There is a certain element of risk in anything you do, but the potential risks in a microbiology course are greater. Persons who work in a microbiology lab may handle infectious agents in additional to other hazards such as chemicals and radioactive materials. There have been many documented cases of lab personnel acquiring diseases due to their work. About 20% of these cases have been attributed to a specific incident, while the rest have been attributed to work practices in the lab. It is possible that you can be exposed to potentially harmful microbes when you isolate bacteria from environmental materials. So, you should consider environmental samples potentially hazardous and use BSL2 containment practices (see below). If you are immunocompromised or immunosuppressed, then you may be at greater risk of acquiring infections in this class than most students and should carefully consider whether you should enroll in this course.

A microbiology laboratory is a unique environment that requires special practices and containment facilities in order to properly protect persons working with microorganisms. Safety in the laboratory is the primary concern. The three main elements of safe containment of microorganisms are (1) good laboratory practices and technique, (2) safety equipment, and (3) facility design.

Microbiology Lab Practices and Safety Rules

1. Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.

2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.

3. Purchase a lab coat and safety glasses, bring them to class, and use them. Alternatively, a long sleeved shirt that buttons or snaps closed is acceptable protective clothing. This garment must cover your arms and be able to be removed without pulling it over your head. Leave protective clothing in the lab and do not wear it to other non-lab areas.

4. Avoid loose fitting items of clothing. Wear appropriate shoes (sandals are not allowed) in the laboratory.

5. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in the cubbyholes by the front door of the lab. Place needed items on the floor near your feet, but not in the aisle.

6. Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.

7. Label everything clearly.

8. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.

9. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.

10. Turn off Bunsen burners when not is use. Long hair must be restrained if Bunsen burners are in use.

11. When you flame sterilize with alcohol, be sure that you do not have any papers under you.

12. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.

13. Wear disposable gloves when working with potentially infectious microbes or samples (e.g., sewage). If you are working with a sample that may contain a pathogen, then be extremely careful to use good bacteriological technique.

14. Sterilize equipment and materials.

15. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors. [In the distant past, some lab personnel were taught to mouth pipette. This practice has been known to result in many laboratory-acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited.]

16. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.

17. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.

18. Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station, shower, sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve).

19. Dispose of broken glass in the broken glass container.

20. Dispose of razor blades, syringe needles, and sharp metal objects in the "sharps" container.

21. Report spills and accidents immediately to your instructor. Clean small spills with care (see instructions below). Seek help for large spills.

22. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

Laboratory Safety Equipment

Biological Safety Cabinet

A biological safety cabinet (BSC) is used as a primary barrier against exposure to infectious biological agents. A BSC has High Efficiency Particulate Air (HEPA) filters. The airflow in a BSC is laminar, i.e. the air moves with uniform velocity in one direction along parallel flow lines. Depending on the design, a BSC may be vented to the outside or the air may be exhausted into the room. BSCs are not chemical fume hoods. A percentage of the air is recirculated in most types of

BSCs. HEPA filters only trap particulates, allowing any contaminant in non-particulate form to pass through the filter.

Proper Use of BSCs:

1. Operate the cabinet for five minutes before and after performing any work in it in order to purge airborne contaminants.
2. Before and after use, wipe the surface of the BSC with a suitable disinfectant, e.g., 70% alcohol or a 10% bleach solution.
3. Place everything you will need inside the cabinet before beginning work, including a waste container. You should not have to penetrate the air barrier of the cabinet once work has begun.
4. Do not place anything on the air intake grills, as this will block the air supply.
5. You should prevent unnecessary opening and closing of door because this will disrupt the airflow of the cabinet.
6. Always wear a lab coat while using the cabinet and conduct your work at least four inches inside the cabinet.
7. Place burners to the rear of the cabinet to reduce air turbulence.
8. Do not work in the BSC while the ultraviolet light is on. Ultraviolet light can quickly injure the eye.
9. When finished with your work procedure, decontaminate the surfaces of any equipment.
10. Remove the equipment from the cabinet and decontaminate the work surface.
11. Thoroughly wash your hands and arms.

Eyewash and shower

Fire Extinguisher

First Aid Kit

Emergency Gas Valve

Cleaning Small Spills

First, contact your instructor or the Biology Department Safety Officer. If it is a small spill of a low hazard microorganism or sample, then you should clean the spill yourself.

