

BioSide Lines

January 2006

The Newsletter of the Office of Biological Safety, UW-Madison Safety Department
www.fpm.wisc.edu/biosafety

Autoclave Safety

An autoclave is familiar equipment in the clinical and research facility. The autoclave utilizes saturated steam under pressure to kill bacteria, viruses, and fungi, including spore forms. The steam is heated many degrees past boiling under pressure; the increased pressure allows this steam to penetrate the contents to be autoclaved. Autoclaving is an effective way to inactivate microbes in lab waste, decontaminate lab equipment, and sterilize growth media.

An autoclave is an efficient killing machine when used properly, but things can go wrong because of poor maintenance and improper use, which can lead to harmful, if not dangerous, situations for the user. Burns can be avoided by following simple precautions. Here are some autoclave tips to prevent dangerous situations.

Before you start a run:

- Get appropriate training before operating an autoclave.
- Make sure nothing was left inside the autoclave by a previous user.
- Check the drain strainer at the bottom of the autoclave and remove obstructions.
- Check the gaskets around the door for damage and the floor for leaks. Do not run the autoclave if a problem is detected. Report problems immediately so repairs can be initiated.
- Use appropriate containers. Plastic containers must be heat resistant. Do not autoclave labware made of the following plastics: polystyrene, polyvinyl chloride (except PVC tubing), styrene acrylonitrile, acrylic, polyethylene and polyurethane.
- Medical sharps should be placed in appropriate rigid sharps disposal containers that can withstand autoclaving. Other sharp items, such as pipette tips should be placed in a rigid container to prevent injuries or broken bags.
- Never put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave. Consult the Laboratory Safety Guide if you have questions about proper disposal of these materials..
- Use shallow trays to catch leakage. Spilled agar could plug the drain pipe when it cools and solidifies. Place glassware in a secondary tray; never on the floor of the autoclave.
- Add water (about ½ inch) to the bottom of the tray to aid in even heating and to prevent bottles from breaking.
- Bags should be closed but not too tightly so that steam can enter. If the load is dry, some water should be added.
- Containers of liquid must not be sealed to prevent internal pressure that could cause them to break. Containers should be capped loosely or covered with foil or cotton plugs. Large bottles with narrow necks, in particular, can explode if they contain too much liquid.

Loading the autoclave:

- Use caution when loading bags of infectious waste into the autoclave. Never lift a bag from the bottom to load it into the chamber. Handle the bag from the top.
- Do not overload an autoclave. An over-packed autoclave chamber does not allow efficient steam penetration. Leave space between items so that steam will distribute evenly. Considerably longer sterilization times may be required to achieve decontamination if an autoclave is tightly packed.

- The cycle length will depend on the load – the density and amount of material, and how the chamber is packed. For example, the cycle time for materials in plastic containers may be longer than glass or metal since heat is transferred more slowly. The time to sterilize a large volume of liquid will be reduced if it is divided into several smaller containers. Stacking containers also may result in poor performance. Adequate cycle times are best determined by experience.
- Use heat sensitive tape with each load to verify that it has been autoclaved. Monitor efficacy periodically by inserting an indicator (e.g., *G. stearothermophilus* spores) into the center of a load. See the Jan. 2005 BioSide Lines for more information on efficacy monitoring.

Running and unloading the autoclave:

