



Biosafety Practices for Teaching Laboratories

Introduction

In 2012, the American Society for Microbiology (ASM) published a document titled, *Guidelines for Teaching Laboratories*.⁽¹⁾ These guidelines recommend procedures for working at Biological Safety Level 1 (BSL-1) and Biological Safety Level 2 (BSL-2) in teaching laboratories. The ASM publication was influenced by the lack of safety guidelines for microbiology teaching laboratories and a multistate outbreak of *Salmonella typhimurium* originating in teaching and clinical laboratories in 2011.⁽²⁾ A major culprit identified during an investigation of the outbreak and a similar one in 2014,⁽³⁾ was a lack of biosafety training and awareness for staff and students.

Arizona State University (ASU) has many teaching labs at the introductory, intermediate, and advanced undergraduate levels, as well as graduate levels. ASU Environmental Health and Safety (EH&S) has compiled these *Biosafety Practices for Teaching Laboratories* with input from the ASU Institutional Biosafety Committee (IBC), instructors, and other key stakeholders, to ensure ASU teaching laboratories are safe for students, staff, visitors, and inspectors. The primary purpose of this document is to prevent pathogen exposure to personnel and the community, and to prevent releases to the environment. Specifically, this document contains biosafety requirements for teaching laboratories operating at BSL-1 and BSL-2 and is intended to supplement the detailed resources provided in the [ASU Biological Safety Manual](#).

The ASU IBC must approve all teaching laboratories using any materials requiring BSL-2 containment. It is important to note that not all teaching laboratories are designed or equipped to safely operate at BSL-2. This is one reason why it is so important to have an IBC protocol submitted before any BSL-2 work is performed in a teaching laboratory. Please contact the Office of Research Integrity and Assurance (ORIA) at IBC@asu.edu or at 480-965-4387 with any questions or clarifications regarding assigned biosafety levels.

Subculturing Unknown Samples

Students are permitted to culture organisms from soil, water, food materials, and the air in the teaching laboratory. If the samples will be used to only count and understand the types of organisms in a particular environment, and no subculturing is performed, then IBC approval is not required.

Subculturing from an initial culture plate requires IBC review and approval, especially if differential media is used in the experiment. In addition, if the laboratory will include subculturing and isolation from environments such as water fountains, door handles, wastewater treatment outfalls, or other areas that could harbor pathogens, review and approval by the IBC must be obtained. Samples must never be cultured from the students' bodies without approval from the IBC, and possibly the ASU Institutional Review Board (IRB), as there may be the potential to grow microorganisms that require BSL-2, or even BSL3 containment.

Note: Instructors are encouraged to create “unknown” samples for students from a mixture of known microorganisms (selected by the instructor), or from a culture where the instructor knows the contents, instead of using samples from the environment. Please contact the ASU Biosafety and Biosecurity team at biosafety@asu.edu or 480-965-5389 for recommendations for surrogate microorganisms.



Minors Working in Biological Laboratories

All minors working in research and teaching laboratories must have their research projects approved by the ASU IBC, EH&S, and all procedures must adhere to the University policy for [Minors in the Laboratory](#). Minors in these laboratories are permitted to work with well-established BSL-1 materials only, unless approved by the IBC and EH&S.

Biological Safety Level One (BSL-1) Requirements

All information regarding biosafety and biosecurity should be included in the course syllabus. This will help ensure easy access to this important information at any time during the course.

BSL-1 Laboratory Facility Requirements

BSL-1 includes microorganisms that are not known to cause human disease, and that may be handled safely on bench tops. The use of BSL-1 containment is the most appropriate for most teaching laboratories. However, some facilities may not meet these requirements due to the original design requirements for the laboratory space. Any facility renovation or new construction must include the following requirements:

- Non-porous flooring, bench tops, chairs, and stools
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- Recommended: Separate storage area for personal belongings
- Recommended: Access to a working and validated autoclave (see the [ASU Autoclave Safety Manual](#) for more information)

BSL-1 Stock Culture Requirements

Always provide students with an exceptional learning experience by ensuring that they are working with known and well-documented stocks.

