



Biosafety Guidelines
Frostburg State University
Department of Biology
Date of Preparation- March 27, 2023

Contents:

Biosafety Committee (3)

Important Telephone Numbers (4)

Useful Websites (5)

1. Purpose, Policy, and Responsibilities (6)
2. Classification of organisms (7)
3. Summary of Biosafety Levels (7)
4. BSL requirements (8)
 - A. Risk assessment (8)
 - B. Personal protection (9)
 - C. Laboratory physical space (10)
 - D. Handling stock cultures (10)
 - E. Standard laboratory practices (10)
 - F. Training (11)
 - G. Waste handling (11)
 - H. Documentation (11)

5. Project approval process of the Biosafety Committee (12)

6. References (12)

Application to work with BSL-2 required organisms (13)

Biosafety Committee:

Dr. Kumudini Munasinghe (Chair)
Associate Professor
Biology Department
Compton Science Center 203
kamunasinghe@frostburg.edu
301.687.4299

Dr. Franklin Hughes (Vice Chair)
Associate Professor
Biology Department
Compton Science Center 313
fphughes@frostburg.edu
301.687.4177

Dr. David Puthoff (Secretary)
Professor
Biology Department
Compton Science Center 212
dpputhoff@frostburg.edu
301.687.4172

Mr. Marvin J. Parsons
Env. Safety & Sust. Officer
University Police
University Police 144
mjparsons@frostburg.edu
301.687.3976

Important Telephone Numbers:

Emergency Fire, Police, Emergency Medical Service 911

University Police – 301-687-4222 (Emergency), 301-687-4223 (non-emergency)

FSU Brady Health Center - 301-687-4310

MedExpress Urgent Care Clinic - 301-729-0529

Useful Websites:

The Occupational Safety and Health Association (OSHA)

<https://www.osha.gov/>

American Society for Microbiology (ASM) Guidelines for Biosafety in Teaching Laboratories

<https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator>

Biosafety in Microbiological and Biomedical Laboratories (BABL)

<http://www.cdc.gov/biosafety/publications/bmb15/>

Department of Environmental Safety Biosafety Manual for University of Maryland

<https://essr.umd.edu/about/research-safety/biological-safety> (UMD biosafety manual in the resources)

Laboratory biosafety manual (4th edition) World Health Organization

<https://www.who.int/publications/i/item/9789240011311>

National Selected Agent Program

<http://www.selectagents.gov/>

World Federation for Culture Collections

<http://www.wfcc.info>

American Type Culture Collection (ATCC)

<https://www.atcc.org/>

BEI Resources Library, National Institute of Allergy, and Infectious Diseases (NIAID)

<http://www.beiresources.org/home.aspx>

1. Purpose, Policy, and Responsibilities

Purpose

This is the official biosafety policy guideline for Frostburg State University to establish a process for handling potentially pathogenic organisms in teaching and research laboratories.

Policy

Frostburg State University is committed to providing a healthy and safe environment for its students, faculty, and staff while protecting the environment and the community. It has been recognized that using potential pathogens in many teaching and research laboratories is necessary. To ensure the safe handling of those pathogenic organisms, Frostburg State University complies with American Society of Microbiology (ASM) guidelines, the Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines from the Centers for Disease Control and Prevention, and University of Maryland biosafety manual recommendations.

Responsibilities

Biosafety is a collaborative task of all the individuals working in the laboratory, including students. However, the Principal Investigator/Professor is the primary person responsible for the decision-making of safe handling of potential pathogens. The PI must ensure that all laboratory personnel are trained to provide a safe work environment. The PI must apply (Pg.13) to the Biosafety Committee and receive approval before starting any research or class activity involving a pathogenic organism. The work will be reviewed and approved or provide recommendations to improvise the project plan or disapproved by the Biosafety Committee.

2. Classification of organisms

BABL has published four levels of safety requirements: Biosafety Level 1 (BSL-1), BSL-2, BSL-3, and BSL-4, while the National Institutes of Health (NIH) classifies pathogens in four groups based on their pathogenicity and the treatments available to control their infections.