- Be sure that the autoclave door is fully closed, the radial interlock bars are in their slots, and the correct cycle is selected. Slow exhaust settings should be used when autoclaving liquids; fast exhaust is suitable for non-liquids.
- Allow the autoclave to cool for 10 minutes before removing treated items. To prevent steam burns, make sure that the chamber pressure is near zero before opening the door at the end of a cycle. Avoid standing directly in front of the door when opening it. Open the door slowly and allow the steam to escape gradually. Bottles containing superheated liquids might boil over and splash personnel with scalding liquid if handled too soon.
- Use personal protective equipment such as heat resistant gloves, protective eyewear, and a rubber apron or lab coat when unloading the autoclave.
- Check the autoclave chamber for spilled material and clean it up after the unit is cool. If glass has broken in the autoclave, use tongs, forceps or other mechanical means to recover fragments. Do not use bare or gloved hands to pick up broken glassware.
- Deface the biohazard symbol before putting autoclaved waste into trash bins. Medical sharps require additional processing (MERI) and may not be discarded as routine trash.
- Do not leave an autoclave operating unattended for a long period of time. Always be sure someone is in the vicinity while an autoclave is cycling in case there is a problem.
- Routinely inspect the autoclave to determine the need for maintenance and repair. Perform preventive maintenance according to the manufacturer's instructions.

Following these simple rules and the manufacturer's instructions will lessen the chance of an injury when using an autoclave.

Tips on the Biosafety Protocol Process

Tips can come in handy in situations such as how to drive safely after a fresh snow. In the spirit of saving you time and energy, the Office of Biological Safety (OBS) offers the following pointers in navigating the biological safety protocol process. The information is presented as responses to frequently-asked questions.

Q: Why does OBS keep on changing its blankety-blank protocol form?

A: We change the biosafety protocol form to improve collection of the information needed to do an independent risk assessment. We also change the form to make it more user-friendly. An example of this type of change is the table of the precautions and containment (Section V.C) that is filled out instead of the previous narrative format.

Q: How do I know if I even need to submit a biosafety protocol?

A: There are six criteria for submission of a protocol, outlined on our website (www.fpm.wisc.edu/biosafety). The Principal Investigator (PI) has the responsibility for making this determination and OBS will gladly provide guidance in interpreting the criteria. The most common question regards low-risk recombinant organisms for which a biosafety protocol is required to comply with federal regulations. The consideration of which recombinant activities are exempt from oversight is complicated by exceptions to the exemptions.

Q: What chemicals need to be included in the biosafety protocol?

A: Only include hazardous or potentially-hazardous chemicals that are administered to biological systems (e.g., animals, plants, cells) to elicit a biological response. OBS can provide feedback on which chemicals need to be included in your protocol. One good source for toxicity information is toxnet (<http://toxnet.nlm.nih.gov/>). The Particularly Hazardous Substance (PHS) form attached to the protocol may be completed instead of describing precautions for handling hazardous chemicals in Section V.C.

Q: What are the practical differences between a renewal and an amendment?

A: The protocol expires after 3 years at which time it must be updated and resubmitted using the current protocol form. Amendments are submitted if your protocol changes significantly during the 3 years. When submitting a renewal, be sure to list all current awards and pending proposals.

Q: What constitutes a change for which an amendment must be filed?

A: Our focus is whether risks might change with the new materials or procedures. Many items (e.g., cell lines, vectors, or pharmaceutical compounds) can be entered in your protocol as categories with representative examples rather than as exhaustive lists. Amendments are then not needed for items that fit into an existing category. However, OBS must be notified if adding a strain of a pathogen already in your protocol if that strain has increased risks. Also important is to make sure the biosafety and animal care protocols are consistent in describing materials administered to animals. Location changes must be submitted to allow assessment of the suitability of the new facilities for the intended purposes. Amendments for changes in personnel, however, are not needed.

Q: How should I submit an amendment?

A: When awards and grant proposals need to be associated with a protocol and the materials or procedures are already described, you only need to submit the first section of the form. Amendments with changes in materials and methods, however, should be done by adding new information to the existing protocol using a distinct character such as bold. This process is made easier if you save it electronically. The amendment, like an update, may be submitted as an attachment to email, by fax (265-8700), or by campus mail.

Q: How quickly will my protocol be processed?

A: Review of protocols is ongoing. Protocol registration forms are finalized and sent by campus mail to PIs on a monthly basis, shortly after the Institutional Biosafety Committee (IBC) meeting which is generally held the first Wednesday of each month. In the meantime, registration forms can be faxed to you upon request if the protocol has already been reviewed and registered. You may request expedited protocol processing and we will do our best to accommodate you.