- Stock cultures must be from approved and reputable sources.
- Subculturing microbes isolated from the environment, clinical samples, or other unknown locations is discouraged as BSL-2 (or higher) microbes may be isolated.
- Subculturing from the environment must be reviewed and approved by the ASU IBC.
- When possible, only well-characterized microbes should be used (e.g., identified with an ATCC number) and examples are provided in [Table 1](#).
- The laboratory instructor must maintain safety documentation for all stock organisms, sources, and procedures for handling stock cultures.
- Fresh stock cultures of microorganisms must be obtained on a regular basis (at least annually) to be certain of the source culture, to minimize spontaneous mutations, and to reduce contamination.

Table 1. Recommended BSL-1 Agents for Teaching Laboratories

Microorganism	BSL	ATCC Number (if known)
<i>Alcaligenes faecalis</i>	1	8750
<i>Aspergillus niger</i>	1	16888
<i>Bacillus globigii</i>	1	69510
<i>Bacillus stearothermophilus</i>	1	7953
<i>Bacillus subtilis</i>	1	23857
<i>Bacillus megaterium</i>	1	
<i>Citrobacter freundii</i>	1	8090
<i>Clostridium sporogenes</i>	1	3584
<i>Enterobacter aerogenes</i>	1	13048
<i>Enterobacter cloacae</i>	1	
<i>Enterococcus casseliflavus</i>	1	700327
<i>Enterococcus durans</i>	1	
<i>Escherichia coli B</i>	1	11303
<i>Escherichia coli K12</i>	1	10798
<i>Geobacillus stearothermophilus</i>	1	12980
<i>Halobacterium salinarum</i>	1	
<i>Klebsiella oxytoca</i>	1	
<i>Lactobacillus acidophilus</i>	1	
<i>Micrococcus luteus</i>	1	4698
<i>Neurospora crassa</i>	1	
<i>Penicillium chrysogenum</i>	1	10106
<i>Providencia alcalifaciens</i>	1	
<i>Pseudomonas fluorescens</i>	1	
<i>Rhanella aquatilis</i>	1	
<i>Rhizopus stolonifer</i>	1	14037
<i>Rhodococcus rhodocus</i>	1	
<i>Saccharomyces cerevisiae</i>	1	9763
<i>Sarcinia aurantiaca</i>	1	
<i>Serratia liquefacens</i>	1	
<i>Serratia marcescens</i> Bizio	1	13880
<i>Staphylococcus epidermidis</i>	1	14990
<i>Staphylococcus saprophyticus</i>	1	15305

Examples of BSL-1 Experiments (Using Non-Infectious Agents)

ASU instructors have many years of experience in determining which experiments are appropriate for novice students. The following experiments are those deemed most appropriate for introductory microbiology teaching laboratories:

- Anaerobic growth
- Bacterial enumeration
- Bacterial transformation
- Capsule stain
- Carbohydrate fermentation
- Casein hydrolase
- Catalase and oxidase test
- Endospore stain
- Eosin methylene blue plate
- Flagella stain
- Gel electrophoresis
- Gelatin hydrolysis
- Gram stain
- Hanging drop
- Indole methyl red Vogues-Proskauer and Citrate (IMViC)
- Kirby-Bauer
- Litmus milk
- Luria broth
- MacConkey Agar
- Mannitol, nitrate reduction
- 4-Methylumbelliferyl- β -D-glucuronide *Escherichia coli* broth medium (*E. coli* MUG)
- Plasmid DNA isolation
- Pour and quadrant streak plate
- Restriction endonuclease digestion
- Spread
- Starch hydrolysis
- Transformation assay
- Triple sugar iron
- Urease
- Use of lambda bacteriophage

BSL-1 Personal Protective Equipment Requirements

- Safety goggles or safety glasses (with side shields) must be worn when handling stocks and liquid cultures, spreading plates, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use. Note that the disinfectant to be used and disinfection time must be documented in the syllabus.
- Long pants or long skirts (ankle length) or other clothing to cover exposed skin must be worn.
- Closed toe and closed heel shoes covering the top of the foot must be worn.



