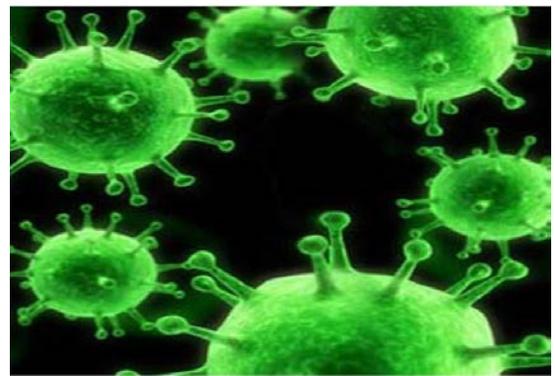




Guidelines For Microbiology Teaching Laboratories



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Introduction

The American Society for Microbiology (ASM) Education Board published Guidelines for Teaching Laboratories in 2012.¹ The ASM publication was influenced by the lack of clear safety guidelines for microbiology teaching labs and a multistate outbreak of *Salmonella typhimurium* originating in teaching and clinical laboratories in 2011.² Unfortunately, similar incidents occurred in 2014 and 2017, thus reinforcing the need for these guidelines.^{3,4} The ASM guidelines include recommendations for working at Biosafety Level (BSL) 1 and BSL2, and are based on the safety requirements found in the Centers for Disease Control and Prevention's (CDC)'s Biosafety in Microbiological and Biomedical Laboratories (BMBL).⁵ A major finding of the epidemiological investigation of the outbreak was deficiencies in biosafety awareness and proper training of staff and students. The University of Utah Department of Occupational and Environmental Health and Safety (OEHS) has compiled guidelines, based on the ASM recommendations, with input from the University of Utah Institutional Biosafety Committee (IBC), in order to ensure our teaching labs are safe for students and to prevent pathogen exposure to persons and the environment. Explanatory notes, sample documents, and additional resources are in Appendices 2 and 3.

This document contains biosafety requirements for teaching laboratories operating at BSL1 and BSL2. This document is meant to supplement the detailed resources outlined in the University of Utah Biosafety Manual, which can be accessed [here](#). Not all teaching laboratories are equipped to safely operate at BSL2. Any and all use of Risk Group 2 (RG2) or higher organisms must be preapproved by the University of Utah IBC: an IBC protocol must be submitted through the BioRAFT system, which can be accessed [here](#). Please contact the biosafety group in OEHS at 801-581-6590 or biosafety@oehs.utah.edu with any questions or clarifications.

Subculturing “unknown” samples and teaching about differential and selective media:

The procedures needed to identify unknown microorganisms can be performed safely, and with little to no risk to the students. Students are permitted to culture organisms from soil, water, food materials, and the air. Subculturing from the initial culture plate is permitted for the above samples, but IBC review and approval must be obtained if differential media used in the experiment could select for the growth of organisms listed at RG2 or higher. If the samples will be used to only count and understand the types of organisms in a particular environment, and no subculturing performed, then IBC approval will not be required. If the laboratory will include subculturing and isolation from environments such as water fountains, door handles or other areas that could harbor pathogens, review and approval by the IBC must be obtained.

Additionally, samples must never be cultured from the students themselves without approval from the IBC, and possibly the Institutional Review Board, as there is the potential to grow microorganisms that require BSL2, or even BSL3 containment.

It is recommended that testing of unknowns should be performed from a mixture of known microorganisms (to the instructor), or from a culture where the contents are known to the instructor, instead of from the environment.

For recommendations on surrogate microorganisms, please contact the OEHS biosafety office at biosafety@oehs.utah.edu.

Minors Working in Biological Labs at the University of Utah

All minors and their parent/legal guardian must sign the “Minor Participant Informed Consent Document” prepared by University of Utah Risk Management. Minors in laboratories are permitted to work with well-established BSL1 materials only, unless approved by OEHS. Many classes, activities, and events require a liability waiver. U of U events or activities which are planned, organized, controlled or supervised by U of U employees or authorized volunteers for minors must contact Risk Management to complete the Minor Participant Informed Consent & Parenting/Guardian Consent to Treatment, Waiver and Release for U of U Event or Activity form, <https://riskmanagement.utah.edu/intranet/contracts/liability-field-trip-waiver.php>.

Risk Assessment and Biosafety Levels

Laboratories must assess the hazards of working with microorganisms and the need to practice safe handling, containment, and disposal of microorganisms. A risk assessment for each laboratory activity and organism is necessary in order to identify the proper procedures and safety equipment needed. Risk assessment determines the biosafety level of the workspace. A thorough risk assessment takes into account the microorganism being used, the manipulations performed with the organism, and the risks inherent in performing the lab activity. Although microbes are commonly handled at a particular biosafety level, the microbe alone does not determine the biosafety level of the lab. For example, manipulations that generate aerosols, create splash potential, or require large volumes of culture increase the risk associated with a particular microbe. Lab members should consult the website of the [CDC](#) or [Public Health Agency Canada](#) to clarify safety requirement information about a particular organism.

Biosafety Level 1

Educators need to be aware of the risks inherent in using microorganisms in the laboratory and must use best practices to minimize the risk to students and the community. The following guidelines are designed to encourage awareness of the risks, promote uniformity in best teaching practices, and protect the health and wellness of their students. These guidelines are not mandatory, but are designed to promote best practices in the teaching laboratory. 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. Many best practices should be adopted to minimize the risk of laboratory acquired infections and to train students in the proper handling of microorganisms. The practices set forth in these guidelines fall into six major categories: personal protection, laboratory facilities, stock cultures, standard laboratory practices, training practices, and documentation.

Personal Protective Equipment Requirements:

- Safety goggles or safety glasses (with side shields) must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash. If glasses are reused (e.g., by students in another semester), they must be sanitized with an appropriate disinfectant after use.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of contamination, damage or degradation. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared. Lab coats must be laundered by an approved laundry facility. Do not take lab clothing home to launder.
- Long pants/ long skirts (ankle length), or other clothing (such as scrubs) to cover exposed skin must be worn.
- Closed shoes that cover the entire foot must be worn.
- Gloves must be worn when the student has fresh cuts or abrasions on the hands, or any time when cultures are handled, 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, or, if none is available, with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

Laboratory Facility Requirements:

- Non-porous floor, bench tops, chairs and stools*
 - Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
 - Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
 - If the laboratory has windows that open, they are fitted with fly screens.
- *Recommended:* Separate storage area for personal belongings
- *Recommended:* Use a working and validated autoclave**

**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 address these requirements.*

***Please refer to the University of Utah Biosafety Manual, which can be accessed [here](#), for details and contact OEHS with questions (biosafety@oehs.utah.edu).*

Stock Culture Requirements:

- Only use cultures from authorized, commercial, or reputable sources (e.g., an academic laboratory or state health department). Do not subculture unknown microbes isolated from the environment because they may be organisms that require BSL2 practices and facilities.
 - Examples of Recommended Microbes for work at BSL1

