



---

## Supplemental Materials

for

### Guidelines for Biosafety in Teaching Laboratories 2.0: A Revised and Updated Manual for 2019

Jeffrey J. Byrd<sup>1\*</sup>, Elizabeth Emmert<sup>2</sup>, Robert Maxwell<sup>3</sup>, and Heather Townsend<sup>4</sup>

<sup>1</sup>*Department of Biology, St. Mary's College of Maryland, St. Mary's City, MD 20686;*

<sup>2</sup>*Department of Biological Sciences, Salisbury University, Salisbury, MD 21801;*

<sup>3</sup>*Department of Biology, Georgia State University, Atlanta, GA 30303;*

<sup>4</sup>*Department of Biology, Community College of Rhode Island, Newport, RI 02840*

## Table of Contents

(Total pages 10)

Appendix     Guidelines for Biosafety in Teaching Laboratories (2019)

\*Corresponding author. Mailing address: Department of Biology, St. Mary's College of Maryland, 47645 College Drive, St. Mary's City, MD 20686. Phone: 240-895-2973.  
E-mail: [jjbyrd@smcm.edu](mailto:jjbyrd@smcm.edu)  
Received: 1 October 2019; Accepted: 28 October 2019;  
Published: 18 December 2019

©2019 Author(s). Published by the American Society for Microbiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Noncommercial-NoDerivatives 4.0 International license (<https://creativecommons.org/licenses/by-nc-nd/4.0/> and <https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode>), which grants the public the nonexclusive right to copy, distribute, or display the published work.

# Guidelines for Biosafety in Teaching Laboratories



# CONTRIBUTING AUTHORS

## ASM Task Committee on Laboratory Biosafety – 2019 Edition

**Jeffrey J Byrd, Chair**  
Department of Biology  
St. Mary's College of  
Maryland

**Robert A. Maxwell**  
Department of Biology  
Georgia State University

**Heather M. Townsend**  
Department of Biology  
Community College of Rhode  
Island

**Elizabeth A. B. Emmert,**  
Department of Biological  
Sciences  
Salisbury University

## ASM Task Committee on Laboratory Biosafety – 2012 Edition

**Elizabeth A. B. Emmert, Chair**  
Department of Biological  
Sciences  
Salisbury University

**Diane Hartman**  
Department of Biology  
Baylor University

*Ex Officio*

**Jeffrey J. Byrd**  
Department of Biology  
St. Mary's College of Maryland

**Amy White**  
Department of Biology  
Virginia Western Community  
College

**Ron Atlas, Co-Chair, ASM**  
Committee on Biodefense,  
Public and Scientific Affairs  
University of Louisville

**Ruth A. Gyure**  
Department of Biological and  
Environmental Sciences  
Western Connecticut State  
University

**Neil Baker, Chair, ASM**  
Education Board  
The Ohio State University  
(Professor Emeritus)

**Amy Chang, Director, ASM**  
Education

## Ad Hoc Reviewers – 2012 Edition

**Cristina Bressler**  
Centers for Disease Control  
and Prevention

**Gary E. Kaiser**  
The Community College of  
Baltimore County

**Susan Merkel**  
Cornell University

**Diane O. Fleming**  
Biological Safety Professions

**Sue Katz**  
Roger State University

**Melanie Popa**  
University of Pittsburgh

**Roxana B. Hughes**  
University of North Texas

**Donald Lehman**  
University of Delaware

**Robert J. Wolff**  
South University

**Kai Hung**  
Eastern Illinois University

**Tracey Meilander**  
Notre Dame College

**Christopher Woolverton**  
Kent State University

**Michael J. Imperiale**  
University of Michigan

**Paul Meechan**  
Centers for Disease Control  
and Prevention

# TABLE OF CONTENTS

<b>BIOSAFETY GUIDELINES.....</b>	<b>1</b>
----------------------------------	----------

<i>Preamble.....</i>	<i>1</i>
<i>Risk Assessment .....</i>	<i>1-2</i>
<i>Personal Protection Requirements .....</i>	<i>2</i>
<i>Laboratory Physical Space Requirements ...</i>	<i>2-3</i>
<i>Stock Culture Requirements .....</i>	<i>3</i>

<i>Standard Lab Practices.....</i>	<i>3-4</i>
<i>Training Practices .....</i>	<i>4</i>
<i>Document Practices.....</i>	<i>4-5</i>
<i>Guidelines for Service Animals in Lab .....</i>	<i>5</i>

## **Preamble:**

Educators must maintain awareness of the inherent risks of using microorganisms in the laboratory and use best practices to minimize the risk to students and the community. A core principle of biosafety is the containment of microorganisms; therefore, organisms being used in the 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 BSL1 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 risk associated with using a particular microbe.