- Gloves must be worn when the student has fresh cuts or abrasions on the hands, when staining microbes, and when handling hazardous chemicals.
- Gloves must be worn when handling cultures.
- Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin.
- Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.
- Recommended: Laboratory coats should be worn when handling cultures.

BSL-1 Work Practices

- Hands must be washed after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. EH&S Biosafety and Biosecurity may be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Students should not touch their faces, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
- All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets, and other personal electronic devices is discouraged.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly with the contents.
- The laboratory door must remain closed at all times when the laboratory is in session. The laboratory instructor must approve all persons entering the laboratory.
- Sharps usage must be minimized. Needles and scalpels are to be used according to ASU guidelines. All sharps (includes coverslips, slides and Pasteur pipets) must be disposed in a sharps container. Please refer to the [ASU Fact Sheet for Handling and Disposal of Sharps](#) for more information.
- Waste materials from the laboratory must be disposed properly. Please refer to the [ASU Biohazardous Waste Handling Procedures](#) for more information.
- Test tube racks or other secondary containers must be used to move cultures in the laboratory.
- Stocks and other cultures must be stored in a leak-proof container when work is complete.
- Broken glass must be handled using a dustpan and broom or forceps and tongs. Students and laboratory personnel must not pick up broken glass with their hands. If contaminated, broom and other tools used to pick up broken glass must be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. Spills or injuries must then be documented with the EH&S [online incident reporting form](#).
- Teach, practice, and enforce the proper wearing and use of personal protective equipment.
- Advise immune-compromised students and those living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. Students should not be asked to reveal if they are immuno-compromised. A general announcement should be made that students with a reduced immune status should consult with ASU Health Services. A note from ASU Health Services is sufficient to excuse a student from laboratory work.
- Recommended: Supply pens and pencils for students and keep separate from personal items.



- Recommended: Keep note taking and discussions separate from work with laboratory materials.
- Recommended: Use micro-incinerators rather than Bunsen burners.

BSL-1 Training Practices

- Faculty and teaching assistants must complete all required safety trainings prior to beginning the first day of class and annually thereafter. These trainings include the [ASU Biosafety and Bloodborne Pathogens Training](#), [EH&S Laboratory Safety Training](#), [Fire Safety Training](#), [Autoclave Safety Training](#), and [EH&S Hazardous Waste Training](#).
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the laboratory. The requirements listed in this document should be included in this training session. The training session must be documented with a sign-in sheet maintained by the instructor. This may be performed using an online system such as Blackboard.
- Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the [ASU Biological Safety Manual](#).
- Students must be informed of safety precautions applicable to each exercise before the procedure is performed.

BSL-1 Documentation

- Safety Data Sheets (SDS) must be available for all chemicals in the laboratory.
- Students must sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. This information should be included in the syllabus for the class so it can be reviewed at any point during the course. The laboratory instructor must maintain student signed agreements in the laboratory. Alternatively, this may be performed and maintained online within Blackboard.
- Instructors must prepare, maintain, and post caution signs on laboratory doors (complete with biohazard symbol).
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list may be included in the syllabus, a laboratory-specific biosafety manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.
- Annual submission of course manual and list of microorganisms used in the laboratory to the [ASU Institutional Biosafety Committee](#). Any major deviation from the material submitted must be updated and approved before the new semester as appropriate.

Biological Safety Level Two (BSL-2) Requirements

All information regarding biosafety and biosecurity should be included in the course syllabus. This will help ensure easy access to this important information at any time during the course.

BSL-2 General Requirements

BSL-2 laboratories are suitable for working with microbes posing a moderate risk to the individual and a low community risk for infection. There are many microorganisms handled in BSL-2 containment that may cause disease in humans via ingestion or inoculation. The BSL-2 requirements build upon those for BSL-1 facilities, and typically include additional engineering controls to protect students. For example,

possible engineering controls include biological safety cabinets, centrifuge safety cups, and safety needle devices.