Microorganism	ATCC number
<i>Acinetobacter baylyi</i>	33304
<i>Alcaligenes faecalis</i>	8750
<i>Aspergillus niger</i>	16888
<i>Bacillus stearothermophilus</i>	7953
<i>Bacillus subtilis</i>	23857
<i>Citrobacter freundii</i>	8090
<i>Clostridium sporogenes</i>	3584

<i>Enterobacter aerogenes</i>	13048
<i>Enterococcus casseliflavus</i>	700327
<i>Enterococcus raffinosus</i>	49427
<i>Escherichia coli</i> B	11303
<i>Escherichia coli</i> K12	10798
<i>Geobacillus stearothermophilus</i>	12980
<i>Halobacterium salinarum</i>	33170
<i>Lactobacillus acidophilus</i>	4356
<i>Micrococcus luteus</i>	4698
<i>Neurospora crassa</i>	44318
<i>Penicillium chrysoenum</i>	10106
<i>Providencia alcalifaciens</i>	9886
<i>Pseudomonas fluorescens</i>	13525
<i>Pseudomonas putida</i>	12633
<i>Rhizopus stolonifer</i>	14037
<i>Saccharomyces cerevisiae</i>	9763
<i>Serratia liquefacens</i>	27592
<i>Serratia marcescens</i> Bizio	13880
<i>Staphylococcus epidermidis</i>	14990
<i>Staphylococcus saprophyticus</i>	15305

- Laboratory instructor must maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis (at least annually) to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Protocols that can be performed easily at BSL1: anaerobic growth, Gram stain, capsule stain, endospore stain, flagella stain, carbohydrate fermentation, casein hydrolase, catalase and oxidase test, bacterial enumeration, eosin methylene blue plate, gelatin hydrolysis, hanging drop, indole methyl red Vogues-Proskauer and Citrate (IMViC), Kirby-Bauer, Luria broth, litmus milk, 4-methylumbelliferyl- β -D-glucuronide *Escherichia coli* broth medium (*E. coli* MUG), MacConkey Agar, mannitol, nitrate reduction, spread, pour and quadrant streak plate, starch hydrolysis, transformation assay, urease, triple sugar iron, use of lambda bacteriophage, bacterial transformation, plasmid DNA isolation, restriction endonuclease digestion, polymerase chain reaction (PCR) and gel electrophoresis.

Standard Laboratory Work Practices:

- Wash hands after entering and before leaving the laboratory.

- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Lab 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. OEHS can be consulted for disinfectant recommendations.
- Teach, practice and enforce the proper wearing and use of personal protective equipment.
- Food, water bottles, gum, and drinks of any kind are not permitted in the laboratory.
- Do not touch your face, 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.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly.
- The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor must approve all persons entering.
- Minimize use of sharps. Needles and scalpels are to be used according to institutional guidelines: do not re-cap needles. Most sharps should be discarded in sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms. However, non-contaminated pipets and pipet tips should be disposed of in broken glass receptacles.
- Contaminated sharps, including coverslips, slides, glass and plastic pipets and pipet tips, and Pasteur pipets, are discarded immediately or as soon as possible in biohazard sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms, and labeled or color-coded appropriately.
- 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. A sealed, leak-proof container, labeled with a biohazard symbol, must be used to transport stocks and cultures from one room to another.
- Cultures should be disinfected/inactivated prior to disposal, either by chemical disinfection or autoclaving.
- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container labeled with a biohazard symbol, which is closed before being removed from the laboratory. Hazardous waste can be picked up by OEHS, arranged through the OEHS Lab Management System

<http://oehs.utah.edu/topics/lab-management-system>).

- Broken glass must be handled using a dustpan and broom or forceps/tongs, not picked up by students or laboratory personnel with their hands. Broken glass must be disposed of in a broken glass box, unless it is contaminated and should be disposed of in a biohazard sharps container. If contaminated, broom will need to be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. When contaminated material is spilled, inform the laboratory assistant immediately. Proper procedure require the instructor and student to secure area, deny entry to non-authorized people. The instructor should assume everything spilled is infectious, wear personal protective equipment (lab coat, eye protection, shoe covers and 2 pairs of gloves), cover spill with paper towels, prepare fresh disinfectant (e.g., 1:10 dilution of bleach) and pour slowly onto spill from outside to in, leave for at least 20 minutes, use tongs to pick up objects and place in sharps containers, place other waste in biohazard waste containers, remove PPE and wash hands. Spills or injuries must then be documented with OEHS, who can be reached at 801-581-6590.
- Should an exposure occur, immediately wash the affected areas with soap and water, or if exposure to eyes or mucous membranes occurred, immediately flush affected area with water for 10-15 minutes. Go directly to the Student Health Center, Madsen Clinic, 555 South Foothill Boulevard, for medical evaluation and follow-up (**see Appendix 1**). For serious or life threatening injury or illness call emergency medical services by calling 911. Clinic addresses and maps are in Appendix 1 and should be incorporated into training documents. Complete and submit the Incident/Accident Report form to Risk Management within 24 hours of the incident. The form can be downloaded from the Risk Management [website](#).
- Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. (Students shall 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 Student Health Services. A note from Student 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 or disposable loops rather than Bunsen burners.

Training Practices:

- Faculty and teaching assistants must complete University of Utah laboratory safety, bloodborne pathogens and biosafety trainings, as applicable. 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 teaching assistants to cover the safety hazards of each laboratory.
 - The microbiologist in charge of the laboratories or the University of Utah Biosafety Officer should conduct the training.
 - Conduct training for instructors whenever a new procedural change is required.
 - Conduct training for teaching assistants annually.
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign-in sheet maintained by the instructor.
- Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the University of Utah Biosafety Manual.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.
- Emphasize to students the importance of reporting accidental spills and exposures.

Documentation:

- Safety Data Sheets (SDS) must be available in the laboratory for all chemicals.
- Require students to 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. The laboratory instructor must maintain student signed agreements in the laboratory.
- Maintain and post caution signs on lab doors (complete with biohazard symbol). These should be obtained from OEHS. <https://oehs.utah.edu/resource-center/forms/hazard-warning-signage-questionnaire>.
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Emergency phone numbers and information must be posted in the laboratory.

Biosafety Level Two

Biosafety Level Two (BSL2) 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 at BSL2 that can cause disease in humans via ingestion or inoculation. When good microbiological techniques are used, these organisms rarely cause serious disease, and effective treatment for laboratory-acquired infections is available. Best practices must be adopted to minimize the risk of laboratory-acquired infections and to train students in the proper handling of organisms that require BSL2 procedures. The guidelines for BSL2 laboratories build upon those for BSL1 facilities, and typically include additional engineering controls to protect students, such as biological safety cabinets, centrifuge safety cups and safety needle devices. The practices set forth in these guidelines fall into six major categories: personal protection, laboratory facilities, stock cultures, standard laboratory practices, training practices, and documentation.

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 shoes that cover the entire foot must be worn.
- Long pants/ long skirts (ankle length), or other clothing (such as scrubs) to cover exposed skin must be worn.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of contamination, damage or degradation. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared. Lab coats must be laundered by an approved laundry facility. Do not take lab clothing home to launder.
- 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.

