

**Biosafety Level 1**

**Standard Operating Procedures**

**(Lab Location Information)**

* 1. **Lab Practices and Safety Rules**

1. Wash your hands with disinfectant soap when you arrive at the lab, after working with any potentially hazardous materials, and again before you leave.
2. Absolutely no food, drinks, chewing gum, application of cosmetics or contacts is allowed in the lab. Absolutely no 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 lab, 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 should be flame sterilized in a Bunsen burner before you lay them down.
10. Tum off Bunsen burners when not in use. Long hair must be restrained if Bunsen burners are in use.
11. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.
12. 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. Do not wash or reuse gloves. Dispose of used gloves in the appropriate biosafety bag.
13. Sterilize equipment and materials.
14. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors.
15. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.
16. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.
17. 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).
18. Dispose of broken glass in the broken glass container.
19. No razor blades, syringe needles, or sharp metal objects will be used in this laboratory.
20. Report spills and accidents immediately to your instructor. Clean small spills with care (see instructions below). Seek help for large spills.
21. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

Cleaning Spills

When a spill occurs, first, contact your instructor. 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 (BSLl):

1. Wear a lab coat, disposable gloves, safety glasses or a face shield, and jf 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 IO 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 soap.

If it is a large spill and your instructor is not available, then call Risk Management and Safety (RMS). Each lab should come equipped with a spill response kit.

* 1. **Laboratory Specific Biosafety Policies**

Biosafety Level 1 (BLI)

Good Microbial Practices:

1. All bacteria and chemicals in the laboratory are to be considered dangerous. Do not touch, taste or smell any bacterial culture or chemical unless specifically told to do so.
2. For bacteria or chemicals ingested, see the lab instructor immediately.
3. Check the label on cultures and chemical bottles twice before removing any of the contents. Take only as much of the bacterial culture or chemical as you need.
4. Never return unused chemicals to their original containers.
5. Never use mouth suction to fill a pipet. Use a rubber bulb or pipet pump. Always keep the pipet pointed away from your body.
6. Never dispense flammable liquids such as ethanol anywhere near an open flame or source of heat.
7. Perform all procedures to minimize the creation of splashes and/or aerosols.
8. Never remove bacteria, chemicals or other equipment from the laboratory.
9. Take great care when transporting cultures and chemicals from one part of the laboratory to other. Hold them securely and walk carefully. All cultures should be in a test tube rack.

Spill Response and Decontamination Procedures:

Use the guidelines below for response to spills of biological materials outside of the biosafety cabinet. The laboratory will be equipped with a spill kit containing necessary materials for cleaning up a spill. All lab personnel will know where it is stored so that it can be retrieved quickly. Spill kit components will be replaced as they are used to prepare for the next incident.

Spill kit contents:

* Gloves
* Safety glasses or goggles
* Paper towels or pads to absorb contaminated liquids
* Biohazard waste bag
* Outline of the spill response SOP

**Surface contamination:**

1. Notify everyone else working in the room that there has been a spill and not to walk through the contaminated area. Notify your Principal Investigator (PI) as soon as possible.
2. Put on necessary protective equipment: gloves and lab coat at a minimum.
3. Cover the spill with paper towels and pour 10% freshly diluted bleach or other effective disinfectant over spill. Do not spray.
4. Allow to sit for at least 10 minutes or the recommended contact time depending on the disinfectant.
5. Wipe with paper towels, and discard towels into autoclave bag.
6. Decontaminate surrounding floor and work surface areas where splashes or larger aerosols may have settled around the spill.
7. Repeat the decontamination procedure.
8. Remove contaminated clothing and place in autoclave bag.
9. Remove gloves and put in autoclave bag.
10. Wash your hands thoroughly.

**Personal contamination:**

1. Notify everyone else who is working in the room of the exposure. Notify your Principal Investigator (PI) as soon as possible.
2. Flush the exposed surface (eyes, mouth, nose or skin) with water for 15 minutes
3. Apply first aid if necessary and treat as an emergency
4. Notify supervisor or Police/RMS if after hours
5. Report to a medical clinic for treatment or counseling

Waste Disposal Procedures:

All personnel are responsible for maintaining a clean work area. Only trained individuals should operate the autoclave.

1. Solid materials

**Solid infectious** materials (used pipettes, flasks, Petri dishes, etc.) must be disposed of in autoclave waste bags. Waste should be placed in a plastic or metal pan to contain any leaks. The autoclave should be run for one hour or sufficient time to fully decontaminate the waste. To request a biological indicator to test the autoclave's effectiveness, contact the biosafety officer.

1. Liquid waste

Liquid infectious wastes, such as spent media, can be autoclaved and poured down the sink or decontaminated by adding household bleach to a final concentration of 10%, allowed to sit for at least 30 minutes, then poured down the sink in the laboratory.

1. Uncontaminated waste

Uncontaminated non-sharp waste should be disposed of in the general lab waste stream. Uncontaminated broken glass is disposed of in a sturdy cardboard box, preferably lined with a plastic bag. When full, the box should be taped closed and disposed of in the dumpster. Housekeeping will not dispose of broken glass.

1. Sharps disposal

Sharps are items which pose a puncture or cutting hazard, such as glass, needles, and razors. No sharps will be used in this lab.

