Biosafety Level 2 Training

This training is required by the National Institutes of Health.
The Aim Of Biosafety Training Is To Increase Your Ability To Recognize And Reduce Hazards In a BSL2 Lab

• Think before you do anything...
  • What could possibly happen? What is the worst thing that could happen?
  • What can I do to prevent it?
  • What will I do if I can’t prevent it?
What is Biosafety?

• Biosafety describes the practices and containment under which potentially biohazardous materials can be used safely.

• What are potentially biohazardous materials?
  – **Infectious agents** or pathogens such as bacteria, viruses, fungi & protozoa
  – **Recombinant DNA** (rDNA) such as genes cloned into plasmid or viral vectors
    • Do the genes encode toxins, antibiotic resistance or oncogenes?
    • Will they be expressed or used to generate transgenic organisms?
  – **Toxins** such as tetrodotoxin, ricin and botulism
What is Biosafety?

• Biosafety describes the practices and containment under which potentially biohazardous materials can be used safely.

• The goal is to protect or ensure the safety of
  – individuals who work with potentially biohazardous agents.
  – others in the work area, the community and the environment.

• Biosafety defines a code of safe working practices and facility design.
What is a Biological Safety Level?

- A Biosafety Level can be assigned to laboratory work
  - Each Biosafety Level describes the practices and containment that will reduce the risk of exposure to potential biohazards.
  - There are 4 levels of biosafety
    - **Level 1** represents the practices and containment required for biohazards that pose the *lowest* hazard.
    - **Level 4** is reserved for labs using materials that pose the *greatest* hazard.
What Is Meant By Each Biosafety Level Designation?

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<tr>
<td>How the biohazardous material will be manipulated</td>
</tr>
<tr>
<td>The primary protective barriers and safety equipment</td>
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<tr>
<td>The secondary protective barriers or the facility design</td>
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These 4 elements are considered during a risk assessment that is used to determine the biosafety level that will be applied to a lab.
The Practices And Containment Required in BSL1 Labs are the Basis for Safety in BSL2, BSL3 and BSL4 Labs

This training will describe the practices and containment required in a BSL1 lab and the additional requirements for a BSL2 lab.
What is Meant by a **BSL1** Designation?

1. The biohazardous material to be used

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| Facilities design (secondary containment) | Handwashing sink, emergency shower and eyewash, autoclave | Same as BSL1 |

* many agents not ordinarily associated with disease are opportunistic pathogens that may cause infection in the very young, the aged and in immunocompromised individuals.

Biosafety level 1 is appropriate for agents that are generally not pathogenic:

- *E. coli*
- *Saccharomyces cerevisiae* (or yeast)
- plasmid cloning vectors
What is Meant by a BSL2 Designation?

1. The biohazardous material to be used

- BSL2 is required with:
  - Human pathogens such as: *Salmonella sp.*
  - *Staphylococcus sp.*
  - Influenza virus
  - Hepatitis A virus
  - Adenoviral and lentiviral cloning vectors
  - All human cell lines, tissues, body fluids and OPIM (OSHA’s Bloodborne Pathogens)

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What is Meant by a **BSL1** Designation?

2. What are you doing with it?

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Standard Microbiological Practices should be observed.

These are prudent practices that should be observed in all labs.
What is Meant by a **BSL2** Designation?

1. What are you doing with it?

### Characteristics of the biohazardous material

- **BSL1**: Not known to consistently cause disease in healthy adults
- **BSL2**: Associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

### Planned manipulations

- **BSL1**: Standard Microbiological Practices
- **BSL2**: BSL-1 practices plus Biosafety manual defining: Restricted access, Biohazard warning signs, “Sharps” precautions, Biowaste practices, Medical surveillance & Spill Clean-up.

### Primary containment and protection

- **BSL1**: PPE: gloves, lab coat and eye protection are used
- **BSL2**: BSL-1 protection plus: Physical containment for splashes/ aerosolization; Biosafety Cabinets: aka “tissue culture hoods”

### Facilities design (secondary containment)

- **BSL1**: Handwashing sink, emergency shower and eyewash, autoclave
- **BSL2**: Same as BSL1

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BSL2 practices are based on the standard microbiological practices of BSL1 labs **BUT with additional Precautions.**

**First, what are Standard Microbiological Practices?**
Standard Microbiological Practices

are prudent practices that should be included in every laboratory safety program:

• Wash hands after handling biologicals, taking off gloves and before leaving the lab.
• No eating, drinking, smoking, or applying cosmetics in the lab.
• Wear clothing (sleeves, scarves, shoes, jewelry) appropriate to your tasks. Tie hair back.
• Always use mechanical pipetting devices (never mouth pipette).