Laboratory Facility Requirements:

- Non-porous floor, bench tops, chairs and stools*
 - Bench tops are impervious to water and resistant to acids, alkalis, organic solvents,

and moderate heat.

- Laboratory furniture is sturdy with surfaces for easy cleaning and decontamination. No cloth chairs. Spaces between benches, cabinet and equipment are accessible for cleaning.
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- Proper pest control practices
- If the laboratory has windows that open, they are fitted with fly screens.
- Separate storage area for personal belongings
- Working and validated autoclave
- Biohazard signage where cultures are used and stored (e.g. incubators), on the door to the room and on containers used to transport cultures. Contact the OEHS Biosafety team at 1-6590 to request a BSL-2 Warning sign.
- *Recommended:* Biological Safety Cabinet. Please see requirements below. All biological safety cabinets must be certified by an approved vendor annually (contact OEHS at 801-581-6590). Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:
 - a. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, and harvesting infected tissues from animals or eggs.
 - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.
- *Please refer to the University of Utah Biosafety Manual for details and contact OEHS with questions (biosafety@oehs.uath.edu).*

**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 address these requirements.*

Stock Culture Requirements:

- Stocks must be from authorized, commercial or reputable sources. Do not subculture microbes isolated from the environment, clinical samples or other unknown locations because they may be microbes that require BSL2 practices and facilities. 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 for the laboratory.
- Strains resistant to clinically relevant antibiotics shall not be handled in teaching laboratories.
- Maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Store stocks in a secure (locked) area.
- Substitute surrogates for common BSL2 pathogens

Examples of Common Microbes used at BSL2

Microorganism	ATCC Number
<i>Klebsiella oxytoca</i>	13182
<i>Proteus mirabilis</i>	25933,7002
<i>Proteus vulgaris</i>	29905
<i>Salmonella enterica</i>	700720
<i>Staphylococcus aureus</i>	12600

- 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 pathogens should be replaced with “Safe Relatives”. Requests to use ESKAPE pathogens rather than the safer alternatives will need to be justified to the IBC, who may require additional safeguards.

➤ ESKAPE pathogen > Safe Relative

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*

Laboratory Work Practices:

- Instructors/Supervisor limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
- Instructors/Supervisor establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet any specific entry requirements (e.g., immunization) may enter the laboratory
- When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign, incorporating the universal biohazard symbol, is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the Instructor/Supervisor or other responsible person(s) for entering the laboratory.
- An insect and rodent control program is in effect.
- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Teach, practice and enforce the proper wearing, use, donning and doffing of personal protective equipment
- Lab benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Additionally, if any materials are spilled, they will be immediately cleaned-up.
 - Disinfectants used must be effective against microbes used in the laboratory. OEHS can be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
- Do not touch your face, apply cosmetics, adjust contact lenses, bite nails, or

chew on pens/ pencils in the laboratory.

- All personal items must be stowed while in the laboratory. The use of cell phones is prohibited.
- Mouth pipetting is prohibited.
- All containers must be labeled clearly.
- The laboratory door must remain closed at all times when the lab is in session.
- Minimize use of sharps. Needles and scalpels are to be used according to institutional guidelines: do not re-cap needles. Most sharps should be discarded in sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms. However, non-contaminated pipets and pipet tips should be disposed of in broken glass receptacles.
- Contaminated sharps, including coverslips, slides, glass and plastic pipets and pipet tips, and Pasteur pipets, are discarded immediately or as soon as possible in biohazard sharps containers that are closable, puncture-resistant, leakproof on sides and bottoms, and labeled or color-coded appropriately.
- 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. A sealed, leak-proof container, labeled with a biohazard symbol, must be used to transport stocks and cultures from one room to another.
- 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: centrifuging, grinding, blending, shaking, mixing, sonicating, etc., must be performed inside a biological safety cabinet or using appropriate engineering controls (centrifuge safety cups). Biological safety cabinets must also be used when opening a container that can become depressurized when opened, and could release aerosols of the stock culture, and students must be trained in the proper use of biological safety cabinets.
- All waste and cultures are appropriately labeled and must be disinfected/inactivated prior to disposal, either by chemical disinfection or autoclaving.
- Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leakproof container labeled with the biohazard symbol, which is closed before being removed from the laboratory. Hazardous waste can be picked up by OEHS, arranged through the OEHS Lab Management System (<http://oehs.utah.edu/topics/lab-management-system>).

- Broken glass must be handled using a dustpan and broom or forceps/tongs, not picked up by students or laboratory personnel with their hands. Broken glass must be disposed of in a broken glass box, unless it is contaminated and should be disposed of in a biohazard sharps container. If contaminated, broom will need to be disposed or sterilized.
- All spills or injuries must be immediately reported to the laboratory instructor. When contaminated material is spilled, inform the laboratory assistant immediately. Proper procedures require the instructor and student to secure area, deny entry to non-authorized people. The instructor should assume everything spilled is infectious, wear personal protective equipment (lab coat, eye protection, shoe covers and 2 pairs of gloves), cover spill with paper towels, prepare fresh disinfectant (e.g., 1:10 dilution of bleach) and pour slowly onto spill from outside to in, leave for at least 20 minutes, use tongs to pick up objects and place in sharps containers, place other waste in biohazard waste containers, remove PPE and wash hands. Spills or injuries must then be documented with OEHS, who can be reached at 801-581-6590.
- Should an exposure occur, immediately wash the affected areas with soap and water, or if exposure to eyes or mucous membranes occurred, immediately flush affected area with water for 10-15 minutes. Go directly to the Student Health Center, Madsen Clinic, 555 South Foothill Boulevard, for medical evaluation and follow-up (**See Appendix 1**). For serious or life threatening injury or illness call emergency medical services by calling 911. Clinic addresses and maps are in Appendix 1 and should be incorporated into training documents. Complete and submit the Incident/Accident Report form to Risk Management within 24 hours of the incident. The form can be downloaded from the Risk Management [website](#).
- Advise immune-compromised students and students 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 student health services. A note from Student Health Services is sufficient to excuse a student from laboratory work.)
- Supply pens and pencils for students, and keep separate from personal items.
- Keep note taking and discussions separate from work with laboratory materials. Note taking can be performed on a pull out desk shelf, if available, but must be taken away from the work area. If this is not available, lecture must be performed before any materials are brought to the bench areas.
- Use micro-incinerators or disposable loops rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators can 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.

Training Practices:

- Teaching assistants must complete OEHS laboratory safety, bloodborne pathogen and BSL2 biosafety trainings.
- Conduct extensive initial training for instructors and teaching assistants to cover the safety hazards of each laboratory.
 - The microbiologist in charge of the laboratories or the University of Utah Biosafety Officer should conduct the training.
 - Conduct training for instructors whenever a new procedural change is required.
 - Conduct training for teaching assistants annually.
- Instructors and/ or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign in sheet maintained by the instructor.
- Require students and instructors to handle microorganisms safely and in conjunction with requirements outlined in the University of Utah Biosafety Manual.
- Inform students of safety precautions applicable to each exercise before the procedure is performed.
- Require students to demonstrate proficiency in standard aseptic technique and BSL1 practices before allowing them to work at BSL2.
- Emphasize to students the importance of reporting accidental spills and exposures.

