Appendix to the Guidelines for Biosafety in Teaching Laboratories

AMERICAN SOCIETY FOR MICROBIOLOGY

2012

CONTRIBUTING AUTHORS

ASM Task Committee on Laboratory Biosafety

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

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

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

Diane Hartman
Department of Biology
Baylor University

Amy White
Department of Biology
Virginia Western Community College

Ex Officio

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

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

Amy Chang, Director, ASM Education

Ad Hoc Reviewers

Cristina Bressler
Centers for Disease Control and Prevention

Diane O. Fleming
Biological Safety Professions

Roxana B. Hughes
University of North Texas

Kai Hung
Eastern Illinois University

Michael J. Imperiale
University of Michigan

Gary E. Kaiser
The Community College of Baltimore County

Sue Katz
Roger State University

Donald Lehman
University of Delaware

Tracey Meilander
Notre Dame College

Paul Meechan
Centers for Disease Control and Prevention

Susan Merkel
Cornell University

Melanie Popa
University of Pittsburgh

Robert J. Wolff
South University

Christopher Woolverton
Kent State University

Please send comments to education@asmusa.org

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APPENDIX

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 (see Table 1), the microbe alone does **not** determine the biosafety level of the lab. For example, manipulations that general 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 (http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf) or Public Health Agency Canada (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php) to clarify safety requirement information about a particular organism.

Explanatory Notes

For ease of use, the BSL1 and BSL2 Guidelines for Biosafety in Teaching Laboratories 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, nonporous lab furniture, culture preservation, note-taking areas, generation of aerosols, biological safety cabinet requirements and certification, microincinerators, disinfectants, proper decontamination and disposal procedures, isolation of unknowns, autoclave validation, pest controls, substitutions of organisms needing BSL1 containment for those needing BSL2 containment, and additional resources.

Personal Protective Equipment

- **Safety goggles or safety glasses when handling liquids or when plating.** If safety glasses (rather than goggles) are used, they should have side panels. Goggles and glasses should not be shared and should remain in the laboratory at all times. If they are reused, e.g., by students in another semester, they must be sanitized. Commercially available UV cabinets are available for this purpose. Alternatively the items can be sanitized by wiping them with 70% ethanol. Use lens paper or a soft cloth for wiping to minimize scratching the items.

- **Lab coats.** Protective coats or covering must be worn in the BSL2 lab. Coats may be purchased by students 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 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 can take them home or before laundering if they are loaned items. 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-toe shoes.** Hard-sole shoes without open toes are required in all laboratories to protect against heavy objects, hot liquids, or broken glass. The closed-toe 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.** Gloves should be worn when microbial cultures are handled at BSL2. 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). 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. Gloves worn in a BSL1 or BSL2 laboratory must be discarded in a waste container for biohazardous materials.

Nonporous Lab Furniture

All furniture in the lab (chairs, stools, etc.) must be nonporous or non-pervious so it can be cleaned and disinfected in case of a spill. Wooden stools should be coated with a sealant (shellac, varnish, etc.) so the wood is nonporous and easily cleaned.

Laboratories Used for Multiple Purposes

When these guidelines are followed, it is safe to use the same room as a microbiology lab and a teaching room for other classes. All cultures should be stored properly (e.g., in incubators or refrigerators) and not left out in common areas. BSL2 biohazardous waste should be removed before the room is used for other purposes. If a prep room or disposal room is shared with other labs, BSL2 cultures and waste should be decontaminated immediately or stored in a separate, non-shared location that is not accessed by non-BSL2 personnel.

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 non-dominant 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.

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, 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 technician and is not something lab personnel are expected or required 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. To search for a biosafety cabinet certifier in your area, go to http://www.nsf.org/Certified/Biosafety-Certifier.

  Class II BSCs are not fume hoods. 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. See http://www.nsf.org/business/biosafety_cabinetry/index.asp?program=BiosafetyCab for details. Here is a link to video of airflow in a BSC versus that in a laminar flow hood: http://www.youtube.com/watch?v=Wg61LdngWlQ&feature=related.