These safety practices should be familiar to you! There are additional practices required in BSL2 labs that may not be as familiar.
BSL2 Practices and Containment Must be Documented in SOPs and a Biosafety Manual

• Standard Operating Procedures or SOPs include the “BSL2 rules” for specific procedures.
  – These documents should be in a “Safety Notebook”
  – Know where it is in your lab and be familiar with the information

• A copy of the F&M Biosafety Manual is in every lab
  – It is also available at www.fandm.edu/bisafety
BSL2 Labs Must Have “Restricted Access”

• Restricted Access at BSL2 means:
  – Immunocompromised individuals are advised of the increased risk.
  – There are “entry requirements” for the lab, such as specific training and Hepatitis B immunization.
  – Doors are kept closed during experiments
  – An approved biohazard sign must be posted at all access areas of the BSL-2 lab.
Communicating Biohazards

• Be aware of warning signs: the biohazard symbol
  • has a fluorescent red background with the universal biohazard symbol in black
  • is used to alert workers to the presence of potentially biohazardous materials

• Be aware of labeling procedures
  • Lab doors
  • Waste containers
  • Any equipment that could be contaminated
Decontaminate Work Surfaces

• Work surfaces must be decontaminated....
  • Before you begin to work every day
  • After a spill, splash or any contamination
  • After you are finished with your work or at the end of the day
• Use Simple Green, Lysol or 10% bleach
  • When you apply the disinfectant, be careful not to generate splashes
  • Allow the disinfectant to remain on the bench for a few minutes before you wipe it up
• Dispose of the paper towel in the biohazard waste bag
• Wash your hands
Sharps Precautions

• Extreme caution should be taken with contaminated sharps.
  • Percutaneous exposure (through the skin) is a primary route of transmission of many pathogens
• Sharps include anything that can pierce your skin (or a sheet of paper!)
  • syringe needles
  • razor blades and scalpel blades
  • broken glass
  • microscope slides
  • plastic pipettes and tips
Sharps Precautions: Broken Glass

• To clean up **Broken Glass**
  – Wear gloves
  – Do NOT attempt to pick up pieces of broken glass with your gloved hands. The risk of being cut is too great.
  – Use tongs or a brush and dust pan-or even 2 pieces of stiff cardboard- to collect broken glassware.
Disposal of Sharps

• **Proper Disposal of Needles, Sharps and Broken Glass**
  
  • Contaminated sharps may be placed in hard walled plastic containers labeled with the biohazard symbol.
  
  • Some types of sharps – tips and microscope slides – may be disposed of in waxed cardboard containers.
  
  • If the sharps or pieces of broken glass are too large, the items must be placed in a puncture resistant resistant container (such as a cardboard box) lined with a biohazard bag, clearly marked "GLASS AND SHARPS".
Biohazardous Waste Management Practices

- All solid biohazardous waste must be placed in red biohazard bags
  • These will be incinerated or steam sterilized before disposal.

- All liquid biohazardous waste must be sterilized by autoclaving or treatment with approved disinfectant – 10% bleach for >20 minutes before drain disposal.
What To Do In The Event Of A Spill

• Use appropriate PPE: gloves, goggles and lab coat
• If there is broken glass, it should be cleaned up using tongs or a dust pan and placed into either a sharps container or a biohazard bag that is inside a cardboard box.
What to do in the event of a spill, cont’d

• Spills should be treated with disinfectant (10% bleach) for >20 minutes.
  – Use absorbent material soaked in disinfectant to cover the spill
  – Cover the spill with paper towels and then pour on disinfectant
  – Be careful not to generate splashes and aerosols!
  – The paper towels should be placed in the biohazard waste.

• Repeat the disinfection process.
Spill Clean Up Procedures, con’t

- All PPE should be placed into biohazard waste and hands washed thoroughly.
- Report the incident to your instructor immediately.
  - Complete an Incident and Accident Report Form
  - These are in the back pocket of the Biosafety Manual and at fandm.edu/biosafety
What To Do In The Event Of An Exposure

• **Immediate** First aid
  • Wash the affected area with disinfectant for >15 minutes.
  • For membrane exposure (eyes, nose, mouth) flush with water for >15 minutes.
  • Cover the wound.

• Exposure may require an immediate evaluation by a medical practitioner.
  – If you feel that an evaluation is appropriate, go to Appel Health or Lancaster General Hospital
What to do in the event of an exposure, cont’d

• Report the incident to your PI immediately and to the Biosafety Officer

• Complete a Laboratory Incident and Accident Report Form
  – These are in the back pocket of the Biosafety Manual and at fandm.edu/biosafety

• Complete a Blood and Body Fluid Exposure form
  – These are in Appendix C of the F&M Exposure Control Plan, in the back pocket of the Biosafety Manual notebook
What is Meant by a **BSL1** Designation?

