Biosafety Level 2 (BSL2) Training

BIOHAZARD

Biosafety Level 2

AUTHORIZED PERSONNEL ONLY

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For information contact the Dept. of Environmental Health & Safety (ext: 962-5367)
This training will help you to recognize potential hazards and reduce your risk.

- 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 is the application of practices and containment necessary to reduce the risks associated with biological hazards, such as potentially pathogenic organisms and toxic agents.

• Biosafety describes the conditions under which such agents can be safely used to prevent the:
  – release of potentially biohazardous agents to the environment
  – exposure of lab personnel to potentially biohazardous agents
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?

#### What are the elements that determine a Biosafety level?

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<th>BSL Level</th>
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<td>BSL1, 2</td>
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<tr>
<td>How the biohazardous material will be manipulated</td>
<td>BSL3, 4</td>
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All of these elements are considered during a risk assessment that is used to determine the biosafety level that will be applied to a lab.
Resources for Risk Assessment and Determination of Biological Safety Level

In the U.S., biosafety levels (BSL) are defined in two documents:

1. **Biosafety in Microbiological and Biomedical Research Laboratories** (the BMBL) from the Centers for Disease Control and Prevention (CDC).

2. **The NIH Guidelines for Research Involving Synthetic and Recombinant Nucleic Acid Molecules** (the NIH Guidelines) from the National Institutes of Health (NIH).
What is a BSL1 Lab?

Biosafety level 1 is appropriate when agents that are generally not pathogenic are in use:

- *E. coli*
- *Saccharomyces cerevisiae* (or yeast)
- plasmid cloning vectors

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What is a BSL1 Lab?

Standard Microbiological Practices should be observed.

The practices followed at higher biosafety levels are based on these standards.

What are Standard Microbiological Practices?

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Standard Microbiological Practices

Daily practice of these activities is critical 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.
- Use personal protective equipment (e.g., lab gowns, coats, and gloves).
- Always use mechanical pipetting devices (never mouth pipette).
- Decontaminate work surfaces daily and after spills.
- Avoid using hypodermic needles. Refer to the Sharps Handling and Disposal section of your lab’s Standard Operating Procedure.
- Use procedures that minimize the formation of aerosols.
- Place all solid biological waste in red autoclavable bags for disposal. Liquids must be disinfected before sink disposal.
What is a BSL1 Lab?

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

- **Secondary Containment**
  - A hand washing sink, safety shower and eyewash station must be available
Biosafety Levels 2, 3 And 4 Build On The Practices And Containment Required in BSL1 Labs

The remainder of this course will describe BSL-2 practices and regulatory requirements.

There are no BSL3 or BSL4 labs at F&M!
What is Biological Safety Level 2?

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![Diagram showing BSL levels from 1 to 4 with increasing hazard from BSL1 to BSL4]
Biosafety Level 2 at F&M

• Working at BSL-2 requires strict adherence to biosafety requirements and proficiency in performing various procedures.

• Resources
  – Your lab’s Standard Operating Procedures
    • [www.fandm.edu/ehs/bloodborne-pathogens](http://www.fandm.edu/ehs/bloodborne-pathogens)
  – The F&M Biosafety Manual
    • [www.fandm.edu/bisafety/biosafety_manual](http://www.fandm.edu/bisafety/biosafety_manual)
### What is a Biological Safety Level 2 Lab?

- **BSL2** is required when using:
  - Human pathogens such as:
    - *Salmonella sp.*
    - *E. coli sp.*
    - *Staphylococcus sp.*
    - Influenza virus
    - Hepatitis A virus
  - Adenoviral and lentiviral cloning vectors
  - All human cell lines, tissues and body fluids

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What is a Biological Safety Level 2 Lab?

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

These additional BSL2-specific practices will be discussed in the next few slides.
“Restricted Access”

• Restricted Access at BSL2 means:
  
  – Immunocompromised individuals are advised of the increased risk and an individual risk assessment can be conducted for these individuals.
  – There are entry requirements for workers in the lab, such as Bloodborne Pathogen Training and Hepatitis B immunization.
  – Doors are kept closed during experiments
  – An approved sign indicating the presence of biohazards must be posted at all access areas of the BSL-2 lab.
Communicating Biohazards

All **equipment** - hoods, freezers, incubators, centrifuges, etc. that are used with biohazardous materials in a BSL-2 lab must have a biohazard label.

Also, **transport containers and biohazard waste containers** must display a biohazard label on the outermost part.
Standard Operating Procedures

- The BSL2-specific practices are detailed in Standard Operating Procedure documents.
  - Description of the “rules of the lab”
  - A copy is in your lab – ask where it is and review it!
  - It will help you to recognize and reduce hazards
- SOP’s serve as a resource to train new lab staff, supplement recurring training and as a valuable reference in the event of an emergency.
What information is in the Standard Operating Procedures?

Standard Microbiological Practices

Daily practice of these activities is critical 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 – not even water).

• Decontaminate work surfaces daily and after spills.

• Use personal protective equipment (e.g., buttoned lab coats, goggles and gloves).

• Use procedures that minimize the formation of aerosols.

• Avoid using hypodermic needles. Refer to the Sharps Handling and Disposal section of your lab’s Standard Operating Procedure.

• Place all solid biological waste in red autoclavable bags for autoclaving and disposal. Liquids must be disinfected before sink disposal.
What information is in the Standard Operating Procedures?

• **Needles and Sharps Precautions**
  
  • Because percutaneous exposure (through the skin) is a primary route of transmission of bloodborne pathogens, **extreme caution** should be taken with contaminated needles or sharps.
  
