

BioSide Lines

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

Ice Machines: Laboratory Uses versus Personal Consumption

Ice from laboratory ice machines should be used only for cooling lab reagents and chemical reactions. The ice from these machines is not fit for human consumption or cooling food and beverages. People who use the ice out of these machines for personal consumption put themselves at risk of becoming severely ill. The routine cleaning and maintenance that is mandatory in the food industry does not typically occur in the laboratory units. Laboratory ice machines can easily become contaminated with pathogens, chemicals, and even radionuclides.

Using ice that is produced by laboratory equipment for personal consumption is obviously not a safe practice. The likelihood of getting sick because of this bad practice may be perceived as low, but the consequences are highly unpleasant and easily avoided. The following relevant facts may help convince you. Several food-borne pathogens, particularly *E. coli*, *Salmonella*, and *Shigella* survived in ice within drinks. *Listeria* is known to multiply at cold temperatures. During an outbreak in 1987 over 5000 people from Pennsylvania and Delaware became ill after consuming norovirus-contaminated ice in soft drinks. In another case, contaminated laboratory ice was documented to be the source of bacterial contamination in cell lines.

To reduce the incidence of contamination of laboratory ice:

1. Use a scoop when obtaining ice, not cups, glassware, or hands.
2. Store the scoop on a holder outside of the ice machine, and clean the holder weekly.
3. Wash the ice scoop daily.
4. Make sure the scoop handle does not contact the ice.
5. Clean and sanitize the ice machine periodically.
6. Keep the lid to the ice machine closed when not in use.

Post signage that the ice is not intended for human consumption and enforce that rule. "Not for Human Consumption" signs are available upon request from the Safety Department (262-8769). The laboratory ice machine is research-related equipment, and like other laboratory equipment, should never be utilized for personal use. Ice for personal consumption should be kept in the same cooler where food is stored, outside of the laboratory areas.

Bunsen Burners and Alcohol Flaming – Not in a BSC

A traditional tool in microbiology is the Bunsen burner. The flame of a burner has been used to sterilize loops and flame the opening of culture tubes for over 150 years. Dipping slides and cover-slips in alcohol and flaming is an old routine sterilization practice.

All too often, researchers think it makes sense to marry the use of a Bunsen burner and flaming to the modern containment device known as a Biological Safety Cabinet (BSC). What might seem to be a good idea is actually just the opposite. The flame and heat naturally produce an upward air current, which prevents contaminants from entering a tube or flask, but in a BSC the flame disrupts the laminar air flow that serves to protect the user and the product. The resultant air turbulence interferes with the air flow that maintains sterility in the BSC, and thus the use of a burner actually increases the likelihood of contamination.

Excessive heat from a burner flame may build up in the BSC because air is recirculated within the cabinet. Heat may damage a HEPA filter and melt the adhesive that holds it in place. The HEPA filter is the key to maintaining sterility of the cabinet work surface and the exhausted air.

The potential for a fire or an explosion increases with natural gas and/or alcohol use in the BSC. The electrical components in a biosafety cabinet are not spark-proof or designed to operate in a flammable atmosphere. If a leak occurred and gas accumulated to flash point, a fire or explosion could happen. Spilled alcohol and flames contacting a container of alcohol have resulted in fires that significantly damaged BSCs. Prevention of injuries is an even more important consideration.



Don't let this happen to your biosafety cabinet!

The following procedures will lessen the chance of a BSC fire while maintaining sterility within the cabinet:

- Use an electric incinerator such as a Bactincinerator® rather than a flame to sterilize materials.
- Replace flame-requiring tools such as loops and spreaders with pre-sterilized disposables. Sterilize supplies like slides, cover-slips, loops and spreaders by autoclaving prior to use in a BSC.
- Keep flammable chemicals out of the BSC, if possible. If use of flammable chemicals is unavoidable, minimize the amount to that which is needed for immediate use.
- If a flame is considered essential, use an on-demand burner like the Touch-O-Matic®. This burner shuts off the flame when your hand is not on the touch plate. It has a very small pilot light that produces little heat and shuts off the gas supply when that pilot light is out.
- Use only butyl rubber tubing to connect the burner with the gas supply. Other types of tubing will degrade with gas exposure, allowing leaks in the tubing to occur.