Documentation:

- A laboratory-specific (or course-specific) biosafety manual is prepared and adopted. Students are advised of special hazards and are required to read instructions on practices and procedures and how to follow them
- Safety Data Sheets (SDS) sheets must be available in the laboratory for all chemicals.
- If available, Pathogen Safety Data Sheets (PSDSs) (previously titled Material Safety Data Sheets for infectious substances) are technical documents that describe the hazardous properties of a human pathogen and recommendations for work involving these agents in a laboratory setting. These documents have been produced by the Public Health Agency of Canada (the Agency) as educational and informational resources for laboratory personnel working with these infectious substances and can be accessed at

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>.

- Spills and Post-Exposure Procedures must be available in the laboratory.
- Require students to 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. Maintain student signed agreements at the institution.
- Prepare, maintain and post caution signs to the laboratory, complete with biohazard symbol.
- Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.
- Register all work at BSL2 with the Institutional Biosafety Committee.
- Maintain an inventory of the quantity and location of all RG2 agents, in line with ASM recommendations.⁶ Create a record of RG2 agents to include the following: (1) identification (name and species of agent), (2) quantity (e.g., approximate number of vials for each agent), (3) location (building, room and cold storage unit ID), (4) name of person familiar with that agent, (5) date entry created, and (6) other related information, such as source, and variant/strain.
- Follow all requirements for BSL2 as outlined in the University of Utah Biosafety Manual.
- Provide access to the current version of Biosafety in Microbiological and Biomedical Laboratories (BMBL) in the laboratory (printed copy or computer access).
- Emergency numbers and information must be posted in the laboratory.

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/2011/lab-exposure-1-17-2012.html>
3. CDC report regarding 2014 *Salmonella typhimurium* outbreak: <http://www.cdc.gov/salmonella/typhimurium-labs-06-14/index.html>
4. CDC report regarding 2017 *Salmonella typhimurium* outbreak: <https://www.cdc.gov/salmonella/typhimurium->

[07-17/index.html](#)

5. Biosafety in Microbiology and Biomedical Laboratories (BMBL), 5th Edition:

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

6. ASM Statement: What is in your laboratory freezer?

<http://www.asm.org/index.php/public-policy/99-policy/policy/93059-freezer-8-14>

Document adapted from the American Society of Microbiology Guidelines for Biosafety in teaching Laboratories, the University of Utah Biosafety Manual and Rutgers University Teaching Laboratory Guidelines.

APPENDIX 1

Medical Assistance

Student Health Center at the Madsen Clinic

Location



555 Foothill Dr. Level 1
Salt Lake City, UT 84112
Phone: 801-581-6431
Fax: 801-585-5294

Hours

- Operating Hours: Monday-Friday, 7:30 am to 5 pm
- Appointment Hours: Monday-Friday, 8 am to 4 pm
- Walk-in (vaccines, lab tests) Hours: Monday-Friday, 9 am to 4 pm

Note: Clinic is closed on Wednesdays, 12-2pm.

Extended Hours

- Tuesdays, evening appointments to 6:30 pm
- Saturdays, appointments from 9 am to 11:30 am
- Fall and Spring Semesters only
 - Tuesday, 7:30 am to 7:30 pm
 - Saturday, 9:00 am to 12:00 pm

Note: Extended hours do not apply to Tuesdays or Saturdays during or near breaks/holidays.

Collegiate Assistance Program

If you need to speak to a nurse when the Student Health Center is closed, call 1-877-643-5139 for the Collegiate Assistance Program. You need to be enrolled in the university's Student Health Insurance Plan through United HealthCare. You will also need the PIN number on the back of your insurance card for the call.

General and After-Hours Care**Student Health Insurance Plan Preferred Provider Network**

University of Utah Health Care Urgent Care centers provide extended hours for general care (<http://healthcare.utah.edu/primarycare/urgent.php>). Or call 801-581-6431 for recorded directory information.

APPENDIX 2

Explanatory Notes:

For ease of use, the BSL1 and BSL2 Guidelines described above have been kept brief. The following sections clarify some of the requirements and practices, and provide practical tips for adapting the guidelines for teaching laboratories. This section covers personal protective equipment, culture preservation, note-taking areas, generation of aerosols, biological safety cabinet requirements and certification, microincinerators, disinfectants, proper decontamination and disposal procedures, isolation of unknowns, cultivation of fungi, autoclave validation, pest control, and additional resources. These are adapted from the ASM Guidelines for Biosafety in Teaching Laboratories

(https://www.asm.org/images/Education/FINAL_Biosafety_Guidelines_Appendix_Only.pdf).

Personal Protective Equipment

- **Lab coats.** Coats will be provided by the University of Utah and can be of either the disposable or the cloth variety. Disposable coats are not sturdy and should be discarded and repurchased during the semester if they become contaminated or show signs of degradation, e.g., holes, rips, or missing snaps. If cloth coats are used, they should fit each student properly, be made of flame-resistant cloth, e.g., cotton and polyester blends, and be at least three-quarters in length to cover the lap when a student is sitting. Lab coats must be used by individual students, never shared, and always stay in the laboratory. To conserve space, individual lab coats may be stored in Ziploc bags in the laboratory between lab sessions. All the bags containing coats for one lab section may be stored in plastic tubs in the lab to conserve space. Students should be instructed to wear their coats properly at all times. For example, the coat should button at all points in a way that completely covers the front of the body to a reasonable point at the lower neck. The sleeves of the lab coat should completely cover the sleeves of street clothes. At the end of the semester, coats must be sterilized, e.g., autoclaved, before students they are laundered for the next semester. Any exposed street clothes and shoes are subject to contamination. Students must be warned that if their clothing or shoes become contaminated with a spill, then such contaminated items must remain in the lab until they have been decontaminated. A good practice is to keep spare sets of scrubs/clogs along with lab coats on the premises in case of spills on street clothes, shoes, or the lab coat itself.
- **Closed shoes.** Hard-sole shoes are required in all laboratories to protect against heavy

objects, hot liquids, or broken glass. Shoes should enclose the entire foot. The closed style is necessary due to the additional risk of contamination in the microbiology lab (see “Lab Coats” above).

