Welcome to General Microbiology~ BSCI 223

In this course we will discuss the physiology, genetics, ecology, and pathogenicity of microorganisms. The labs are designed to complement lecture discussions.

Understanding laboratory experiments will help you understand and conceptualize lecture topics.

There are many aspects to learning:
- attending lectures,
- reading the text,
- group discussions,
- Web CT course support material,
- writing reports,
- and lab exercises.

All of these will be used in this course to help you learn and enjoy microbiology. If you have any questions about lab or lecture material please discuss these with your Teaching Assistant or make an appointment to visit your course instructor.

Throughout this lab manual reference will be made to Alexander and Strete’s 2001 edition of Microbiology, a photographic Atlas for the Laboratory.

The symbol \( \odot \) will direct you to appropriate pages and figures in the atlas.

INTRODUCTION

When working in a microbiology lab, it is important to understand safety considerations. Microorganisms are capable of causing disease. Special precautions must be taken to inhibit the spread of microbes from the contained environment of the test tube or petri plate, to the human host. Depending upon the characteristics of the microbe, the possibility of disease after contact with the host will vary. Those microbes that are particularly capable of causing disease are referred to as pathogens. Biological labs are rated by the Center for Disease Control (a branch of the U.S. Department of Health and Human Services located in Atlanta Georgia). The rating, or Biosafety Level, reflects the pathogenicity of microbes studied in the lab and dictates the precautions that must be taken to ensure the safety of the lab worker (Table 1).

Agents used in General Microbiology labs fall into the category of Level 1 agents. Work may be done on an open bench top, and containment is achieved through the use of standard microbiological practices. One goal of the microbiology laboratory is to teach students the use of these standard practices.
Table 1: Summary of recommended biosafety levels for infectious agents.

<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Microbial Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to cause disease in health animals</td>
<td>Standard microbiological practices</td>
<td>none</td>
<td>open sink easily cleaned area bench top impervious to water, acids, alkalis, organic solvent and moderate heat</td>
</tr>
<tr>
<td>2</td>
<td>Associated with human disease</td>
<td>Standard procedures + -limited access -biohazard warning signs -sharps precautions -biosafety manual with instructions for specific agents</td>
<td>Biosafety cabinets for any manipulations that cause splashes or aerosols of infectious materials. As needed: -lab coats -gloves -face protection</td>
<td>BSL-1 + autoclave available</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents with potential for aerosol transmission, disease may have serious or lethal consequences</td>
<td>BSL2 practices + -controlled access -decontamination of all waste -decontamination of all clothing prior to laundering -baseline serum</td>
<td>Biosafety cabinets used for all manipulations. Protective lab clothing; gloves; respiratory protection as needed</td>
<td>BSL-2 + -Physical separation from access corridors -Self-closing double door access -exhausted air not circulated -negative air flow</td>
</tr>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents of unknown risk or transmission.</td>
<td>BSL3 practices+ -clothing change before entering -shower at exit -all material decontaminated on exit from facility</td>
<td>All procedures conducted in biosafety cabinets in combination with full-body, air supplied positive pressure personnel suit.</td>
<td>BSL-3 + -separate building or isolated zone -dedicated supply/exhaust and decon systems -other requirements as needed</td>
</tr>
</tbody>
</table>

(Reference: Biosafety in Microbiological and Biomedical Laboratories. HHS Publication No. (CDC) 93-8395, third edition, 1993.)

Although we will not discuss the pathogenicity of microbes until the later third of the course, it is important to understand now that microbes have the potential to cause disease, and without precaution in handling, students are at risk. Microbes can cause infectious disease. The likelihood of disease depends upon

- the characteristics of the microbe,

  (Some have attributes - virulence factors- that readily contribute to disease, while others are poorly suited for growth in an animal, and will never cause disease),

- the characteristics of the host,

  (With the immune system intact, many microbes cannot establish an infection),

- and the exposure to the infectious agent.

  (Microbes will only cause disease if they enter into the system at an appropriate site--portal of entry.)

The reservoir of an infectious agent consists of all of the places where the agent may be found. The goal of standard practices is to ensure that the microbe is not transmitted from that source. Without transmission from source to human host, infection cannot be established.

The use of Standard Microbiological Practices in a laboratory is akin to the use of Universal Precautions in a clinical setting. Universal Precautions are a set of guidelines for the handling all blood and body fluids. According to Universal Precautions, all body fluids are treated as potential sources for infectious agents. These precautions place roadblocks in the trip
a microbe might take from the possibly contaminated source to a health care worker. As microbes can enter the human body via a cut, gloves are always worn when working with body fluids. Additionally there is no eating, drinking or smoking in a lab where body fluids are handled. In hospitals, when leaving the room of one patient to visit another, hands are always washed. These are examples of Universal Precautions. Standard Microbiological Practices serve the same purpose. In the lab, we work to ensure that no microbes are spread from the petri dish or test tube to the human worker. Our lab safety rules are listed below failure to follow these rules will result in your expulsion from the laboratory and failure in the course.