3. Summary of Biosafety Levels

Table 1. Summary of Biosafety for selected infectious agents

BSL categories	Health risk	Practices	Primary barriers	Secondary barriers	Example organisms
1	Not known to cause disease in healthy individuals	Open bench microbiology	None required	Open benchtops and sinks	<i>Micrococcus luteus</i> and <i>Bacillus megaterium</i>
2	Can cause disease in healthy people, but organisms are easily contained	Limited lab access, and biohazard warning signs	Gloves, lab coat, eye protection, and face shield as needed	BSL-1 plus Access to autoclave	<i>Escherichia coli</i> and <i>Staphylococcus aureus</i> (most human pathogens)
3	Can cause severe disease, especially when inhaled	BSL-2 plus Controlled access to lab No unsterilized material can leave the lab. Decontamination of clothes prior to laundering	BSL-2 plus Biosafety cabinets used for all manipulations	BSL-2 plus Access to self-closing double doors Negative pressure (air flows into lab from outside) Exhausted air not recirculated	<i>Mycobacterium tuberculosis</i> , HIV, and <i>Yersinia pestis</i>
4	Highly virulent microbes posing extreme risk to humans, especially when inhaled	BSL-3 plus Clothing must be changed before entering and personnel must shower upon exiting the lab. All material is decontaminated prior to leaving the facility	BSL-3 plus All procedures are conducted in complete isolation. Biosafety cabinets along with full body, positive pressure suits with supplied air	BSL-3 plus Isolated building or lab Isolated laboratory systems (air supply and exhaust, vacuum, and decontamination)	Lassa fever virus Ebola virus, and Marburg virus

A BSL-1 laboratory has no risks of causing diseases in healthy and immunocompetent adults. The American Society of Microbiology (ASM) recommends using organisms that require BSL-1 practices unless it is necessary to use organisms requiring BSL-2 in demonstrating testing results and research labs. According to the ASM, BSL-1 and BSL-2 practices are acceptable for teaching labs, and BSL-3 and BSL-4 practices are strictly reserved for research purposes.

People using BSL organisms must be trained well in laboratory protocols about high-density cultures and aerosolization. Once isolated, samples containing unknown organisms, including environmental samples, must require BSL-2 practices.

It is imperative to have prior approval from the Biosafety committee of FSU before working with BSL-2 organisms.

4. BSL requirements

A. Risk assessment

Risk assessment and planning are essential tools for setting up labs each day. It is imperative to train all novices to handle the BSL-1 and BSL-2 required organisms due to their high-density growth cultures and aerosolization.

Risk assessment requires careful judgment. PI or the Professor needs to identify potential risk factors of the organism used and should plan the procedures to control the risks.

In addition to these guidelines, other valuable resources such as The Occupational Safety and Health Association (OSHA), ASM safety guidelines, and BMBL need to be considered in decision-making regarding procedures and protocols for handling BSL-2 organisms.

B. Personal protection

BSL-1 requirements

- ❖ Laboratory coats are strongly recommended for working in the lab.
- ❖ Wear closed-toe shoes.
- ❖ Tie back hair in the lab.
- ❖ Practice proper hand washing with soap and water prior to and after finishing lab work.
- ❖ Wear safety goggles when handling liquid cultures.
- ❖ If proper hand hygiene practices are performed, gloves are not required to work with BSL-1 organisms unless student's hands have cuts or any other abrasions, **this guideline recommends that novices wear gloves while handling BSL-1 organisms while working in teaching labs and research labs.**

- ❖ Properly discard waste materials into either regular trash, glass disposal, or culture bin. Trash from the culture bin must be autoclaved prior to putting them into the regular trash bin.
- ❖ Properly handle spills.
 - Immediately inform the instructor
 - Cover the spill with paper towels.
 - Pour in disinfectant liquid and leave it for 15 min.
 - Wipe off the spill using paper towels and wash your hands.
- ❖ Use waste beakers with disinfectant to discard microscope glass slides, cotton swabs, wooden applicators, and filter papers after transferring organisms and then later discard them properly to relevant waste bins.
- ❖ Only the PI or Professor should handle stock cultures.
- ❖ Do not discard any organism that requires BSL -1 without autoclaving them.
- ❖ Do not wear lab coats and gloves outside the lab.

BSL-2 requirements (in addition to those for BSL-1)

- ❖ Lab coats, gloves, closed-toe shoes, and safety goggles are required to work with BSL-2 required organisms.
- ❖ Using a biological safety cabinet is recommended to work with these organisms.
- ❖ Hand hygiene is a must for working with these organisms.
- ❖ Properly handle spills.
- ❖ Do not discard these organisms without autoclaving them.
- ❖ Do not wear lab coats and gloves outside the lab.

C. Laboratory physical space

BSL-1 requirements

- ❖ nonporous floor, bench tops, chairs, and stools.
- ❖ sink with soap for hand washing.
- ❖ eyewash station.
- ❖ lockable door to the room.
- ❖ Keep all personal belongings (including cell phones) separate from the work area.
- ❖ Use a working and validated autoclave.