- **Gloves when handling hazardous and/or infectious materials.** It is imperative to teach students the practices of proper glove use (see “Resources for More Information” below) and to constantly reinforce and remind them of these practices (see the “Note-Taking Area” below for details on how to structure lab procedures and work areas to allow for safe and proper use of gloves). Practices of proper glove use include checking the integrity of the gloves, not re-using single use disposable gloves, aseptic techniques for glove removal, and that hands must be washed immediately after glove removal. When a microscope and prepared slides are used or when a procedure is not actively being performed, gloves are not necessary. However, gloves are recommended for all procedures if a student has any open wounds such as a fresh cut. Providing non-latex gloves (some students have latex allergies) in all sizes to ensure a proper fit for all students is also recommended: ensure that the glove selected will provide protection against the hazards (biological and chemical) present in the laboratory. Gloves worn in a BSL1 or BSL2 laboratory must be discarded in a waste container for biohazardous materials.
- **Safety Glasses and Goggles.** In the United States, the federal government establishes safety guidelines for workplaces, to decrease the risk of on-the-job injuries. The Occupational Safety and Health Administration (OSHA) within the U.S. Department of Labor oversees safety practices in the workplace and in educational settings. OSHA has adopted safety eyewear standards established by the American National Standards Institute (ANSI), a private, non-profit organization that creates quality and safety standards for a wide variety of products. The University of Utah also follows these standards.

Updated ANSI safety eyewear standards include the following key features:

- Safety lenses now have two classifications of performance: basic impact and high impact. For most biology laboratory environments, lenses meeting the basic impact standard are sufficient.
- For the basic impact tests, lenses are tested separately (not mounted in a frame). For the high impact classification, the frame and lenses are tested together as a unit.
- Non-prescription lenses used for high impact testing are considered to be structurally weaker than prescription lenses made of the same material; the

prescription lenses are generally thicker. Non-prescription safety eyewear with non-removable lenses must be permanently marked with the manufacturer's trademark and "Z87" (basic impact) or "Z87+" (high impact) on either the front of the frame or on one temple.

- Prescription safety lenses are allowed if they meet the high impact testing requirements. Prescription safety frames must be permanently marked with the manufacturer's trademark and "Z87-2" on the front of the frame and on both temples.
- Indirectly vented, chemical-splash goggles, Z87+, should be the standard eye protection when using chemicals (solids and liquids), glassware, heating sources, preserved specimens, or dust/solid particles. An indirectly vented, chemical-splash goggle should fit snugly on the face surrounding the eyes, and the soft, pliable flange seals should extend around the eye. Since goggles need ventilation to reduce fogging, indirectly vented chemical-splash goggles are required to have hoods or caps over the vent openings to prevent chemical splashes from entering the inside of the goggle and causing injury to the eye.
- Face shields alone do not protect employees from impact hazards. Face shields may be used in addition to safety goggles or safety glasses if additional splash protection is required: see [OSHA FAQs](#).

Culture Preservation

When new stock cultures are obtained, they can be frozen for long-term storage and then revived annually or whenever needed. Common culture preservation methods include inoculating CryoBeads (Hardy Diagnostics) or freezing liquid cultures in 15% glycerol or 10% DMSO. CryoBeads maintain long-term culture viability when frozen at -20°C. Freezing cultures in glycerol or DMSO requires storage at -80°C for long-term viability.

Note-Taking Area

While conducting their experiments, students should never contaminate items that will leave the laboratory. The area for culturing and working with microorganisms should be as separate as possible from the area for taking notes. Absolutely no cell phone or personal electronic device use is permitted in the laboratory. Students should write with laboratory-use-only pens and pencils while taking notes in lab. These items should be provided by the institution, be used only for microbiology laboratories, and never leave the laboratory. Always minimize the

number of notebooks and/or lab manuals on the lab bench. Of their belongings, students should keep out only what is necessary for the day's work. All other personal belongings, e.g., backpacks, purses, books, etc., should be stored way from the work area in spaces approved by the instructor. Papers that students will take home should be protected from contamination during the lab period. Optional approaches for taking notes in the lab are dependent upon the design of the facility, and practices will vary from institution to institution. Here are some options:

- Note Taking:
 - If a pull-out desk shelf is available, all notes should be taken on that shelf, away from the work area.
 - If a pull-out desk is NOT available, the instructor should lecture, allowing students to take notes first, then have the students put away their notebooks and conduct the experiment that is assigned that day.

Optional approaches for conducting experiments, accessing the laboratory protocol, recording results and notes during the experiment are available:

- Protocols may be:
 - Shown to the entire class using a projector.
 - Separated from the lab manual that is printed in loose-leaf form and laminated; these laminated pages are disinfected after each lab and stored in the lab.
 - Separated from the lab manual that is printed in loose-leaf form and inserted into plastic report covers; these covers are disinfected at the end of each lab.
 - Available in a lab manual that has been designated as a desk copy that remains in the lab at all times and is the property of the institution; the desk copy can be kept at each lab table for use during the lab.
- Recording results and taking notes during the experiment:
 - One person per lab group can be the recorder for the day; the student recorder does not actually conduct the experiment, but rather takes notes for the day and shares

these notes with other students at the end of the lab session.

- Students can use the one-glove method to take notes. Students wear a glove on the nondominant hand, and only that hand touches any surface that potentially contains microorganisms. The student uses the dominant, ungloved hand to write notes in his or her laboratory notebook. Instruction on proper glove use must be provided and enforced.
- Notes and results can be recorded on a computer in the lab and emailed to all students.
- Notes and results can be scanned using handheld scanners that remain in the lab. Scanned documents can be emailed to all students.
- Notes and results can be sealed in a Ziploc bag. The outside of the bag can be bleached, removed from the lab and photocopied. Copies can be given to students as they leave the lab.
- Any papers or notebooks that become contaminated during the experiment must remain in the lab and be properly decontaminated and discarded.
- Data can be recorded on a tablet device sealed in a Ziploc bag. The outside of the bag can be bleached and the device removed from the lab. Data can be shared electronically.

Generation of Aerosols

Most laboratory-acquired infections are believed to be due to inhalation of microbial aerosols. Use of proper techniques to minimize aerosols must be emphasized when teaching microbiology. For example, when pipetting, hold the pipet tip against the edge of the culture tube to allow the liquid to run down the inside of the tube (rather than dripping liquid into the tube) and stop before the final drop of culture is blown out of the pipet. When using heat to sterilize loops, separate sterile plates with agar media for students to use as a “sizzle plate.” In this case, hot loops always touch the sterile agar sizzle plate before touching the working culture. In a BSL2 lab, any procedure known to generate aerosols (centrifuging, grinding, blending, shaking, mixing, sonicating, etc.) must be performed in a biological safety cabinet.

Biological Safety Cabinets (BSCs)

- **Requirements:** A BSC is not required for handling or working with organisms at BSL1. In

microbiology teaching laboratories that handle organisms at BSL2, most standard pipetting and plating protocols, if done properly, do not generate aerosols such that a BSC is necessary for student use. In these cases, PPE, including eye protection, and proper handling of materials are sufficient.

Whenever procedures have a potential for creating infectious aerosols, properly maintained BSCs (inspected and certified annually), other appropriate personal protective equipment, or other physical containment devices must be used. These procedures are standard laboratory procedures and include some pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, and opening containers of infectious materials. In addition, the BSC is required when using high concentrations or large volumes of infectious agents or when opening sealed containers of organisms in a BSL2 lab that become depressurized upon opening and can result in the release of concentrated stock culture.