**Laboratory Administration, Procedure, and Safety**

I. Your laboratory instructor has complete control over the laboratory. Failure to follow his or her instructions may result in your dismissal from the course.

II. **Laboratory attendance is mandatory.** Should circumstances beyond your control arise that prevent your attendance, you must contact your lab instructor and explain your absence. If at all possible, this should be done prior to the lab session you are going to miss--arrangements can then be made for your attendance in a different lab section. Three un-excused absences from Laboratory will result in failure in the course.

III. You are required to provide:
   a. waterproof marker,
   b. a three ring binder for lab protocols,
   c. copies of lab protocols printed from the web,
   d. and a lab coat or lab apron.
   Goggles if you are wearing contact lenses.

   In accordance with the University’s Manual on BioSafety, students wearing contact lenses are encouraged to wear protective goggles.

IV. The microbiology laboratory is serious business. All microbial cultures must be considered potential pathogens. All chemical reagents must be considered potentially hazardous. Therefore, your attitude in the laboratory must be attentive, intelligent, and professional. To ensure this, you must read each laboratory protocol before the lab meets, write down lab instructor's notes given at the beginning of each lab, and follow the safety rules listed below.
SAFETY RULES

PERSONAL CONDUCT

1. No eating, drinking, smoking in the lab application of cosmetics or chap stick.

2. Lab coats or aprons must be worn at all times.

3. Proper attire required. No open-toed shoes, lab coats must cover lap.

4. With the exception of your lab notebook, and pen or pencil, no personal items may be placed on the lab bench. Aisles between the benches must remain clear. Personal items must be left at the coat hanger area of the lab.

FIRE

5. Bunsen burners must be turned off when not in use.

6. Long hair must be tied back.

7. In the event of a fire, or if the fire alarm sounds, vacate the lab in an orderly manner. Leave the building via the nearest exit, and wait for further instructions.

ASEPTIC TECHNIQUE

8. Lab benches must be scrubbed with disinfectant at the beginning and the end of each lab session. Microscope eyepieces must be wiped with alcohol-soaked lens paper before and after use.

9. Aseptic technique must be followed at all times. Under no circumstances is anything to be pipetted by mouth.

10. Keep pens, pencils, and fingers out of your mouth. Do not rub eyes with fingers or hands.

11. Wash your hands with soap and water after each lab session, and each time you accidentally touch a culture or something that has come into contact with a culture.

MICROBIAL CULTURES

12. Label all material to be incubated with your name, date, lab section, and culture.

13. Used pipettes must be placed in the pipette receptacles on each bench. All other items containing or exposed to a microbial culture must be disposed in a kill pan.

ACCIDENTS
14. Personal accidents, such as cuts and burns, must be reported immediately to the lab instructor, who will then personally accompany you to the Student Health Center. If you refuse to go to the Health Center, you must sign a release so stating.

15. If you spill a microbial culture, cover it with disinfectant and immediately inform your lab instructor. If you spill a chemical reagent, inform your lab instructor before doing anything.

**Transmission via a handshake.**

How easily is an infectious agent transmitted? Good question! Take the example where you have spilled a culture onto your hand. Or, consider the scenario where you are ill with a bacterial infection such as bacterial pneumonia. You sneeze, cover your nose with your hand and where do those microbes go??

Work with the six students at your bench to design an experiment to answer these questions:
- How easily is a microbe spread, via a handshake, from a contaminated individual to the six students of your lab bench?
- Must the contaminated individual shake your hand directly for you to be exposed?
- How could you stop the transmission of the microbe?

Hint:
Microbes unseen on a glove will grow to visible colonies on appropriate media.

**Materials:**

- 2 gloves/student
- 1 culture *Serratia marcescens* /lab section
- 1 nutrient agar plate/student
- 1 cotton swab/lab bench

*Serratia marcescens* is an organism that produces a red pigment when grown at room temperature. Historically this organism has been associated with observations of “bloody” hosts or bread!
Lab Report 0

Introductory Lab

Draw the plate showing results of thumb print exercise.

1. How easily is a microbe spread, via a handshake, from a contaminated individual to the six students of your lab bench? Explain.

2. Which steps in the safety rules for BSCI 223 involve steps that inhibit transmission of microbes.

3. Give an example that you employ in your kitchen to stop transmission of microbes.

4. Define:
   - Infectious Disease
   - Biosafety level