

# Guidelines for Biosafety in Teaching Laboratories



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## Preamble

Educators must maintain awareness of the inherent risks of using microorganisms in the laboratory and employ best practices to minimize the risk to students and the community. A core principle of biosafety is the containment of microorganisms; therefore, organisms used in the teaching laboratory must remain in the laboratory and instructors must guard against inadvertent passage of the microbes out of the laboratory by a student or assistant. Even though organisms manipulated in a BSL-1 laboratory pose a low level of risk to the community and are unlikely to cause disease in healthy adults, most of the microorganisms used in the microbiology teaching laboratory are capable of causing an infection given the appropriate circumstances. A risk assessment for each laboratory activity and organism used is necessary to identify the proper procedures and safety equipment needed. A thorough risk assessment considers the microorganism(s) used, the manipulations performed with the organisms, and the risks inherent in performing the lab activity. Although specific microorganisms are usually handled at a specific biosafety level, the microbe alone does not determine the biosafety level of the lab. Manipulations that generate aerosols, create splash hazards, propagate organisms, or require large volumes of culture increase the risks associated with using a particular microbe.

The designation BSL implies that there is institutional oversight of the lab and that there are facility safeguards in place for containment and prevention of accidental release of organisms. An informal setting such as a science fair or camp should not be considered an appropriate setting for BSL designation. Instead, individual activities to be conducted in an informal setting should be assessed for risks, and safety measures should be put in place to ensure containment. K-12 facilities are encouraged to reach out to and partner with microbiologists at local higher educational institutions or government-sponsored labs as they assess the risks of using live microorganisms.

ASM put these biosafety guidelines in place in response to the ongoing release of *Salmonella* from teaching/clinical laboratories as documented by the CDC in 2011, 2014, and 2017. As such, instructors working with microorganisms in teaching/clinical labs are expected to adhere to the guidelines set forth in this document. To ensure safety, educators must adequately train their students/assistants and should continually assess the hazards of working with microorganisms in all situations to address the importance and need for safe handling, containment, and disposal of the microorganisms.

To help provide a safe environment, ASM recommends that organisms requiring BSL-1 practices be used at all times unless a specific result can only be demonstrated with organisms requiring BSL-2 practices and such a result is deemed necessary for the student to observe with a live culture. All samples containing unknown organisms (e.g., any environmental sampling), once cultured, must be handled as BSL-2. To make sure that students work in a safe manner, students must be trained and demonstrate proficiency with an organism requiring BSL-1 practices, using a specific technique, prior to using that same technique with an organism requiring BSL-2 practices. The ASM Biosafety Guidelines are for teaching labs and, since BSL-3 and BSL-4 techniques are reserved for research settings, only BSL-1 and BSL-2 practices are acceptable for teaching labs.

The practices set forth in these guidelines fall into eight major categories: risk assessment, personal protection, laboratory physical space, stock cultures, standard laboratory practices, training, documents, and service animals. For ease of use, these requirements and practices are brief. Explanatory notes, sample documents, and additional resources are provided in the Appendix, and instructors are encouraged to refer to these additional resources often.

## **Risk Assessment**

Prior to setting up a lab, it is good practice to conduct a risk assessment of the laboratory activities and procedures you plan to use. During a risk assessment, the instructor will need to identify potential hazards, critical processes or procedures, and needed or required protective measures to control the risk. In an instructional lab, there are three common biological risk factors:

- *Novices.* The CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL; <https://www.cdc.gov/biosafety/publications/bmbl5/>) manual BSL criteria work under the assumption that the people working in the lab are well trained and competent in the procedures used in the lab. By definition, instructional labs are designed to train people in procedures. Working with novices increases the risk, and it is best to assume the student knows little to nothing about the laboratory's culture of safety. Safety training as well as documentation of that training are therefore essential.
- *High Cell Density Cultures.* During instructional labs, students grow organisms. The organisms used at BSL-1 are not consistently associated with a disease, but these organisms may pose a health risk once grown to a high cell density. Training students to recognize this potential risk is another aspect of their training.
- *Aerosolization.* A significant risk factor associated with all BSL microorganisms is aerosolization of a culture. While aerosolization at BSL-1 may not cause disease, it has a high risk of contaminating objects and materials that will leave with students, e.g., backpacks, thus posing a community risk of the inadvertent release of an organism.