BSL-2 Laboratory Facility Requirements

- Non-porous floor (e.g., tile or epoxy), bench tops, chairs and stools*
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- Separate storage area for personal belongings*
- Working and validated autoclave (using biological spore indicators as instructed in the [ASU Autoclave Safety Manual](#)).
- Biohazard signage where cultures are used and stored (e.g., incubators), on the door to the room, and on containers used to transport cultures.
- An approved vendor must certify all biological safety cabinets used to handle infectious materials annually.

** It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to include these requirements.*

BSL-2 Stock Culture Requirements

- Stock cultures must be from approved and reputable sources.
- Subculturing microbes isolated from the environment, clinical samples, or other unknown locations is discouraged as BSL-2 (or higher) microbes may be isolated. Subculturing from the environment must be approved by the ASU IBC.
- Samples must never be obtained from clinical sites unless a full description of strain antibiotic susceptibility and resistance is provided, and the IBC has approved the use of these strains.
- Strains resistant to clinically relevant antibiotics must not be handled in teaching labs.
- Documentation must be maintained for all stock organisms, sources, and stock cultures.
- Fresh stock cultures of microorganisms must be obtained on a regular basis to be certain of the source culture, minimize spontaneous mutations, and reduce contamination.
- When possible, surrogates should be substituted for common pathogens (see the substitutes for ESKAPE pathogens provided in this document for recommendations).
- Stocks must be stored in a secure (locked) area.

Common BSL-2 Microbes and Ordering Information from ATCC

- When choosing a test organism, many instructors want to choose organisms that are clinically relevant (i.e., pathogens). There are six microorganisms that are considered major threats, not because they cause the most devastating illnesses but because they comprise the majority of antibiotic-resistant infections observed in health care settings. These are referred to as ESKAPE pathogens and include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and species of *Enterobacter* (ESKAPE).

- ESKAPE pathogen > Safe Relatives
 - *Enterococcus faecium* > *Enterococcus raffinosus* or *Enterococcus casseliflavus*
 - *Staphylococcus aureus* > *Staphylococcus epidermidis*
 - *Klebsiella pneumonia* > *Escherichia coli*
 - *Acinetobacter baumannii* > *Acinetobacter baylyi*
 - *Pseudomonas aeruginosa* > *Pseudomonas putida*
 - *Enterobacter species* > *Enterobacter aerogenes*

BSL-2 Personal Protective Equipment Requirements

- Safety goggles or safety glasses must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash.
- Closed toe and heel shoes that cover the top of the foot must be worn.
- Wear long pants/long skirts (ankle length) to minimize potential for exposure to biological and chemical hazards.
- Laboratory coats must be worn. These may be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of damage or degradation. Laboratory coats must be stored within the laboratory and must be assigned to individual students, not shared.
- Gloves must be worn when handling cultures, when staining microbes and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

BSL-2 Laboratory Work Practices

- Hands must be washed after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. EH&S Biosafety and Biosecurity may be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Students should not touch their faces, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
- All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets, and other personal electronic devices is prohibited unless students are required to use their cell phone cameras to record data to include in class presentations (e.g., biochemical test results, Gram stains). This photographic record is very helpful in troubleshooting experiments. Students are required to remove their gloves and wash their hands prior to handling their personal electronic equipment.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly with the contents.
- The laboratory door must remain closed at all times when the laboratory is in session. The laboratory instructor must approve all persons entering the laboratory.