BSL-2 requirements (in addition to those for BSL-1)

- ❖ Post biohazard signage in the laboratory.
- ❖ The biological safety cabinet is required when a procedure will create aerosols.

D. Handling stock cultures

- ❖ Maintain a curated culture collection that contains cultures from authorized or commercial sources (e.g., ATCC, NCIMB, or Carolina Biological Supply Company), a full list of cultures can be found at the World Federation for Culture Collections (WFCC) <http://www.wfcc.info>
- ❖ Cultures collected from nonauthorized, or noncommercial cultures should not be purchased (Isolating organisms from field samples in labs does not prevent this statement).
- ❖ If a clear chain of ownership exists, cultures can be obtained from other institutions. Clinical cultures received from hospitals/clinical labs should not be maintained in the lab.
- ❖ All extractions from environmental samples, such as soil and water, should be considered as BSL-2 required cultures.

E. Standard laboratory practices

BSL-1 requirements

- ❖ Use only institution-provided marking pens and writing instruments. These always remain in the lab and must be disinfected on a regular basis.
- ❖ Do not handle personal items (cosmetics, cell phones, laptops, calculators, pens, pencils, etc.) while in the laboratory. Cell phone usage is sometimes permitted but disinfect the cell phone with disinfected wipes.
- ❖ Tie back long hair.
- ❖ Wash hands after entering and before exiting the laboratory.
- ❖ Do not eat or drink in the laboratory.
 - ❖ Do not touch the face, apply cosmetics, adjust contact lenses, or bite nails.
- ❖ Disinfect the bench before and after the laboratory session with disinfectant.
- ❖ Properly wear and remove gloves.
- ❖ Notify the instructor of all spills or injuries and document them.
- ❖ Do not handle broken glass with your fingers; use a dustpan and a broom.
- ❖ Do not mouth pipette.
- ❖ Label all containers clearly.
- ❖ Transport test tubes in racks.
- ❖ Arrange for licensed waste removal in accordance with local, state, and federal guidelines.
- ❖ Advise immune-compromised students including those who are pregnant or may become pregnant to consult physicians to determine the appropriate level of participation in the laboratory.

BSL-2 requirements (in addition to those for BSL-1)

- ❖ Keep taking notes while working with BSL-2 required organisms.

F. Training

BSL-1

- ❖ Students must be trained in the safe use of microorganisms.
- ❖ Inform students of safety precautions relevant to each exercise before beginning the exercise.
- ❖ Require students and instructors to handle microorganisms safely and responsibly.
- ❖ Emphasize to students the importance of reporting accidental spills and exposures.
- ❖ Be aware that student assistants may be employees who are subject to OSHA.
- ❖ Conduct training for instructors whenever a procedural change is required.

BSL-2 (in addition to requirements for BSL-1)

- ❖ Students must demonstrate competency with BSL-1 organisms for each technique prior to using a BSL-2 organism for that specific technique.

G. Waste handling

- ❖ BSL-1 and BSL-2 required organisms should be autoclaved or incinerated within the laboratory before disposal.
- ❖ Train students in properly discarding waste generated from the laboratory. Use the regular trash bin, glass disposal bin, and autoclave/culture bin appropriately.
- ❖ All used gloves should be put into the autoclave/culture bin.

H. Documentation

- ❖ Make the Biosafety Manual available to students and staff.
- ❖ Students should sign safety agreements explaining that they have been informed about safety precautions and the safety sheets should be kept for one year.
- ❖ Keep records of Department and institution-required safety training.
- ❖ Make available a list of all cultures for students.
- ❖ Post proper signage.
- ❖ Document all injuries and spills.
- ❖ Make Safety Data Sheets (SDS) available in the laboratory.
- ❖ Keep updated contact information in the laboratory.

5. Approval process to work with organisms classified as BSL-2 in FSU laboratories.

The Biosafety Committee (BSC) will review and approve the work. The Biosafety Committee should consult an expert in the relevant field if needed. The review results could be approved, approved after making changes, or rejected. Once approved, the procedure can only be changed with permission from the Biosafety Committee.

6. References:

American Society for Microbiology guidelines for biosafety in teaching laboratories.

Biosafety in Microbiological and Biomedical Laboratories (BABL).

Laboratory Biosafety Manual (4th edition) World Health Organization.

Biosafety Manual for University of Maryland, College Park MD.

Kumudini Munasinghe. 2022. Essential Topics in Microbiology lab manual.

Barry Chess. Microbiology lab manual. Summary of biosafety levels. Table 1.

Prepared by Dr. Kumudini Munasinghe and approved by the Biology Department Chair, Dr. Rebekah Taylor and the Biosafety Committee on 9/10/2023.