Many protocols are reviewed in-house by OBS staff but some require approval by the IBC. Protocols to be reviewed by the IBC must be submitted at least 2 weeks in advance of the IBC meeting. This allows sufficient time for OBS to provide feedback and for you to do revisions if necessary prior to distribution to the committee.

Q: Can someone other than the PI sign the protocol form?

A: No, we need to have the signature of the PI, which gives us assurance that s/he accepts responsibility for the information submitted. However, protocols received as an attachment to email from the PI will be accepted as the electronic equivalent of the signature.

The biosafety protocol serves several functions. It is the basis for risk assessments performed by OBS and the IBC. PIs have the primary responsibility to provide information to personnel (e.g., lab, animal care, custodial) who may be exposed to hazards from the PI's research. As such, the biosafety protocol can serve as a valuable tool for training staff regarding potential hazards and precautions to take to mitigate those hazards. The protocol serves as a means to demonstrate that the campus is in compliance with local, state, and federal regulations that pertain to biological safety. We sincerely appreciate receiving your suggestions on ways we can improve the biosafety protocol process.

Appropriate Containment

A good starting point for risk assessments and determination of appropriate precautions is the risk group (RG) listing of pathogens provided in the NIH *Guidelines* (Appendix B). Included in this listing are representative genera and species that are known to be pathogenic. Microorganisms that normally do not cause disease in healthy human adults are categorized as Risk Group 1 and are not explicitly listed. The next step up in hazardous nature is Risk Group 2; these organisms have the ability to cause disease in healthy human adults but the infections typically are not severe and are treatable.

Agents that are not listed in Appendix B, however, are not automatically or implicitly classified in RG1. The listing is not meant to be all-inclusive and it does not include normal flora of humans that are pathogenic when they gain access to other than normal sites, such as might happen during a laboratory accident. Work with microorganisms in the laboratory setting may create situations whereby the normal route of transmission is circumvented. In the lab, the concentration and volume are typically higher than encountered outside of the lab and procedures provide “opportunities” to infect in an abnormal manner such as splash to mucosa or needle stick injury. Decisions regarding precautions for handling such organisms should be based on a risk assessment that considers the laboratory activities.

Microbes do not naturally segregate into discrete risk group categories but cover the spectrum of pathogenicity. Certain opportunistic pathogens, although ubiquitous in the environment or normally commensal, may cause significant disease in individuals whose immune system is stressed. The immunocompromised condition is not uncommon in our population and the principal investigator cannot reliably expect an individual to inform them of a change in health status. This change may occur for many reasons, including routine illness, pregnancy, and chemotherapy to treat underlying illness. It is important to protect these individuals.

Some microbes seem to fall between the RG1 and RG2 categories. Risk assessments are needed that evaluate the pathogenicity of specific strains and the lab procedures involving such borderline pathogens. Clinical isolates are of greater concern than those collected from environmental samples.

Based on risk assessments, the Institutional Biosafety Committee has determined that biosafety level 2 (BL2) precautions are appropriate for handling the following microorganisms:

Adeno-associated virus (AAV) – This virus is generally considered RG1 because it usually requires another virus for replication. However, this virus can become latent by integrating into the genome and be expressed during infections with helper viruses. There is some evidence of AAV infection of the human embryo in pregnancy and AAV has been associated with male infertility.

Aspergillus flavus and *A. fumigatus* cause opportunistic infections in the immuno-compromised individual.

Bacillus anthracis (Sterne) is a vaccine strain and is missing an important virulence factor. There are some reports of pathogenicity of this strain in animals. Furthermore, per guidance from CDC, BL2 practices will avoid contamination of the lab, which could cause problems in confounding detection of anthrax.

Bacillus cereus - This foodborne pathogen produces toxin and causes cutaneous infections in healthy adults.