While these guidelines focus on BSL criteria as set out in the BMBL manual, there are other guidelines, regulations, and rules you may need to consider. The Occupational Safety and Health Association (OSHA) has several regulations regarding work in a biology lab, e.g., eye protection, gloves, and bloodborne pathogens (<https://www.osha.gov/law-regs.html>). As you perform your risk assessment, make sure that you conform and align with all local, state, and federal regulations.

## **Personal Protection Requirements**

### **BSL-1 requirements**

- Wear safety goggles or safety glasses when handling liquid cultures, when performing procedures that may create a splash hazard, or when spread plating.
- Wear closed-toe shoes that cover the top of the foot. Ballet flat-style shoes are unacceptable. The material of the shoe should not quickly absorb liquids nor allow the liquid to easily pass through.
- Instruct students in the proper use and safe removal of gloves.
- Wear gloves when the student's hands have fresh cuts or abrasions, when staining microbes, and when handling hazardous chemicals. Gloves are not required for standard laboratory procedures using BSL-1 organisms if proper hand hygiene is performed. Proper hand hygiene involves thorough hand cleansing prior to and immediately after finishing handling microorganisms and any time that microbes accidentally contact the skin. Hand cleansing is performed by washing with soap and water or rubbing with an alcohol-based hand sanitizer.
- See the Appendix for detailed guidelines on safety goggles/glasses, shoes, gloves, and lab coats.
- *Recommended: To build a culture of laboratory safety, the use of laboratory coats and gloves is strongly recommended and encouraged.*

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

- Use safety goggles or safety glasses when working with BSL-2 organisms. Use face shields or safety goggles and masks when performing procedures that may create a splash hazard. If work is performed in a biological safety cabinet, goggles and face shields/masks do not need to be worn.
- Wear gloves when handling microorganisms or hazardous chemicals
- Wear laboratory coats

## **Laboratory Physical Space Requirements**

### **BSL-1 requirements**

- All laboratory space must include:
  - nonporous floor, bench tops, chairs, and stools
  - sink with soap for hand washing
  - eyewash station
  - lockable door to the room
- Follow proper pest control practices as detailed in the Appendix
- *Recommended: Keep all personal belongings (including cell phones) in an area separate from the work area*
- *Recommended: Use a working and validated autoclave*

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

- Keep storage area for personal belongings (including cell phones, pens, calculators, etc.) separate from work area.
- Keep a working and validated autoclave in the building or arrange for licensed waste removal according to local, state, and federal regulations. See the Appendix for more guidelines on autoclave validation.
- Post biohazard signage (sample biohazard signage is available in the Appendix)
  - wherever cultures are used and stored
  - on the door to the room
  - on any containers used to transport cultures
- *Recommended: Have a biological safety cabinet. The biological safety cabinet is required when large volumes of culture are used or when a procedure will create aerosols.*

### **Stock Culture Requirements (same requirements for BSL-1 and BSL-2)**

- 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 authorized culture collections can be found at World Federation for Culture Collections (<http://www.wfcc.info>)
  - Cultures from nonauthorized or noncommercial culture collections should not be used in an instructional lab.
  - Cultures acquired from other institutions can be used if there is clear provenance (a clear chain of ownership) and the culture was originally obtained from an authorized or commercial source.
  - Clinical cultures acquired from hospitals or clinical labs should never be maintained or used in an instructional setting.
- A well-maintained and cataloged culture collection containing authenticated stock cultures with clear provenance (clear chain of ownership). This includes maintaining records for each culture, including:
  - where the culture came from
  - the stock number from the originating culture collection
  - when the culture was first acquired
  - when the culture was first subcultured
  - any subsequent subculturing for stock or storage purposes
  - BSL as defined by the commercial source of the culture
- Ensure that the cultures you acquire are suited to your BSL
  - Most culture collections state the appropriate BSL required to work with the organism
  - The American Biosafety Association also maintains a Risk Group database that has BSL information on common strains (<https://my.absa.org/tiki-index.php?page=Riskgroups>)