1. Disposal of waste into dumpsters

Lab staff is responsible for transporting autoclaved waste to the dumpsters in a timely manner. Waste bags should not be left sitting in the laboratory or autoclave room for more than a few hours. If the dumpster is full, trash bags may NOT be discarded outside the dumpster. Bags must be returned to the lab and disposed of when the dumpster has been emptied.

* 1. **Use of the Centrifuge**

Aerosol containment for procedures done outside of the BSC is important. Centrifugation is a common type of lab procedures with a risk of generating aerosols. Aerosols may be generated if liquid leaks from the tube or container while the centrifuge is running, since this liquid will get splattered around the rotor and/or chamber. Leaks often happen if a tube or container cracks or breaks during the run. They can also happen if the cap is not secured to the container properly. Unfortunately, there is no way to know if such accidents have occurred during the run, until after you open the centrifuge and see the leaks. At that point, exposure to infectious aerosols has already occurred. To prevent exposures to infectious aerosols, it is expected that lab personnel use the following precautions:

* Always use a sealed rotor lid with fixed-angle rotors. These are typically screw-on lids, rather than
* Snap-on lids, and they have an O-ring to ensure an airtight seal on the rotor. Note, the centrifuge lid is not the same as the rotor lid and does not provide the necessary personal protection from aerosols.
* For swinging bucket rotors, use safety cups or buckets. These are the same type of tube holder or multi­ well plate holder that are typically used in tabletop centrifuges, except they have lids fitted with O-rings to ensure an airtight seal.
* Always inspect the O-rings for integrity prior to use. Replace any that are showing signs of wear.
* Whenever possible, use tubes with screw-on caps. Those with O-rings are an even better option.
* Never overfill the containers, and always be sure to balance your samples before starting the centrifuge.

Vortexing will be done with a capped or closed container.

Accidental Spills in the Centrifuge:

Spills or breakage of containers inside of an operating centrifuge poses a serious potential for exposure due to the creation of aerosols. If a primary container has broken in a centrifuge without a closed rotor or bucket, immediately suspend use, notify lab staff and Instructor.

For suspected or confirmed spills/breakage in any centrifuge, wait at least 30 minutes after the centrifuge has stopped operating to initiate clean up.

1. Put on lab coat and gloves prior to opening centrifuge. Open carefully to assess the damage.
2. If the spill is contained within a closed cup, bucket or rotor, spray the exterior with disinfectant (bleach) and allow at least 10 minutes of contact time. Remove the carrier to the nearest biosafety cabinet (BSC). If a biosafety cabinet is not available, close the centrifuge, post a sign to indicate it cannot be used. Notify the Instructor.
3. If a BSC is available, gather supplies needed, such as a sharps container for broken glass and bins filled with disinfectant and place into the BSC. Use forceps to remove broken glass and place directly into sharps container. Carefully remove any unbroken tubes and place into a bin filled with disinfectant for 20 minutes. Wipe carrier/bucket with disinfectant.
4. After disinfection, carrier, bucket or rotor should be washed with a mild soap and water.
5. Spray the interior of the centrifuge chamber with a disinfectant, let sit for 20 minutes and then wipe down
6. Remove protective clothing and wash hands.
   1. **Use of the Autoclave**
7. Only persons trained and approved by laboratory supervisor can operate autoclave.
8. Suggested temperatures and exposure times for autoclaving from NIH Biohazards Guidelines are:
9. Liquids 121°C (250°F) 1 hour, (each gallon)
10. Laundry 121°C (250°F) 30 minutes
11. Trash 121°C (250°F) 1 hour
12. Glassware 121°C (250°F) or 160°C (320°F) 1 hour to 4 hours (dry heat)
13. The autoclave is operated in (insert Building and Room # information).

The autoclave will be tested biweekly using a commercially available test indicator kit that uses bacterial spores (Geobacillus stearothermophilus). Test results and date are recorded in Autoclave log.

1. Testing Frequency: (Building and Room Number): (Frequency (e.g. Once a month, Biweekly)
2. Biohazardous waste will be documented on an autoclave waste treatment record. The record should contain the date of treatment, the amount of waste treated, the method/conditions of treatment, and the printed name and initials of the person performing the treatment. If provided for, charts or printout strips should be kept with the record as documentation. Additionally, documentation of the date and results of all verification tests using biological indicators is required.
   1. **Emergency Procedures**

Fire evacuation procedures

During a fire emergency, lab staff should prioritize life safety. Walk to the nearest exit. Pull the fire alarm if necessary, and call 911 once outside the building.

Power outage

In the event of a power outage, put away cultures. Remove PPE and exit the lab normally. Emergency lighting within the buildings should provide adequate visibility to exit the building. Notify the PI immediately.

Medical emergency

In the event of a medical emergency in the lab, follow appropriate procedures depending on the hazards present. If the emergency involves a spill of hazardous materials onto the clothing or body, assist the victim to the shower or eyewash station. If the victim requires medical attention, call 911.

Accidental exposure

For splashes to the eyes, rinse the eyes under the eyewash for 15 minutes. If the victim requires medical attention, call 911. Report to Risk Management Safety and follow up by contacting Occupational Health Partners.

Potential Health Risks

Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to their healthcare provider for appropriate counseling and guidance.