of oil-coated cover-slips in the metal container designated for discarded slides.

   Contaminated glassware – Contaminated beakers, flasks, slide culture chambers and other miscellaneous glassware can be placed on the top shelf inside the discard area, but **not** within the metal bins. Please prevent contaminants from touching the shelf.

   NEVER DISCARD ANY CONTAMINATED SUBSTANCES IN SINKS OR TRASH BASKETS. All potentially pathogenic microorganisms must be terminated (autoclaved) prior to their disposal.

Thank you for your cooperation throughout the semester.