- **Certification:** Biosafety cabinet certification is crucial to maintaining primary containment to keep the lab and personnel as safe as possible. Class I and II biological safety cabinets are tested and certified when they are installed in a lab, any time they are moved to a new location, or following any maintenance procedure. The BSC should be certified annually. This requires a knowledgeable NSF-certified technician and is not something lab personnel are able to perform. There should be a sticker prominently displayed on the front of the BSC that tells when the BSC was last certified and when it is due for recertification. Users should be in the habit of checking the certification each time they use the hood. If you need assistance with cabinet re-certification please contact OEHS (801-581-6590).

Class II BSCs are not fume hoods and must not be used with volatile chemicals, unless they are a Type B2 cabinet that is vented to the outside. The BSC is designed to contain biological specimens. Class II BSCs have an inward airflow that will protect the user, a HEPA-filtered downward laminar airflow to protect the specimen, and a HEPA-filtered exhaust that protects the lab. National Sanitation Foundation International in Ann Arbor, Michigan, has developed standards for BSC design, construction, and performance as well as a list of products that meet these [standards](#). [Here](#) is a link to a video of airflow in a BSC versus that in a laminar flow hood.

- **Care and Use:** Education of personnel is critical. The function of the BSC can be compromised by inappropriate actions or an unsuitable location within the lab. Inward flow of air may be disrupted by movement of others in the room, improper placement of materials within the hood, opening and closing doors to the lab, movement of arms

into and out of the BSC, and sideways motion of hands in the cabinet.

BSCs should be surface decontaminated with an appropriate disinfectant prior to and at the conclusion of any work in the cabinet: 70% ethanol is commonly used but is not appropriate after handling blood or other potentially infectious material as defined in the [OSHA Bloodborne Pathogen Standard](#), in which case an EPA-registered disinfectant must be used. The entire cabinet should be thoroughly cleaned at least once a month. This includes removal and disinfection of the bottom pan as well as thoroughly cleaning the front and back of the sash and all interior surfaces of the BSC.

Microincinerators

Due to the danger posed by open flames in the laboratory, microincinerators are recommended for the teaching lab. Sterilization of loops or needles takes only a few seconds in the incinerator, and the chance of splattering the culture when sterilizing a contaminated loop is eliminated. Heat-fixing cultures and flaming the open end of a tube can be accomplished over the entrance to the microincinerator.

Disinfectants

A wide array of disinfectants are commercially available. Disinfection of blood or other potentially infectious material, as defined in the [OSHA Bloodborne Pathogen Standard](#), require the use of [EPA-registered disinfectant](#): alcohol is not EPA-registered and should not be used in these cases.

The instructor is responsible for assessing the risk level of microbes used in his or her lab and determining the most appropriate disinfectant for routine decontamination of laboratory surfaces and equipment. Consideration should be given to the specific microbes used in a lab, the concentrations of microbes handled by people working in the lab, and the types of surfaces to be decontaminated.

Commonly used disinfectants for microbiology labs include 1:10 dilutions of bleach (approximately 0.5% sodium hypochlorite), 70% ethanol, and 70% isopropanol. Many others are available; efficacy and cost are considerations. This is not an endorsement of any one commercial product. Squirt bottles stamped with the ethanol or sodium hypochlorite and the NFPA symbol are available. Instructors and student assistants should be familiar with the proper concentrations utilized for each disinfectant and follow the manufacturer's instructions for proper application techniques and required contact times.

Sodium hypochlorite is readily available and inexpensive. Commercial bleach products are typically 5-6% aqueous solutions of sodium hypochlorite. Sodium hypochlorite is used to

decontaminate surfaces; in waste containers for used pipettes, tips and swabs; and to clean up spills. Bleach is corrosive to metals and should be used sparingly on stainless steel. Metal surfaces that have been treated with bleach should be “rinsed” with 70% ethanol or water after a 20 minute contact time.

- Routine benchtop disinfection:

Freshly prepared 1: 10 (10%) dilutions of commercially available bleach are suitable for general use to disinfect tabletops and work areas. Spray the 1:10 dilution of bleach solution on the benchtop, wipe the entire surface, and allow to air dry. Mix 100 ml bleach with 900 ml dwater: the solution should be prepared fresh daily.

- Disinfecting a spill

- A 1:10 solution of bleach should be used to clean up spills and in discard containers for used pipettes, tips and swabs: for larger spills (>10ml, use undiluted to 1:4 diluted bleach).
- Following a spill, everyone in the lab should be made aware that there is a spill and the area evacuated.
- Allow aerosols to settle for at least 60 minutes.
- If splashed, remove PPE and don clean PPE (Lab coat, 2 pairs of gloves, eye protection, face mask, shoe covers (if spill is on floor)).
- Cover the spill with paper towels and pour disinfectant slowly around and over the spill, starting from the outside and working in.
- Saturate the area with bleach and allow to remain undisturbed for at least 20 minutes.
- Clean up paper towels and place in the biohazard bag to be autoclaved/disposed: if the spill included any items that could cut or abrade your skin (glass, needles, pipet tips) use tongs to pick up the paper towels.
- Spray the area again with bleach solution and allow to air dry
- Remove PPE and dispose in biohazard bag. Wash hands with soap and water.

Sodium hypochlorite solutions should be mixed fresh daily.

Alcohols (ethanol and isopropanol) should not be used to clean up spills because of their volatility which preclude an appropriate contact time. Alcohols are highly flammable and should not be used near an open flame. Alcohols are effective for routine decontamination of stainless steel surfaces, such as those in biosafety cabinets, but not spills. Alcohols can be used to remove residual bleach from metals to minimize corrosion. However, alcohols are not effective against bacterial spores.

Incubators, chemical fume hoods, and biological safety cabinets should be thoroughly disinfected monthly.

Proper Decontamination and Disposal Procedures:

Autoclaving is the gold-standard sterilization method and must be used for all petri dishes contaminated with organisms in a BSL2 lab. Autoclaving is used for contaminated dishes in a BSL1 lab, if available. If not available, disinfection of petri dishes contaminated with organisms in a BSL1 lab may be accomplished by soaking the surface of the contaminated plate in a 1:10 dilution of bleach for 2 hours prior to discarding the plate.¹ Gloves used in a BSL1 lab can also be decontaminated using this bleach soaking protocol if an autoclave is not available. After two hours of soaking in a 1:10 dilution of bleach, solid waste (plates, gloves, etc.) can be disposed of in the regular trash and the bleach solution can go down the drain.

Isolation of Unknown Microbes from the Environment:

A common microbiology exercise is collecting environmental samples and plating the samples for colonies. Whether the sample is from nature (soil, leaves, etc.), inanimate objects (doorknobs, telephones, etc.), or humans (skin swabs, etc.), the isolated colonies could be organisms needing BSL2 containment and in rare cases BSL3 containment. Plating isolates from environmental samples can be performed in a BSL1 lab. These plates should be sealed, stored in a secure location, and only observed, not opened or subcultured. After observation, the plates must be decontaminated by autoclaving and properly disposed of. Subculturing of environmental samples should only be performed in a BSL2 lab.