The proper procedures for cleaning small spills of microorganisms or samples (BSL1 and BSL2 levels):

1. Wear a lab coat, disposable gloves, safety glasses or a face shield, and if needed, approved respiratory equipment.
2. Soak a paper towel(s) in an appropriate disinfectant (70% ethanol or fresh 10% bleach solution) and place around the spill area.
3. Working from the outer edges into the center, clean the spill area with fresh towels soaked in the disinfectant. Be sure to decontaminate any areas or surfaces that you suspect may have been affected by the spill. Allow 10 minutes contact time.
4. Place the paper towels and gloves into a biohazard bag and autoclave these materials to sterilize them.
5. Dispose of any contaminated clothing properly.

6. Wash your hands with a disinfectant soap.

If it is a large spill and your instructor and the Biology Department Safety Officer are not available, then call the UMD Department of Environmental Health and Safety. Each lab is equipped with a spill response kit.

Biosafety Levels and Practices

The Centers for Disease Control (CDC) and the National Institutes of Health (NIH) have developed standard procedures providing protection against biological hazards. The publication, *Biosafety in Microbiological and Biomedical Laboratories* (http://www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm), provides specific descriptions of microbiological practices, laboratory facilities, and safety equipment, and recommends their use in four biosafety levels (BSLs). Biosafety levels are selected to provide the end-user with a description of the minimum containment required for handling different microorganisms safely in a laboratory setting and reduce or eliminate exposure to potentially hazardous agents. Containment refers to safe methods for managing infectious material in the laboratory environment. These biosafety levels are applicable to facilities such as diagnostic, research, clinical, teaching, and production facilities that are working at a laboratory scale. The four biosafety levels are described as:

BSL	Agents	Practices	Safety Equipment	Facilities
1	Not known to cause disease in healthy adults	Standard microbiological practices	None required	Open bench top Hand washing sink required
2	Associated with human disease; main hazard is percutaneous injury, ingestion, and mucous membrane exposure.	BSL-1 plus: • Limited access to lab • Biohazard warning sign • "Sharps" precautions • Biosafety manual defining waste decon and medical surveillance policies	Class I or II BSC or other physical containment devise used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: lab coats; gloves; respiratory protection as needed.	BSL-1 plus: • Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission. Disease may have serious or lethal consequences.	BSL-2 plus: • Controlled access • Decon of all waste • Decon of lab clothing before laundering • Baseline serums obtained	Class I or II BSC or other physical containment device used for all open manipulations of agents. PPE: lab coats; gloves; respiratory protection as needed.	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust not recirculated • Negative airflow into BSL-3 lab
4	Dangerous/exotic agents which pose a risk of life-threatening disease, aerosol-transmitted lab infections, or related agents with unknown risk of transmission	BSL-3 plus: • Clothing change before entering • Shower upon exit • All material decontaminated upon exit from facility	All procedures conducted in Class III BSC or Class I or II BSC **in combination with** full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus: • Lab is in a separate building or isolated zone • Dedicated supply and exhaust system • Other as outlined in text

Biosafety Level 1 (BSL1)

Examples of BSL1 Agents: *Bacillus subtilus*, *Naegleria gruberi*, many *Escherichia coli*, *Infectious Canine Hepatitis Virus*

BSL1 containment is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. A BSL1 lab requires no special design features beyond those suitable for a well-designed and

functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Biosafety Level 2 (BSL2)
Examples of BSL2 Agents: *Bacillus anthracis*, *Bordetella pertussis, Brucella* spp., *Cryptococcus neoformans*, *Clostridium botulinum, Clostridium tetani, Helicobacter pylori,* most *Salmonella* spp., *Yersinia pestis, Mycobacterium leprae*, *Shigella* spp., *Human Immunodeficiency Virus*, *Human blood*

The primary exposure hazards associated with organisms requiring BSL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring BSL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand washing sinks and decontamination facilities (autoclaves).

Biosafety Level 3 (BSL3)
Examples of BSL3 Agents: *Myobacterium tuberculosis, Salmonella typhi, Vesicular Stomatitis Virus*, *Yellow Fever Virus*, *Francisella tularensis*, *Coxiella burnetti*

Laboratory personnel have specific training in handling these pathogenic and potentially lethal agents and are supervised by scientists who are experienced in working with these agents. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. BSL3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of BSL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Biosafety Level 4 (BSL4)
Examples of BSL5 Agents: smallpox virus, Ebola virus, hemorrhagic fever viruses

This is the maximum containment available and is suitable for facilities manipulating agents that are dangerous/exotic agents, which post a risk of life threatening disease. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas. BSL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.