- Do not subculture or handle (e.g., Gram staining) unknown microbes isolated from the environment without the use of BSL-2 practices and facilities
- Obtain fresh stock cultures of microorganisms annually (e.g., purchased, revived from frozen stock cultures, etc.) to be certain of the source culture, minimize accumulation of spontaneous mutations, and reduce contamination

## **Standard Laboratory Practices**

### **BSL-1 requirements**

- Use only institution-provided marking pens and writing instruments. These remain in the lab at all times 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 during lab; however, cell phones **MUST** be kept in a resealable plastic bag. After use, the bag should be disposed of in the proper receptacle.
- Do not wear valuable electronics (smart watches, Fitbits, etc.) or dangling jewelry in the laboratory.
- Tie back long hair.
- Wash hands after entering and before exiting the laboratory.
- Do not bring food, gum, drinks (including water), or water bottles into the laboratory.
- Do not touch the face, apply cosmetics, adjust contact lenses, or bite nails.
- Keep door closed while the laboratory is in session. Laboratory director or instructor approves all personnel entering the laboratory.
- Disinfect bench before and after the laboratory session with a disinfectant known to kill the organisms handled. Use disinfectants according to manufacturer instructions.
- Teach, practice, and enforce proper wearing, removal, and use of gloves.
- Notify instructor of all spills or injuries. Document all injuries according to school, university, or college policy.
- Do not handle broken glass with fingers; use a dust pan and broom.
- Do not mouth pipette.
- Label all containers clearly.
- Minimize the use of sharps. Use needles and scalpels according to appropriate guidelines and precautions.
- Use proper transport vessels (test tube racks) for moving cultures in the laboratory, and store vessels containing cultures in leak-proof containers when work with them is complete.
- Use leak-proof containers for storage and transport of infectious materials.
- Arrange for proper (safe) decontamination and disposal of contaminated material (e.g., in a properly maintained and validated autoclave) or 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) and students living with or caring for an immunocompromised individual to consult physicians to determine the appropriate level of participation in the laboratory.
- *Recommended: Keep note-taking and discussion practices separate from work with hazardous or infectious material.*
- *Recommended: Use microincinerators or disposable loops rather than Bunsen burners.*

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

- Keep note-taking and discussion practices separate from work with hazardous or infectious material.
- Use microincinerators or disposable loops rather than Bunsen burners.

## **Training Practices**

### **BSL-1**

- Students must be trained in the safe use of microorganisms for each technique.
- 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 of the institution and subject to OSHA, state, and/or institutional regulations.
- Conduct extensive initial training for instructors and student assistants to cover the safety hazards of each laboratory. The institution's biosafety officer or microbiologist in charge of the laboratories should conduct the training.
- Conduct training for instructors whenever a procedural change is required.
- Conduct training for student assistants annually.

### **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.
- *Recommended: Students should complete OSHA Bloodborne Pathogen training. Check with your institution, as they may have a training program in place that could be used.*

## **Documentation Practices**

### **BSL-1**

- Develop a lab safety manual and make this available to the students and staff. A one-page lab safety brief can also be used to highlight critically important issues of safety (see the Appendix for an example).
- Require students to sign safety agreements explaining that they have been informed about safety precautions and the hazardous nature of the organisms they will handle throughout the course.
- Maintain student-signed safety agreements at the institution. Check with your institutional safety officer as to how long you must keep these forms. In most cases the signature can be a digitally signed agreement, such as a safety quiz.
- Check to see if your institution requires other safety training, including training regarding blood-borne pathogens, hazardous waste awareness, or right-to-know global harmonized system.
- Maintain and make available (e.g., in a syllabus, in a laboratory manual, or online) to all students a list of all cultures (and their sources) used in the course
- Prepare, maintain, and post proper signage
- Document all injuries and spills; follow school/college/university policy, if available.
- Make Safety Data Sheets (SDS) available at all times; follow institutional documentation guidelines regarding number of copies, availability via print or electronic form, etc.
- Post emergency procedures and updated contact information in the laboratory.