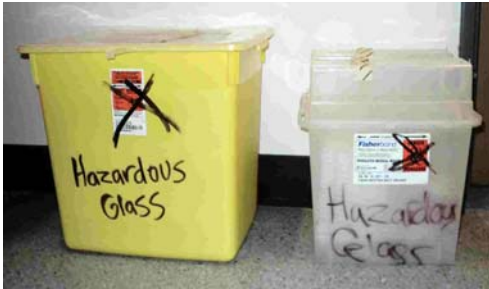
Photo reproduced with permission from Chair of AIHA Laboratory Health & Safety Committee.

Biohazardous Waste- Segregate and Save

Even before biohazardous waste is generated, a plan is needed to safely get rid of it. Key elements are to segregate and package the waste appropriately and to communicate about the nature of it. Alternative options may be considered in selecting the route of disposal for most infectious waste, the exception being medical sharps. All medical sharps must be disposed via the University's medical waste contractor, MERI. MERI stands for Madison Energy Recovery, Inc., a nonprofit organization that handles medical waste from many area medical care facilities. The University is charged \$50 every time medical waste is picked up from a building plus 30¢ per pound. The charges are substantial, especially when compared to the cost of routine trash pickup (less than 2¢ per pound). For biohazardous waste that excludes sharps, disinfection allows the waste to be discarded as routine trash. Your cooperation in minimizing the waste that is routed to MERI will assist the institution during this period of financial constraints.

Several disinfection options are available. Autoclaving is an effective procedure for killing microorganisms. After autoclaving, the biohazard symbol on the bag or container must be defaced, and the waste then can be handled as routine trash. Alternatively, many chemical disinfectants are effective against bacteria, viruses, and fungi. At the point of generation, such as within the work zone of a biological safety cabinet, the contaminated materials can be deposited directly into a container of disinfectant. After soaking, the disinfectant liquid can be discarded via the lab sink and the objects put in a trash container. The disinfectant chosen should be one with proven efficacy against the pathogens that may be present. When in doubt, select a disinfectant that has a broad spectrum of efficacy, such as bleach, Virkon S, or Wescodyne.

The following discussion applies to waste, exclusive of medical sharps. Certain lab wastes have the ability to penetrate skin, yet are not intentionally designed to do so. Examples are plastic pipette tips, Pasteur pipettes, and other fragile glass. They must be packaged in a manner that prevents injury. If these items are contaminated with infectious microbes, recombinant organisms, or potentially infectious materials, they must first be decontaminated before they are discarded as routine trash. Non-contaminated items may be placed in a plastic-lined cardboard box that is taped shut when it is full. However, if these objects will be autoclaved prior to disposal, a heat-resistant plastic container may be used.



**Plastic box used for glass disposal.
Note defaced biohazard symbol.**

Even though this container might look like a medical sharps box, it can be handled as routine lab waste after disinfection, if and only if it does not contain true medical sharps. These containers, whether a plastic-lined cardboard box or plastic container, will be picked up by custodians if the biohazard symbol was defaced after disinfection, the container is marked appropriately, e.g., “Glass for Disposal”, and it is placed in the hallway next to your lab door.

Be sure to segregate the different types of wastes. Do not mix infectious and non-infectious wastes. Also do not mix true medical sharps with any other materials. True medical sharps must be placed in a rigid, puncture-resistant plastic or metal container that is labeled with a biohazard symbol or the words “biohazard”, “sharps” or “infectious waste”. MERI is willing to take just about any waste that gets put into their medical waste collection barrels. Their shredding equipment, which renders medical sharps non-usable, will not handle big chunks of metal, such as an artificial hip joint. Sharps containers that are filled with needles also may cause problems for this equipment, so these containers should be additionally marked as “Metal, Do Not Shred” to allow them to be segregated.