  • Sharps include items such as razor blades, scalpel blades, broken glass and plastic tips
  
  • Needles/contaminated sharps:
    • Must never be bent, recapped or removed unless there is no alternative
What information is in the Standard Operating Procedures?

• **Needles and Sharps Precautions – Broken Glass**
  
  – Large contaminated broken glass items must be autoclaved separately in a hard-walled container (such as a cardboard box) lined with a biohazard bag, clearly marked "GLASS AND SHARPS".
  
  – Always wear gloves and use tongs or a brush and dust pan to collect broken glassware.
What information is in the Standard Operating Procedures?

• **Needles and Sharps Precautions - Disposal**
  
  – Properly dispose of sharps and broken glass that have been exposed to potentially biohazardous materials.

  • Contaminated sharps must be placed in hard walled plastic containers labeled with the biohazard symbol.
What information is in the Standard Operating Procedures?

• Biohazardous Waste Management
  – 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 before disposal.
What should you do in the event of a spill?

- Every lab member working at BSL-2 must follow the proper protocol in the event of a biohazardous spill to
  - contain the contamination
  - minimize exposure to others

- Be familiar with your lab’s Standard Operating Procedure for spills and exposures.

- Household bleach at a 1:10 dilution is a very effective disinfectant. Other effective commercial disinfectants may be available in your labs.
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 inside a box.
What to do in the event of a spill cont’d

• Small spills should be treated with disinfectant for 10 minutes, absorbed with absorbent material like paper towels. The paper towels should be placed in biohazard waste. Repeat the disinfection process.
What to do in the event of a spill, con’t

• All PPE should be placed into biohazard waste and hands thoroughly washed before leaving the lab.
• Report the incident to your PI immediately.
  – Complete an Incident and Accident Report Form
  – If the spill is large and/or you need assistance, contact the Biosafety Office
What to do in the event of an exposure

• Follow the procedure in your lab’s SOP and the F&M Exposure Control Plan.
  • Wash the affected area with disinfectant for 5-15 minutes.
  • For membrane exposure (eyes, nose, mouth) flush with water for 5-15 minutes.
  • Cover the wound.
What to do in the event of an exposure

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

• Exposure may require an immediate evaluation by a medical practitioner.
  – Go to Appel Health or Lancaster General Hospital

• Complete a Laboratory Incident and Accident Report Form - found in the back pocket of the Biosafety Manual notebook and on the Biosafety website.

• Complete a Blood and Body Fluid Exposure form – found in Appendix C of the F&M Exposure Control Plan and in the back pocket of the Biosafety Manual notebook.
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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.
PPE: Personal Protective Equipment

The minimum PPE required for BSL-2 laboratories is gloves, safety glasses (or goggles) and lab coats.

Additional PPE such as surgical masks or faceshields may be required for procedures with a high probability for splashes, sprays, splatters or droplets.
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. phones, keyboards, 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.

• 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.

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

• Lab coats should be BUTTONED.
• You must NOT take lab coats home.
  • Handle soiled lab coats as little as possible, using gloves.
  • Place soiled lab coats in autoclavable bags; they may be laundered and reused after sterilization.
• Eye/face protection should be used if splashes or sprays are a possibility when working outside a biosafety cabinet.
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 in a BSL2 lab to minimize the production of splashes and aerosols!

It has been estimated that 65% of laboratory acquired infections are caused by aerosols.

The use of sealed centrifuge rotors and tubes and biosafety cabinets are required.
Steps to prevent the generation of aerosols in centrifuges and shakers

1. Routinely inspect the device to ensure that gaskets are properly in place to prevent leakage.

2. Wipe the outside of the tubes and flasks with an appropriate disinfectant after they are filled and sealed.

3. Centrifuge inside a biological safety cabinet. If a biological safety cabinet is not available, internal aerosol containment devices (e.g., sealed canisters, safety cups or buckets with covers, heat sealed tubes or sealed rotors) should be used.

4. After you remove tubes or flasks, open them in a biological safety cabinet. If a biological safety cabinet is unavailable, a minimum of 10 minutes settling time should be allowed before opening.
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 a 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

Air is filtered through a HEPA filter before exhausting and before contacting the work surface
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 a Biological Safety Level 2 Lab?

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No additional facilities are required for BSL2 labs.

A handwashing sink, safety shower and eyewash station and autoclave must be available.
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
  – LSP361
  – 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 Exposure Control Plan.
  • Wash the affected area with disinfectant or flush for 5-15 minutes.
• Report the incident to your PI immediately and to the Biosafety Officer
• Exposure may require an immediate evaluation by a medical practitioner.
• Do the paperwork
  – Complete a Laboratory Incident and Accident Report Form
  – Complete a Blood and Body Fluid Exposure form
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 inside a box.
• Small spills should be treated with disinfectant for 10 minutes, absorbed with absorbent material like paper towels. The paper towels should be placed in biohazard waste. Repeat the disinfection process.
What to do in the event of a spill

• All PPE should be placed into biohazard waste and hands thoroughly washed before leaving the lab.
• Report the incident to your PI immediately.
  – Complete an Incident and Accident Report Form
  – If the spill is large and/or you need assistance, contact the Biosafety Office
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
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**
Who can answer your questions?
If you have any questions.... ever

• Ask your research advisor or PI
• Check out the lab SOPs, F&M Biosafety Manual and the F&M Exposure Control Manual
• Ask the Biosafety Officer
  – Dr. Frielle
  – LSP361
  – 358-4600
  – dfrielle@fandm.edu
Take the test!

When you are ready, you can take the Biosafety Level 2 Training test. It can be found on the Biosafety Blackboard in the folder “Tests”. It will be graded automatically and you will be able to determine if you passed – passing requires a 100% test score.

Good luck!