The designation of BSL implies that there is an institutional oversight of the lab and that there are facility safeguards in place for containment and prevention of the accidental release of organisms. As such, 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 risk and safety measures put in place to ensure containment. K-12 facilities are encouraged to reach out to microbiologists at local higher educational institutions or government-sponsored labs to partner with as they assess the risk of using live organisms.

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 need of safe handling, containment, and disposal of the microorganisms.

To help provide a safe environment, ASM recommends that organisms requiring BSL1 practices be used at all times unless a specific result can only be demonstrated by organisms requiring BSL2 practices and that 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 BSL2. To make sure that students work in a safe manner, students must be trained and demonstrate proficiency with an organism requiring BSL1 practices, using a specific technique, prior to using that same technique with an organism requiring BSL2 practices. It should also be noted that the ASM Biosafety Guidelines are for teaching labs and, since BSL3 and BSL4 techniques are reserved for research settings, only BSL1 and BSL2 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, the requirements and practices are brief. Explanatory notes, sample documents, and additional resources are in the **appendix** and instructors are encouraged to refer to these often.

## **Risk Assessment**

Prior to setting up a lab, it is good practice to run 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 Biosafety in Microbiological and Biomedical Laboratories Manual's 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, and the documentation of that training is therefore essential.

- *High Cell Density Cultures:* During instructional labs, students grow organisms. The organisms used at BSL1 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 is aerosolization of a culture. While aerosolization at BSL1 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 with the inadvertent release of an organism

While these guidelines focus on Biosafety Level criteria as set out in the BMBL (<https://www.cdc.gov/biosafety/publications/bmbl5/>), 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 BSL1 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 about 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 level requirements (requirements below are in addition to those for BSL-1)**

- Use safety goggles or safety glasses when working with BSL2 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**

- Require all laboratory space to 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 (requirements below are in addition to those for BSL-1)**

- Keep the 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
    - wherever cultures are used and stored.
    - on the door to the room.
    - on any containers used to transport cultures.
- Note: sample biohazard signage is available in the Appendix.
- *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 non-authorized or non-commercial culture collections should not be used in an instructional lab.
  - Cultures acquired from other institutions can be used if there is clear provenance (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 level as defined by the commercial source of the culture
- Ensure that the cultures you acquire are suited to your BSL level.
  - 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 BSL2 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 (Same requirements for BSL-1 and BSL-2)**

#### **BSL-1 Requirements**

- Use only institution-provided marking pens and writing instruments. These remain in the lab at all times and 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 dangling jewelry or wear valuable electronics (smart watches, Fit bits, etc.) 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 the 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 dustpan 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 a leak-proof container 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 below are 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 new procedural change is required.
- Conduct training for student assistants annually.

**BSL-2 (requirements below are in addition to those 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 complete the OSHA Bloodborne Pathogen training. Check with your institution as they may have a training program in place that could be used.*

**Document 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 example in the appendix).
- 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, such as *Blood Borne Pathogen*, *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 (requirements below are in addition to those 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 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 the student with a Service Animal should work to identify whether there are arrangements that can be made that would make access by a 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 the protection of the community from 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 an animal is out of control and the Service Animal's handler does not take effective action to control it, or 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 animal be observed during a trial period to see if they are safe, controlled, and able to carry out their service function within the lab environment. The instructor should aid to protect the Service Animal from being injured or exposed 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 their paws covered when they are 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 covering - 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 of 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 the animal 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 service animal and human partner. If the animal is not required to be next to their 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 their human partner so that has to be accounted for if necessary.
- If the service animal must be right next to their human partner then location within the lab and at the bench needs to be accessed. 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 - We must always keep in mind the potential of 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 the case of an accident involving a service animal, the lab coordinator will add emergency contact information regarding the service animal to their lab safety manual (e.g., local pet hospital, Poison Control)