### 3. Primary Containment

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- **Primary Containment**
  - Gloves, lab coats and eye protection are used in BSL1 labs to protect workers

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Personal Protective Equipment

The minimum PPE required for BSL-1 laboratories is gloves, safety glasses (or goggles) and lab coats.

Additional PPE such as surgical masks or faceshields may be required for procedures with high probability for splashes, droplets and aerosol generation.
Proper Use of Gloves

• Gloves should NOT to be worn outside of the work area.
  – DO NOT wear gloves to open doors or touch equipment (i.e. keyboards or
door handles) that others will be handling without gloves.

• Gloves must be replaced as soon as possible after they become
  contaminated, torn, punctured or compromised.
  – Be extremely careful when using a flame- do not catch the gloves on fire!

• Disposable gloves cannot be reused.

• Gloves must be removed CORRECTLY prior to washing hands and
  leaving the laboratory.
  – With both hands gloved, pinch the top of one glove, carefully pull it off so
    that it is inside out and hold it in the gloved hand.
  – Using the ungloved hand, grab the inside top of the second glove and pull
    it off so that it is inside the first glove.

• All PPE must be disposed of as biohazardous waste.
Proper Use of Lab Coats and Goggles

- Lab coats should be BUTTONED to protect your street clothing.
- You must NOT take potentially contaminated lab coats home.
  - Handle soiled lab coats as little as possible, using gloves.
  - Place soiled lab coats in autoclavable bags. After steam sterilization, they may be laundered and reused.
- Eye/face protection should be used if splashes or sprays are a possibility when working outside a biosafety cabinet.
What is Meant by a **BSL2** Designation?

### 3. Primary Containment

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Primary containment at BSL2 is based on the use of PPE.

**BUT** additional measures to provide protection from aerosols are required: use of sealed centrifuge rotors and Biosafety Cabinets.
Protection from Aerosols in a BSL2 Lab

What are Aerosols?
Aerosols are solid or liquid particles suspended in the air (generally 1 to 100 μm)

- Larger particles settle more rapidly becoming a risk for surface contamination.
- Smaller particles can remain airborne for a long period of time and spread wide distances.
- Smaller particulates (1 to 10 μm) are also more easily inhaled.
Do I Ever Generate Aerosols?

Here are some common laboratory procedures that may produce aerosols:

- blowing out pipettes
- dropping / breakage of culture containers
- **carelessly removing protective gloves**
- harvesting infected material
- flaming inoculating needles, slides or loops
- inserting a hot loop into a culture
- opening lyophilized cultures, culture plates, tubes and bottles
- **pouring liquids**
- **removing stoppers**
- stirring liquids
- streaking inoculum

In addition, there are many devices that, if used incorrectly, may create aerosols, including:

- blenders and vortexers
- bottles and flasks
- **centrifuges**
- homogenizers
- needles and syringes
- pipettes
- pressurized vessels
- rubber stoppers
- **shakers**
- sonicators
- vacuum and aspirating equipment
It Is Important To Minimize The Production Of Splashes And Aerosols!

• How to control splashes, aerosols and droplet production
  – Pathogens may be transmitted by inhalation and by membrane exposure to droplets and aerosols

Biological safety cabinet or BSC

sealed centrifuge rotors
Proper Use of Biological Safety Cabinets
Contains Aerosols

The biosafety cabinet is relied upon to:

1. protect the **worker** from procedures that may generate an aerosol.
2. protect the **environment** by preventing release or exhaust of aerosols.
3. protect materials in the cabinet: provide a sterile **work environment**.
How Does a Biosafety Cabinet Offer Protection?

A- Room air is pulled into the cabinet by fans.

B- The air is circulated behind the work space to the top of the cabinet.

C- Some of the air is filtered through a HEPA filter and exhausted.

D- Some of the air is filtered through another HEPA filter and this sterile air is directed onto the work space.

Potentially contaminated air from the work space mixes with air pulled in from the room and is directed back to the HEPA filters.

HEPA filtered air is considered to be sterile!
Tips for using a biosafety cabinet correctly!

• Preplanning is important to minimize contamination
  • Every movement in and out of the cabinet disrupts the delicate air barrier.
  • Avoid having materials in the cabinet that are not required for your procedure.
  • Placing items on the grate at the front of the cabinet disrupts the air flow patterns
  • Use of a flame (for sterilization) inside a biosafety cabinet is not allowed.
Samples Being Transporting Outside Of A BSL2 Lab Require Secondary Containment.

