

Biosafety Manual

Everhart Lab

October 9, 2014

Welcome to the lab! Your health and safety is a priority, as is the health and safety of the other lab members that you work with. Since you are new to the lab, there are aspects of working in a lab that we want you to be especially aware of. Below are required activities that should be completed as soon as you've joined the lab:

1. Read this biosafety manual, as it has the most important information
2. Take the required online training <http://ehs.unl.edu/web-based-training>

Required for everyone:

- Core - Injury and Illness Prevention Plan
- Core - Emergency Preparedness
- EHS Chemical Safety

Additional required training for temporary workers:

- EHS Biosafety Basics Training

Additional required training for permanent workers (graduate students, postdocs, technicians):

- EHS Biosafety in the BSL-2 Laboratory
- Personal Protective Equipment Training
- NIH Guidelines Module

Additional recommended training for permanent workers:

- EHS Fire Extinguisher Training
- EHS DOT/IATA Training for shipping biological

3. Review the online virtual lab manual:
<https://scsapps.unl.edu/VirtualManual/AccessYourProfile.aspx>
E-mail address: everhart@unl.edu
4. When in doubt, ask a lab manager what you should do.

Dangers from the organisms we work with

APHIS Regulated Plant Pathogens

In general, the organisms we work with pose no human threat. However, all are pathogens of economically important crops and regulated by the USDA Animal and Plant Health Inspection Service (APHIS). Below are some basic handling procedures for fungi in our lab:

1. Door to lab must remain locked and closed.
2. Transferring cultures must be done in the level-2 biosafety cabinet.
3. All cultures that are no longer needed for research must be disposed of properly, which means autoclaving before disposal in the trash.
Read through the Autoclave Operation guide:
<http://ehs.unl.edu/sop/s-bio-autoclavesafety.pdf>
4. All items that come into contact with these organisms must be autoclaved before disposal.
5. Wash your hands after completing work.
6. All work surfaces should be disinfested using 70% ethanol after use.
7. If a spill occurs, disinfest the area using 70% ethanol →



Opportunistic Human Pathogens

Although the plant pathogens that we work with have not been reported to infect healthy humans, a few of the pathogens in our lab have been reported to infect humans that have compromised immune systems. If you have a compromised immune system, please notify lab managers. Plant pathogens in our lab that have been reported to infect immune-compromised humans:

- *Colletotrichum graminicola*
- *Fusarium verticillioides*
- *Macrophomina phaseolina*



Dangers in working with chemicals

Working with fungicides

There are some basic safety procedures you should follow if you work with fungicidal chemicals (many of these same procedures apply for working with all chemicals):

1. Look up the Material Safety Data Sheet (MSDS) online, which describes the health dangers for the chemical you're going to work with.
2. Based on these dangers, select the appropriate personal protective equipment (PPE).
3. Basic PPE that is required:
 - a. Lab coat
 - b. Gloves
 - c. Eye protection
4. Additional PPE may be necessary, depending on the chemical, such as:
 - a. Working in the fume hood
 - b. Wearing a face shield
5. Know how to use the eye wash. Don't know? Ask! →
6. Do not add fungicides to hot media. Place flask in water bath and wait until it has cooled to the touch (approx. 30 min). This practice will help ensure the chemicals are not volatilized into the air you breathe.
7. If a chemical spill occurs, make sure to take care of your own personal safety first. Then, find the chemical spill kit, located under the sink →
8. Report spills to lab managers
9. For additional information, see:
http://ehs.unl.edu/training/colloquium/2007-09_Presentation.pdf



Working with ethidium bromide (EtBr)

Although our lab strives to avoid work with ethidium bromide (EtBr), there are times when it will be used in running gels. EtBr can be absorbed by the skin and is a known mutagen. For your own protection, please observe the following:

1. Always wear nitrile gloves that are less permeable than latex and help prevent contact of EtBr with bare skin.
2. Do not touch the area where work with EtBr is conducted.
3. When working with a gel that contains EtBr, assume that your gloves are contaminated and remember to remove a glove before touching any non-contaminated work surface.
4. For additional information, see:
http://research.unl.edu/docs/HighlyToxic_Resources.pdf

What would you do?

The following are scenarios that you may encounter while working in the lab.

Q. Someone is using ethanol to sterilize tools used to transfer cultures and accidentally lights the container of ethanol on fire, what do you do?

A. Simply replace the lid to the container to smother the fire.

Q. You're planning to use liquid nitrogen to grind tissue for DNA extraction, but because it's a hot day out, you're wearing shorts and low coverage shoes. What should you do?

A. Delay your plans for the day because your work with liquid nitrogen could easily splash and cause serious burns to any exposed skin. Consider bringing appropriate clothing in a separate bag and changing once you arrived at work to avoid this danger.

Q. You come to the lab, find the door open, and someone you don't recognize is there. What do you do?

A. Ask this person if they need any assistance and find out if they are permitted to be there. No unauthorized access is permitted in the lab because it contains APHIS regulated plant pathogens. Notify lab managers of what happened.

Q. You're working with a culture of one of the plant pathogens in our lab and you accidentally knock cultures off of the counter and onto the floor, where the fungus makes contact with the floor. What do you do?

A. Any culture dishes that opened will likely be contaminated and require disposal, so place those cultures into the autoclave trash. To decontaminate the floor, use 70% ethanol, spread across area and allow drying.

Remember, as a new lab member, you are responsible for your own safety and not compromise the safety of others in the lab. If something has happened that you are concerned about, please don't hesitate to mention this to the lab managers. We want this to be a safe and happy work environment.