Appendix to the Guidelines for Biosafety in Teaching Laboratories

2012

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- 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

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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.**

- **D sdipTd <00d <003TT1 1 Tf -35.94 -1.169 Td ( )Tj EMC /LBody <</MCID 11 >>BDC /C2(l)-1.57 Td ( )Tj**

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,

LABORATORY SAFETY STATEMENT
BIOL 215 MICROBIOLOGY*

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.

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)

MICROBIOLOGY
BIOSAFETY MANUAL FOR BSL2 LABS

_____ University

Rooms _____