- Use secondary containers (e.g. ziploc bag w/ a paper towel or other absorbent material).
- Secondary containers should be leak-proof, closable and labeled with the biohazard symbol.
- Use a cart to help prevent accidents where materials are dropped.
What is Meant by a **BSL1** Designation?

### 4. Facility Design

- A hand washing sink, safety shower, eyewash station and autoclave must be available

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What is Meant by a **BSL2** Designation?

4. **Facility Design**

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No additional facilities are required for BSL2 Labs.
If you have any questions.... ever

• Ask your research advisor
• Check out the lab SOPs, the Biosafety Manual or the F&M Exposure Control Manual
• Ask the Biosafety Officer
  – Dr. Frielle
  – LSP332E
  – 358-4600
  – dfrielle@fandm.edu
Review

The next few slides contain quiz questions (and answers) to help you review for the post-test.
Which is **not** true of both BSL-1 **and** BSL-2?

a. Standard Microbiological Practices are followed.

b. Work is conducted with microbes that are not known to consistently cause disease in healthy adults.

c. A handwashing sink, eyewash & safety shower should be readily available.

d. All of the above
Which is **not** true of both BSL-1 **and** BSL-2?

a. Standard Microbiological Practices are followed.

b. **Work is conducted with microbes that are not known to consistently cause disease in healthy adults.**

c. A handwashing sink, eyewash & safety shower should be readily available.

d. All of the above
Which of the following material(s) must be handled at BSL-2?

a. Well characterized human cell lines.
b. Adenoviral and lentiviral vectors
c. *Saccharomyces cerevisiae* (yeast)
d. A & B only
e. All of the above
Which of the following material(s) must be handled at BSL-2?

a. Well characterized human cell lines.
b. Adenoviral and lentiviral vectors
c. *Saccharomyces cerevisiae* (yeast)
d. A & B only
e. All of the above
True or False?

Biohazard symbols must be present only on the doors to BSL2 labs but not on equipment in the labs.
False!

All equipment used in BSL-2 containment must be posted with a biohazard label. For example, hoods, freezers, incubators, and centrifuges, etc. should be labeled if they are used with biohazardous materials. Also, transport containers and biohazard waste containers must display a biohazard label on the outermost part.
What should you do in the event of an exposure?

a. Run to the shelf, get the lab safety notebook and quickly look up “What to do in the event of an exposure”.

b. Be familiar with the SOP and know what to do in the event of an exposure.
What to do in the event of an exposure

• Follow the procedure in your lab’s SOP and the F&M Biosafety Manual.
  • Wash the affected area with disinfectant for at least 10 minutes.
  • For membrane exposure (eyes, nose, mouth) flush with water for at least 15 minutes.
  • Cover the wound.

• Exposure may require an immediate evaluation by a medical practitioner.

• Report the incident to your research advisor immediately and to the Biosafety Officer (Dr. Stephanie Stoehr 358-5975, sstoehr@fandm.edu)
What should you do in the event of a spill?

a. Run to the shelf, get the SOP and quickly look up “What to do in the event of a spill”.

b. Be familiar with the SOP and know what to do in the event of a spill.
What to do in the event of a spill

• Use appropriate PPE: gloves, goggles and lab coat
• If there is broken glass, it should be cleaned up using tongs or a dust pan and placed into either a sharps container or a biohazard bag.
• Small spills should be treated with disinfectant for 10 minutes, cleaned with absorbent material like paper towels. The paper towels should be placed in biohazard bag. Repeat the disinfection process.
What to do in the event of a spill, con’t

• All PPE should be placed into biohazard bag and hands thoroughly washed before leaving the lab.

• Report the incident to your PI immediately.
A biological safety cabinet is not:

a. A cabinet that provides a sterile work environment for manipulations
b. A cabinet that provides protection of the worker conducting the manipulations
c. A cabinet that provides protection of the surrounding laboratory and the environment
d. A biological safety cabinet does all of these things
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Who can answer your questions?
If you have any questions.... ever

• Ask your research advisor
• Check out the lab SOP, F&M Biosafety Manual and/or the F&M Exposure Control Manual
• Ask the Biosafety Officer
  – Dr. Stephanie Stoehr
  – LSP254D
  – 358-5975
  – ssstoehr@fandm.edu
Take the test!

When you are ready, you can take the Biosafety Level 2 Training Post-test. It will be graded automatically and you will be able to determine if you passed – passing requires a 100% test score.

If at first..... Try again!

Good luck!