- Students must be taught proper technique to minimize production of aerosols. For example: when pipetting, place tip on side of tube and allow liquid to run down the side of the tube, and when flaming a loop to transfer culture, have a sterile agar plate used as a “sizzle” plate so students do not touch a culture with a really hot loop.
- All procedures that generate aerosols, such as centrifuging, grinding, blending, shaking, mixing, and sonicating must be performed inside a biological safety cabinet or using appropriate engineering controls (e.g., centrifuge safety cups). Biological safety cabinets must also be used when opening a container that may become depressurized when opened, and could release aerosols of the stock culture.
- Sharps usage must be minimized. Needles and scalpels are to be used according to ASU guidelines. All sharps (includes coverslips, slides and Pasteur pipets) must be disposed in a sharps container. Please refer to the [ASU Fact Sheet for Handling and Disposal of Sharps](#) for more information.
- Waste materials from the laboratory must be disposed properly. Please refer to the [ASU Biohazardous Waste Handling Procedures](#) for more information.
- Test tube racks or other secondary containers must be used to move cultures in the lab.
- Stocks and other cultures must be stored in a leak-proof container when work is complete.
- Broken glass must be handled using a dustpan and broom or forceps and tongs. Students and laboratory personnel must not pick up broken glass with their hands. If contaminated, broom and other tools used to pick up broken glass must be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. Spills or injuries must then be documented with the EH&S [online incident reporting form](#).
- Teach, practice, and enforce the proper wearing and use of personal protective equipment.
- Advise immune-compromised students and those living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. Students should not be asked to reveal if they are immuno-compromised. A general announcement should be made that students with a reduced immune status should consult with ASU Health Services. A note from ASU Health Services is sufficient to excuse a student from laboratory work.
- Lecture should be performed before materials are brought to the work areas. Note taking should be kept to a minimum when hazardous materials are out. When possible, note taking should be conducted away from the work area and after gloves have been removed.
- Use micro-incinerators rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators may also be used to heat fix bacterial smears on microscope slides and flaming the end of a test tube by passing these items over the entrance to the micro-incinerator.

BSL-2 Training Practices

- Faculty and teaching assistants must complete all required safety trainings prior to beginning the first day of class and annually thereafter. These trainings include the [ASU Biosafety and Bloodborne Pathogens Training](#), [EH&S Laboratory Safety Training](#), [Fire Safety Training](#), [Autoclave Safety Training](#), and [EH&S Hazardous Waste Training](#).
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practices with students on the first day of the laboratory. The requirements listed in this document should be included in this training session. The training session must be documented with a sign-in sheet maintained by the instructor. This may be performed using an online system such as Blackboard.



- Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the [ASU Biological Safety Manual](#).
- Students must be informed of safety precautions applicable to each exercise before the procedure is performed.
- Students must demonstrate proficiency in standard aseptic technique and BSL-1 practices before allowing them to work at BSL-2.

BSL-2 Documentation

- Safety Data Sheets (SDS) must be available for all chemicals in the laboratory.
- Students must sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. This information should be included in the syllabus for the class so it can be reviewed at any point during the course. The laboratory instructor must maintain student signed agreements in the laboratory. Alternatively, this may be performed and maintained online in Blackboard.
- Instructors must prepare, maintain, and post caution signs on laboratory doors (with biohazard symbol).
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list may be included in the syllabus, a laboratory-specific biosafety manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.
- Annual submission of course manual and list of microorganisms used in the laboratory to the [ASU Institutional Biosafety Committee](#). Any major deviation from the material submitted must be updated and approved before the new semester as appropriate.
- All work at BSL-2 must be registered with the Institutional Biosafety Committee.
- All requirements for BSL-2 must be followed as outlined in the [ASU Biological Safety Manual](#).

Questions?

Contact ASU EH&S [Biosafety and Biosecurity](#) at 480-965-5389.

References

- (1) ASM teaching guidelines: <http://www.asm.org/index.php/education-2/22-education/8308-new-version-available-for-comment-guidelines-for-best-biosafety-practices-in-teaching-laboratories>
- (2) CDC report regarding 2011 *Salmonella typhimurium* outbreak: <http://www.cdc.gov/salmonella/typhimurium-laboratory/011712/index.html>
- (3) CDC report regarding 2014 *Salmonella typhimurium* outbreak: <http://www.cdc.gov/salmonella/typhimurium-labs-06-14/index.html>