- **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 70% alcohol (ethanol or isopropanol) prior to and at the conclusion of any work in the cabinet. The entire cabinet should be thoroughly cleaned at least once a month. This includes removal and disinfection of the bottom tray 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. 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 5-10% sodium hypochlorite (bleach), 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 products are 5-6% aqueous solutions. 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.

- **Routine benchtop disinfection**
  Ten percent dilutions of commercially available bleach are suitable for general use to disinfect tabletops and work areas. Spray the 10% bleach solution on the benchtop, wipe the entire surface, and allow to air dry. **Mix 100 ml bleach with 900 ml dwater for a 10% solution.**

- **Disinfecting a spill**
  A stronger solution of bleach (25% dilution) should be used to clean up spills and in discard containers for used pipettes, tips and swabs. Following a spill, everyone in the lab should be made aware that there is a spill. Cover the spill with paper towels and **pour** disinfectant around and over the spill. Saturate the area with bleach and allow to remain undisturbed for 15 to 30 minutes. After that, place paper towels in the biohazard bag to be autoclaved. Then, spray bleach solution over the entire area and wipe it down one more time. **Mix 250 ml of bleach with 750 ml dwater for a 25% solution.**

Sodium hypochlorite solutions should be mixed fresh weekly.

Alcohols (ethanol and isopropanol) are most effective as 70% solutions. Inactivation of organic debris is a hydrolytic reaction. Alcohols are highly flammable and should not be used near an open flame. Alcohols are effective at decontaminating stainless steel surfaces, such as those in biosafety cabinets. Alcohols can be used to remove residual bleach from metals to minimize corrosion.

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. Disinfection of petri dishes contaminated with organisms in a BSL1 lab may be accomplished by soaking the surface of the contaminated plate in 10% 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 10% 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.

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

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. Check with your public health department for requirements pertinent to your area.

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^{o}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.)

   Ideally, any autoclave paper tape would be kept with the waste log to verify autoclave parameters.

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.).
   3. The person in charge of the autoclave operation or a designated safety officer should conduct a monthly performance verification using a biological thermophilic spore former, such as *Bacillus 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 spores from the apathogenic organism *Bacillus stearothermophilus* ATCC 7953. 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.

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.

Substitution of Organisms Handled at BSL1 for Those Handled at BSL2

The use of organisms needing BSL2 containment is required for demonstrating specific microbiology procedures; however, operating within BSL2 guidelines can be challenging for some institutions. A number of standard microbiology tests can be conducted with organisms needing BSL1 rather than BSL2 containment with the same results, thereby minimizing risk to the students. Organisms needing BSL1 containment should be used whenever it is possible to conduct lab activities without compromising learning goals.

- Protocols that can be performed easily with organisms contained at BSL1 include those for anaerobic growth, capsule stain, carbohydrate fermentation, casein hydrolysis, catalase, endospore stain, bacterial enumeration, flagella stain, EMB plate, gelatin hydrolysis, Gram stain, hanging drop, IMViC, Kirby-Bauer, Luria broth, indole, litmus milk, *E. coli* MUG, MacConkey agar, mannitol, nitrate reduction, oxidase, spread plate, starch hydrolysis, streak plate, transformation assay, urease, streak plating, and TSI.
- Protocols that are difficult to perform by substituting organisms handled at BSL1 for organisms handled at BSL2 include tests for acid-fast stain, animal cells in culture, CAMP test, coagulase, and hemolysis.

Table 1 includes a list of frequently used microbes with their ATCC numbers and common BSLs used to handle the organisms. The majority of these organisms can be purchased inexpensively as Preceptrol cultures from the ATCC (www.atcc.org).

Table 1. Frequently Used Microbes with ATCC Numbers and Common BSL for Use

Microbe	BSL	ATCC number
Alcaligenes faecalis	1	8750
Aspergillus niger	1	16888
Bacillus stearothermophilus	1	7953
Bacillus subtilis	1	23857
Citrobacter freundii	1	8090
Clostridium sporogenes	1	3584
Enterobacter aerogenes	1	13048
Enterococcus casseliflavus	1	700327
Enterococcus faecalis	2	19433
Escherichia coli B	1	11303
Escherichia coli K-12	1	10798
Klebsiella pneumoniae	2	13883
Micrococcus luteus	1	4698
Penicillium chrysogenum	1	10106
Proteus vulgaris	2	Multiple
Pseudomonas aeruginosa	2	10145
Rhizopus stolonifer	1	14037
Saccharomyces cerevisiae	1	9763
Salmonella enterica	2	700720
Serratia marcescens Bizio	1	13880
Staphylococcus aureus	2	12600
Staphylococcus epidermidis	1	14990
Staphylococcus saprophyticus	1	15305