Cultivation of Fungi:

If you cultivate fungi in the laboratory, it is highly recommended that you keep them in separate incubators and/or refrigerators dedicated for fungal growth and storage. These storage units should be cleaned frequently with bleach. Fungal cultures should be opened and transferred in a dedicated area or biological safety cabinet, away from bacterial cultures.

Autoclave Validation:

Requirements for autoclave validation vary by state. Utah Administrative Code [R315-316, Infectious Waste Requirements](#), states that sterilization units shall be evaluated for effectiveness with spores of *Bacillus stearothermophilus* at least once each 40 hours of operation or each week, whichever is less frequent.

Autoclaves are used to sterilize and decontaminate biological waste. The key components are:

- A. Appropriate use of the autoclave to decontaminate biological waste
 - Minimal parameters are 121°C at 15 psi for 15 min.
 - Time may need to be increased for larger loads and larger volumes of fluid.
 - Items should be loaded in a manner that ensures that steam can penetrate packages and test tubes.
- B. Recordkeeping – There should be a log or notebook adjacent to the autoclave to indicate:
 - Date
 - Time
 - User name and contact number
 - Type of load (liquids, hard goods, etc.)
 - Items autoclaved (media, waste, pipettes, etc.) and amount
 - The temperature, pressure and duration of the treatment.

Ideally, any autoclave paper tape would be kept with the waste log to verify autoclave parameters. Logs must be maintained for at least 3 years.

- C. Performance verification – threefold
 - 1. If the autoclave has paper tape to record performance, this should be checked prior to opening the door to be sure all temperature, pressure, and/or time parameters were met.
 - 2. Autoclave indicator tape should be clearly visible on each item placed in the

autoclave (one per rack of tubes, one per beaker, one on a bag of used plates, etc.). Some biological waste bags have integrated heat sensitive indicators, e.g., Fisherbrand™ Orange Autoclave Bags With Sterilization Indicator (Product # 01-814).

3. The person in charge of the autoclave operation or a designated safety officer should conduct a monthly performance verification using the biological thermophilic spore former *B. stearothermophilus* (ATCC 7953). There are several different verification methods that employ this organism. One is the Sterikon Plus Bioindicator ampule system, a rapid and easy-to-use method for verifying steam sterilization. Indicators consist of an ampule containing nutrient broth, sugar, a pH indicator, and 2 mL of *B. stearothermophilus* spores. Simply place ampules in the autoclave along with the batch to be sterilized, and incubate afterwards. A color change of the ampule contents clearly indicates whether sterilization was successful. This testing should be documented monthly and readily available for inspection.

Waste shall not be considered sterilized if the tape or equivalent indicator fails to indicate that a temperature of at least 250 °Fahrenheit (121 °Celsius) was reached and a pressure of at least 15 psi was maintained during the process.

D. Annual calibration and maintenance

- An outside maintenance person familiar with the operation of autoclaves should perform this service.

Pest Control:

The microbiology lab is a controlled, regulated, and sanitary environment where only known organisms should be cultured and stored. Just as in the home or commercial kitchen, any contamination with insects, rodents, other pests or unwanted contaminants (such as mold) cannot be allowed. Following all safety guidelines for hygiene should help ensure that these unwanted visitors are reduced or eliminated. However, in older buildings or through any mismanagement, neglect, or careless use of a facility, problems are inevitable.

- **Rodents.** All media should be stored in sealed containers or rodent-proof cabinets. If possible, keep plated cultures in sealed plastic containers or bins when they are not refrigerated or incubated. Never store animal feed or grains in the microbiology laboratory. If there is any sign of rodent invasion, take action immediately with recommend protocols, e.g., traps or consultation with pest-control specialists, for your

institution. Glue traps for mice have been banned in some locations. A more humane approach is to use traps that kill mice instantly. If you set traps, check them frequently.

- **Flies, roaches, and other insects.** Windows in a microbiology lab should not be opened, but if this is necessary, then the windows must be screened. Typical methods of flying-insect or roach control can be used to eliminate these pests. Good hygienic practices are the first defense, and the source of any new infestation should be speedily identified. Fruit flies can present a special problem since they are attracted to microbial cultures and can enter and exit petri plates easily. This makes cross-contamination of cultures and contamination of surfaces possible. Keeping all media sealed is the best method of control. Door guards can prevent flies from entering from another laboratory. In some cases, flies come from a nearby lunchroom. There are many commercially available traps for fruit flies; a simple homemade trap consists of diluted vinegar or apple cider poured into the bottom of an open plastic soda bottle. Flies are attracted to the liquid, enter or fall into it, and drown. Such traps should be changed frequently during an infestation.
- **Mold.** Damp environments, especially those in areas without proper ventilation, cultivate mold. Keeping the laboratory clean and dry with windows closed is the best method of prevention. Report any serious mold infestation in the laboratory because it can pose a human health hazard that should be addressed by professionals.

Resources for More Information

Resources, especially from federal regulatory agencies (OSHA, FDA, EPA, CDC, etc.) and state health departments, are very helpful. Valuable online resources include:

- Biosafety in Microbiological and Biomedical Laboratories (BMBL), version 5 (<https://www.cdc.gov/biosafety/publications/bmb15/>). CDC, Atlanta, GA
- American Biological Safety Association website (www.absa.org)
- Biological Safety Principles and Practices, 4th edition, edited by Fleming and Hunt
- Public Health Agency of Canada, Pathogen Safety Data Sheets (<http://www.phac-aspc.gc.ca/labbio/res/psds-ftss/index-eng.php>)

Another resource may be YouTube videos that demonstrate safe practices, such as [aseptic glove removal](#) and [proper use of a biological safety cabinet](#).

¹ James, D.E., 2008. Nine safe practices for the microbiology laboratory. Carolina Biological Supply, Burlington, NC.

APPENDIX 3

Sample Forms

The following sample forms are provided by [ASM](#). These are available to be edited and revised as appropriate for your laboratory.

- Laboratory Safety Statement and Student Agreement on Laboratory Safety
- Biosafety Manual: the Biosafety Office has detailed BSL2 Biosafety manual templates available on request

LABORATORY SAFETY STATEMENT

*BIOL XXX MICROBIOLOGY**

The lab exercises in this course involve the use of living organisms. Although the microorganisms we use are not considered to be highly virulent, **all microorganisms should be treated as potential pathogens** (organisms capable of causing disease).