In conclusion, due to the significant costs incurred, the MERI waste stream should be reserved for waste such as medical sharps that cannot be rendered harmless by research personnel. Feel free to consult staff of the Office of Biological Safety about options for managing biohazardous waste.

Training FAQs: Don't Learn Safety Lessons the Hard Way

Q: Why should I take the time to learn about biological safety?

A: The main reason to learn about safety is to prevent injuries. Experience shows that safety training prevents many accidents and illnesses. A major benefit of biological safety training is prevention of lab-acquired infections.

Risk assessments are not just tools for safety professionals. Evaluating risks associated with the job should be part of each person’s routine. Make sure to find out what safety precautions to take before you even start a task. The need to plan safety into your daily work habits can’t be emphasized enough. Don’t learn safety lessons the hard way: through exposures to hazards. Instead, learn and take the necessary precautions upfront.

Q: Who is responsible for safety training on the UW-Madison campus?

A: Supervisors are responsible for ensuring that their personnel receive training necessary to do their jobs safely. It is the responsibility of each principal investigator (PI) to make sure that their research is done in a safe manner.

Q: Who needs to get training in biological safety?

A: Function-specific training must be provided to personnel who may be exposed to biological hazards. PIs sometimes focus solely on biological safety training for laboratory staff, but training must also be in place for other staff (e.g., animal care, custodial, or maintenance staff) who may be exposed to biological hazards.

Q: So each PI has to directly train non-laboratory staff such as animal care staff?

A: The PI is responsible for making sure that anyone who might be exposed to hazards from their research receives function-specific safety training, but this training can be delegated to some extent. For example, animal care staff should receive basic safety training from their supervisors. Specific information about the hazards and precautions associated with each PI's project(s) should be communicated to animal care staff by the PI or their delegate. Appropriate signage is one way to convey information about hazards and the precautions necessary when handling hazardous materials.

Q: Doesn't the training offered by the Safety Department fulfill training requirement?

A: Although the training offered by the Safety Dept. provides a valuable foundation of basic safety information, it can't meet job-specific training needs. Job-specific safety training must be provided that describes hazards present and details precautions to be taken to mitigate those hazards at the point of use.

Q: When should a person be given training in biological safety?

A: It is imperative that each person is trained in biological safety issues relevant to their job prior to handling any potentially-infectious material. Risk assessments and job-specific safety training based on those risk assessments need to be done before initiation of each project. The Basic Biosafety class offered by OBS is targeted especially to staff new to biological research and can serve as a starting point. The Advanced Biosafety class goes into more detail on biosafety level 2 precautions. These classes, however, cannot replace job-specific safety training.

Q: Why should training always be documented?

A: One reason is that governmental regulations state not only that staff receive job-specific safety training but also that the training is documented. Another reason is logistical: it is difficult to keep track of who has had what training if it is not documented. It is simply good practice to document training, and it also may protect you from liability.

Q: What should be documented?

A: Document informal as well as formal training: classes, on-line training, orientations, safety info covered in lab meetings, one-on-one training on new techniques, etc. At minimum, document the date, who attended, the topics covered, and the trainer's name.

Q: What training is offered by the Safety Department?

A: See pages 7-8 for a summary of current training offered by OBS with dates for the next classes. This article focuses on biological safety training but much of the discussion above applies to other types of safety training. You can go to the Safety Department website (www.fpm.wisc.edu/safety) to find out more about other available trainings (e.g., Chemical Safety, Occupational Health, Radiation Safety, etc).

Human Cell Lines in Lab and Animal Research

You're working with established human cell lines (e.g., HeLa cells) that have been used for years in your lab and have been tested for major human pathogens in the past. You got the cells originally from American Tissue Culture Collection (ATCC), and the ATCC website actually designates biosafety level 1 (BSL-1) as appropriate for some of these cell lines (e.g., MOLT-3, KG-1). So why does the Office of Biological Safety tell you these cells need to be handled as potentially infectious material (PIM) with BSL-2 precautions and containment?