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 (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm). 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/lab-bio/res/psds-ftss/index-eng.php)

Safety regulations vary by state, and instructors should determine which safety regulations are unique to their state. Another resource may be YouTube videos that demonstrate safe practices, such as proper glove removal. A future activity for ASM may be to identify YouTube videos, images, and other resources that clarify or model safe practices when handling microorganisms.

Sample Forms

The following sample forms are provided. Please revise as appropriate for your institution.

- Laboratory Safety Statement and Student Agreement on Laboratory Safety
- Information for Physicians
- Biohazard Sign
- Emergency Contact Information
- Biosafety Manual

LABORATORY SAFETY STATEMENT
BIOL 215 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. 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-toe 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** should 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

Material Safety Data Sheets (MSDS) 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 Biological Sciences, ABC University,* 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 215 (MICROBIOLOGY) FALL 2012

NAME OF STUDENT (PRINT): _____

SIGNATURE OF STUDENT: _____

DATE: _____

In the highlighted areas, insert applicable information for your institution.

INFORMATION FOR PHYSICIANS

Microbiology labs at ABC University are operated at **Biosafety Level 2** (BSL2). Personal protective equipment (**lab coats, gloves, goggles. etc.**) is required to work in this lab, and access to the laboratory is restricted by the laboratory director when work with infectious agents is in progress.

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 should consult with physicians to determine the appropriate level of participation in the lab. Should your physician discern that you should not participate in this lab, please have him or her write a note stating the concerns. Alternative accommodations may be indicated.

BSL2 agents used in the lab include:

- *Enterococcus faecalis (*formerly *Streptococcus faecalis)*
- *Klebsiella pneumoniae*
- *Morganella morganii*
- *Mycobacterium smegmatis*
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*
- *Salmonella enterica* serovar Typhimurium
- *Staphylococcus aureus*
- *Streptococcus pyogenes*

SAMPLE BIOHAZARD SIGN – Print on orange paper and post on lab door.

BIOHAZARD

ROOM ACCESS IS RESTRICTED
TO AUTHORIZED PERSONNEL ONLY

BSL2 organisms in use

All personnel must wear long pants, closed-toe shoes, lab coats, gloves, and goggles. Long hair must be tied back.

PRIOR TO EXIT

Remove lab coats, gloves, and goggles.

WASH HANDS THOROUGHLY

PRINCIPAL INVESTIGATOR(S)

List name(s) and phone number(s)

SAMPLE EMERGENCY CONTACT INFORMATION FORM

ROOM _____
DEPARTMENT OF BIOLOGY
ADMITTANCE TO AUTHORIZED PERSONNEL ONLY
EATING, DRINKING, SMOKING, AND APPLYING COSMETICS ARE PROHIBITED

EMERGENCY INFORMATION

MSDS NOTEBOOK LOCATION:

_____

FIRST AID KIT LOCATION:

_____

SPILL KIT LOCATION:

_____

EMERGENCY CONTACT INFORMATION
Primary Faculty Contact: _____
Secondary Lab Contact: _____
Risk Management (add phone number): _____
Fire Emergency (add phone number): _____
Housekeeping (add phone number): _____
University Police (add phone number): _____

MICROBIOLOGY
BIOSAFETY MANUAL FOR BSL2 LABS

_____ University

Rooms _____

_____ Building

Date (document last revised): _____
Person responsible for this lab: _____
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/bmbl5/bmbl5toc.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, 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 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. An orange 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, leak-proof 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 15 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. **Material Safety Data Sheets (MSDS)** are located _____.
4. **First aid kits** are located _____.
5. **Eyewash stations** are located _____.
6. **The shower** is located _____.
7. **Fire extinguishers** are mounted on the wall _____.