The following rules must be observed at all times to prevent accidental injury to and infection of yourself and others and to minimize contamination of the lab environment:

1. **Never place books, backpacks, purses, etc.,** on bench tops. Always place these in the assigned cubicles. Keep manuals and pens on pull-out desks.
2. Personal electronic devices should not be brought into the lab. This includes, but is not limited to iPods, MP3 players, radios, cell phones, and calculators.
3. Clean your work area with dilute bleach solution at the **beginning AND end** of each lab.
4. **Wash your hands with soap** and dry with paper towels when entering and leaving the lab.
5. **Wear a lab coat** at all times while working in the lab to prevent contamination or accidental staining of your clothing.
 - a. **Closed shoes** (no sandals) are to be worn in the lab.
 - b. **Long hair must be tied back** to prevent exposure to flame and contamination of cultures.
 - c. **Gloves must be worn** when staining microbes and handling hazardous chemicals.
6. **Do not place anything in your mouth or eyes while in the lab.** This includes pencils, food, and fingers. Keep your hands away from your mouth and eyes.
 - a. Eating and drinking are prohibited in the lab at all times.
 - b. This includes gum, cough drops, and candy.
 - c. Do not apply cosmetics in the lab. This includes Chapstick and Blistex.
 - d. **Never pipet by mouth.** Use a mechanical pipetting device.

7. **Do not remove media, equipment, or bacterial cultures from the laboratory.** This is absolutely prohibited and unnecessary.
8. Do not place contaminated instruments such as inoculating loops, needles, and pipettes on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles of bleach solution.
9. Carry cultures in a test tube rack when moving around the lab or when keeping cultures on bench tops for use. This prevents accidents and contamination of your person or belongings.
10. **Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution.** Notify your instructor that there has been a spill. After 15 minutes, dispose of the towels and broken items as indicated by your instructor.
11. **Report accidental cuts or burns to the instructor immediately.**
12. At the end of each lab session, place all cultures and materials in the proper disposal area.
13. Persons who are immune-compromised (including those who are pregnant or may become pregnant) and students living with or caring for an immune-compromised individual are advised to consult with your physician to determine the appropriate level of participation in the lab. Should your physician determine that you should not participate in this lab, please have him or her write a note stating the concerns. Alternative accommodations may be indicated.

OSHA INFORMATION

Safety Data Sheets (SDS) are located _____.

The first aid kit is located _____.

The eyewash station is located _____.

The shower is located _____.

The fire extinguisher is located _____.

STUDENT AGREEMENT ON LABORATORY SAFETY

I have read the Laboratory Safety Statement of the Department of XXXXXXXX,* University of Utah, and I understand its content. I agree to abide by all laboratory rules set forth by the instructor. I understand that my safety is entirely my own responsibility and that I may be putting myself and others in danger if I do not abide by all the rules set forth by the instructor.

COURSE: *BIO XXX MICROBIOLOGY**

NAME OF STUDENT (PRINT): _____

SIGNATURE OF STUDENT: _____

DATE: _____

*Insert Appropriate Information

SAMPLE BIOSAFETY MANUAL

MICROBIOLOGY BIOSAFETY MANUAL FOR BSL2 LABS

University of Utah

Rooms _____

_____ **Building**

Date (document last revised): _____

Person responsible for this lab and updating this manual: _____

His or her title: _____

I. Authority for Microbiology Lab and Prep Room Regulations

Labs will follow the guidelines posted by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. These guidelines describe acceptable biosafety practices in biomedical and microbiological laboratories and can be found at: <http://www.cdc.gov/OD/ohs/biosfty/bmb15/bmb15toc.htm>.

_____ (room numbers) _____ are multipurpose rooms. BSL1 precautions will be followed during routine media prep, autoclaving, and subculturing.

Whenever a BSL2 agent is in use, biohazard signs will be posted on the doors and the entire room will follow BSL2 practices.

II. Regulations

A. Access, Training and Responsibilities

1. Access is limited to individuals involved directly in media prep, clean up, lab prep, lab work and research.
2. The lab and prep room doors will be closed when a BSL2 agent is in use.
3. All staff and students are required to read, understand, and follow these regulations before working in _____.
4. All staff and students working in _____ will receive training from _____ concerning use of the equipment.
5. _____ will train staff and students on aseptic techniques appropriate for handling pathogenic agents. This will include the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures.
6. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
7. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
8. Any staff or students found in violation of the regulations may have their access to room _____ terminated.

9. The supervising PI/teacher is responsible for seeing that the consequences of student or staff actions are rectified, including correction of damages and violations and take-down of experiments.

B. Apparel

1. Personnel entering room _____ will be required to wear closed-toe shoes and have long hair tied back.
2. Personnel working in _____ at BSL2 must wear lab coats at all times. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., restroom, cafeteria, library, or administrative offices). All protective clothing is either autoclaved or laundered with bleach by the institution before being returned to personnel.
3. Gloves are worn when handling microorganisms or hazardous chemicals. Gloves are disposed of when contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Gloves are placed in a biohazard bag and autoclaved prior to disposal. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Hands are washed following removal of gloves.
4. In a BSL2 lab, safety goggles or safety glasses are worn for normal lab procedures involving liquid cultures that do not generate a splash hazard (e.g., proper pipetting, spread plates, etc.). Safety goggles and face shields or safety goggles and masks are worn when performing procedures that may create a splash hazard.
5. When working in a biosafety cabinet, only lab coats and gloves are needed for personal protection.

C. Standard Microbiological Practices

1. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the lab. Food for human consumption is never stored the lab.
2. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Information to be posted includes the agent(s) in use, biohazard symbol, Biosafety Level 2, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

3. Persons wash their hands upon entering the lab, after they finish working in the lab, after removing gloves, and before leaving the laboratory.
4. Work surfaces are decontaminated prior to beginning any work in these rooms, on completion of work or at the end of the day with 10% bleach solution. Any spill or splash of viable material should be decontaminated with 25% bleach solution.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols. Any procedure that would potentially create aerosols will be performed within the biosafety cabinet.
6. Mouth pipetting is prohibited; mechanical pipetting devices are used.
7. A limited number of needles and syringes are used for reconstituting reagents. After use, these materials are placed in a puncture-proof red sharps container. Do not recap needles.
8. All cultures, swabs, and waste containers are decontaminated before disposal by autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container for transport from the laboratory.

D. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. Persons who are at increased risk of acquiring infection – e.g., those who are immunocompromised or immunosuppressed – or for whom infection may have serious consequences, should consult with their physician to determine the appropriate level of participation in the lab.

E. Transfer of materials

1. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container that prevents leakage during collection, handling, processing, storage, and transport.

F. Disposal of Materials and Decontamination

1. Laboratory equipment and work surfaces should be decontaminated with 10% bleach on a routine basis and after work with infectious materials is finished. Overt spills, splashes, or other contamination by infectious materials should be decontaminated with 25% bleach.

2. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
3. Broken glassware that does not contain live cultures should be swept up with the broom and dust pan and discarded in the glass disposal box.
4. Broken glassware that contains live cultures should be saturated with bleach solution. After 20 minutes, the debris should be “swept” up into an autoclave bin using a plastic beaker and/or paper towels. After being autoclaved, the glassware can go into the glass disposal box and the paper towels can go into the regular trash.

G. Hygiene and Housekeeping

1. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and bleach used to decontaminate the work surfaces.
2. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs used in laboratory work should be covered with a nonporous material that can be easily decontaminated.
3. Safety Data Sheets (SDS) are located _____.
4. First aid kits are located _____.
5. Eyewash stations are located _____.
6. The emergency shower is located _____.
7. Fire extinguishers are mounted on the wall _____.