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

- Keep a biosafety manual specific to the laboratory and/or course in the BSL-2 laboratory
- Keep a copy of the current version of Biosafety in Microbiology and Biomedical Laboratories (BMBL) in the laboratory.



## **Guidelines for Service Animals in Microbiology Laboratories**

A Service Animal is defined by the Americans with Disabilities Act (ADA) as a dog or a miniature horse that is individually trained to do work or perform tasks for people with disabilities. Because having animals in the lab greatly increases the risk of working with microorganisms, only Service Animals should be considered for laboratory access. Allowing a Service Animal to access a teaching laboratory may be reasonable after considering relevant factors, including but not limited to the hazards present in the laboratory, how those hazards could impact the Service Animal, how the presence of the Service Animal could impact other students, and the research/experiments being performed.

It is the goal of institutions of higher education to provide students with a successful and rewarding learning experience. Institutions of higher learning and a student with a Service Animal should work to identify whether there are arrangements that can be made that would make access by the Service Animal reasonable and safe and also ensure the sterility of the environment, the safety of the animal, the safety of all students in the room, and protection of the community from potential breaches in the containment policies of the lab.

The following guidelines have been established to assist faculty and ADA Compliance Officers in identifying potential reasonable accommodations for students or others with Service Animals. Should the Service Animal be admitted to the laboratory, the expectation is that the Service Animal handler and the Service Animal remain in compliance with the predetermined biosafety guidelines at all times while in the laboratory. It is also expected that the Service Animal be under the control of the handler at all times (i.e., voice control or other effective means). If the Service Animal is out of control and its handler does not take effective action to control it, or if an animal is not house broken, the handler may be asked to remove the animal. It should be noted that a poorly controlled animal poses a significant risk to itself, its handler, and other students in the laboratory. It is recommended that the student, instructor, and ADA Compliance Officer discuss the need for the Service Animal to be under control at all times. It is further recommended that the Service Animal be observed during a trial period to see if that it is safe, controlled, and able to carry out its service function within the lab environment. The instructor should aim to protect the Service Animal from injury or exposure to a pathogen while in the lab and to limit the possibility of the Service Animal from inadvertently removing an organism from the lab on their paws/coats.

### **Guidelines to assist instructors**

- *Paws covered.* The service animal should have its paws covered when it is walking in the lab, to protect them from potentially getting cut by glass or picking up pathogens that might be lurking on the floor. This requirement is no different from students having to wear shoes in the lab.
- *Impervious body cover.* If the animal must be at the bench, then the animal should be covered with an impervious covering so as to protect the animal from potential spills. We must always keep in mind that the animal will be below the bench surface, so any item that rolls off or liquids that spill off of the bench could potentially harm the animal.
- *Eye protection.* The service animal should wear goggles/eye covering to protect it from spills (just as we protect the eyes of our students).
- *Location of service animal*
  - It must be determined how much interaction there needs to be between the service animal and its human partner. If the animal is not required to be next to its human partner during the lab, then the animal should be housed in an inactive portion of the lab. The animal may have to be in view of its human partner; this has to be accounted for if necessary.
  - If the service animal must be right next to its human partner, then location within the lab and at the bench needs to be assessed. If appropriate under the circumstances, the service animal may be located underneath the bench for added protection of the animal.
  - In either case, the animal should remain on a surface that can be disinfected before and at the end of each lab. The animal should not lay/sit directly on the floor.
- *Possible trip hazard.* Always keep in mind the potential for a trip hazard. Locating the student in need of the animal at a bench that is out of the standard traffic flow of the lab may help to prevent a trip hazard if the service animal must be at the bench.

- *Exiting the lab.* When the animal is to exit the lab, the coat, paw coverings, and goggles should be removed and disinfected.
- *Emergency contacts.* In case of an accident involving a service animal, the lab coordinator should add to the lab safety manual emergency contact information regarding the service animal (e.g., local pet hospital, poison control).