One major reason to treat established human (and Old World monkey) cells as PIM is that you cannot test for every known and unknown pathogen. Just because the cells are negative for some common pathogens does not mean they are free of all pathogens. Think of handling human cells prior to the discovery of human immunodeficiency virus (HIV), for example.

HeLa cells have been used so long that they are often viewed as innocuous, but HeLa cells carry the full genome of human papilloma virus-18 (HPV-18), a known human tumor virus associated with cervical cancer. Many cell lines have been treated with Epstein-Barr virus (EBV) to immortalize the cells.

HeLa cells have been reported to shed very low levels of HPV, and EBV-transformed cell lines could shed very low levels of EBV.

Another major reason to treat these cells as PIM is because cell lines commonly get contaminated over time, and these contaminating pathogens can be transmitted to staff handling the cells or materials exposed to the cells. A case of a lab-acquired infection due to a contaminated human cell line is described in a January 2005 BioSide Lines article.

Adventitious agents are commonly discovered in established human cell lines. HHV-8 (the herpesvirus associated with Kaposi's sarcoma) has been discovered in cell lines thought previously to be free of viruses. About half of the HeLa cultures tested have been shown to contain the Mason-Pfizer monkey virus (MPMV), which is associated with tumors in primates (effect of MPMV on humans is unclear).

BSL-2 precautions and containment are not onerous. Containment (e.g., a biosafety cabinet) is usually already being used for work with human cell lines to prevent contamination. Personal protective equipment used for BSL-2 is similar to that used for BSL-1: lab coat and protective eyewear. In addition, gloves are used routinely with BSL-2 materials while glove use depends on the circumstances in BSL-1 labs (e.g., used for handling hazardous materials, to prevent allergies, or to protect the material being used). Again, the use of gloves and lab coat also prevents contamination and is most likely already being done in your cell culture facility.

Special risks are presented when human (or Old World monkey) cells are administered to immunocompromised animals, since these animals act as incubators for any pathogens that may be present in the xenograft. Immunocompromised animals are more likely to propagate a pathogen to high titers and more likely to transmit a pathogen to people and to other animals. Therefore, animal BSL-2 precautions and containment are warranted for projects involving administration of PIM to immunocompromised animals. A summary of ABSL-2 precautions and containment are outlined in an October 2005 BioSide Lines article.

See our website or contact OBS for further guidance on appropriate biological safety precautions.

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.

Thursday, April 13, 2006
Union South 1:00 – 3:30 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. Computer-based training is available only for those who attended the class for their initial certification.

HazMat Packaging Workshop NEW

An optional hands-on HazMat packaging workshop will be offered after the regular HazMat Shipping class. Trainees will decide how example materials should be shipped (e.g., Infectious substance; Biological substance, category B; Exempt patient specimens) and then will package the surrogate materials in appropriate containers. Feedback and tips on packaging will be provided. People with current HazMat certification may register for the workshop only.

Thursday, April 13, 2006
Union South 3:30-4:30 p.m.

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

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, April 18, 2006
Union South 1:00 – 3:30 p.m.

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

Advanced Biosafety Training NEW

This class builds on the Basic Biosafety class and focuses on biosafety level 2 (BSL-2) precautions and containment in lab and animal research. Topics include risk assessment, proper use of containment equipment and personal protective equipment, and disinfection procedures for various types of Risk Group 2 pathogens (viruses, bacteria, fungi, parasites, prions). A major goal of the class is to provide safety information on commonly-used viral vectors since viral vectors are becoming a widespread research tool. The Basic Biosafety class should be taken prior to the Advanced Biosafety class either by attending the Basic Biosafety class on April 18th or by reviewing the training available at the OBS website.

Wednesday, May 17, 2006
Union South 1:30-3:30 p.m.

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

OBS Online Training

Online training offered by OBS includes re-certification for Shipping Infectious Substance and Other Biological Materials, Basic Biosafety, and Biosafety Cabinet Training.

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