Candida albicans is an opportunistic pathogen with significant consequences for individuals with stressed immune systems.

Clostridium difficile - The “new” epidemic strain (B1/NAP-1) of this bacterium has substantial clinical consequences, is usually resistant to some antibiotics such as fluoroquinolones, and can infect healthy adults.

Enterococcus faecalis and *E. faecium* - Vancomycin resistant strains pose increased risk because vancomycin is an antibiotic of last resort. Furthermore, containment will minimize contamination of surfaces in the lab, which is important because this pathogen is extremely hardy in its ability to survive for weeks on environmental surfaces.

Pneumocystis carinii causes opportunistic infections in the immunocompromised individual.

Pseudomonas aeruginosa – This pathogen causes chronic respiratory infections among cystic fibrosis patients and eye infections, especially in contact lens wearers.

Salmonella typhimurium LT2 - There is evidence that this strain may cause disease in healthy adults as well as immunocompromised individuals.

Most wet labs will meet the standards for a BL2 facility but there are several key differences in practices between biosafety level 1 and 2. Some of the practices utilized under BL2 containment are:

- Biohazard signs are posted on the door to the laboratory when work with the microorganism is in progress.
- Individuals who may be exposed are informed of the potential risks to their health posed by the pathogen.
- Exposures are treated with first aid and reported to the PI with medical evaluation as deemed prudent.
- The lab is under negative air pressure relative to the corridor and the lab door is kept closed to maintain negative pressure.
- Many procedures can be conducted safely at the open lab bench under BL2 containment, but activities involving high concentrations and/or large volumes or that generate aerosols need to be avoided or done in containment.
- Use of a biological safety cabinet is preferred but is not required; alternative containment equipment may be acceptable.
- Disinfectants are chosen that are effective against the pathogens handled.
- Submission of a biosafety protocol is required.

Additional details concerning what working at BL2 entails can be found in these references:

Biohazard Recognition and Control, UW-Madison biosafety manual

Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH 4th ed.

NIH Guidelines for Activities Involving Recombinant DNA Molecules, Appendix G

This policy regarding opportunistic and borderline pathogens was adopted by the Institutional Biosafety Committee at the December 2005 meeting. It will be posted at the OBS website. This policy will be reviewed routinely and pathogens may be added as relevant information is brought to the IBC's attention.

Eye Protection

Your eyes are extremely vulnerable to injury. Individuals should wear eye protective at all times when they are in a laboratory, not just when their activities involve known hazardous materials. There is no excuse for not wearing eye protection in a laboratory.

Protecting your eyes is easy. Inexpensive safety glasses are available from Material Distribution Services and other laboratory equipment suppliers. Contact lenses may be used with discretion and in combination with additional eye protection. For higher hazard activities, side shields on glasses, goggles, or face masks may be needed.

Prescription safety glasses in a variety of stylish frames may be purchased at minimal cost from the Safety Department (262-8769) under the state contract. Many departments on campus will pay the cost of prescription safety glasses that are required for work. Ask your supervisor for details.

Happy New Year

The Office of Biological Safety wishes you a very happy and prosperous New Year!

Shipping Infectious Substance and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping Infectious Substance and other biological materials, with a focus on safety and regulatory compliance for research laboratories. The Department of Transportation requires that persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials.

Monday, January 9, 2006
Union South 1:30 – 4:00 p.m.
Refreshments will be served.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

All staff are welcome to attend this class for initial training or re-certification. Staff approaching their two-year expiration for certification will receive a notice in advance of that date. Computer-based training is available only for those who attended the class for their initial certification.

Basic Biosafety Class Offered

This class will give an overview of basic biological safety. Topics include: biosafety levels and biohazard containment, good microbiological techniques, waste disposal, risk assessment, and emergency preparedness. It is intended primarily for students and staff who are new to this institution and/or new to working with biological materials in a laboratory. Everyone is welcome to attend.

Tuesday, February 7, 2006
Union South 1:30 – 3:30 p.m.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

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