

# BioSide Lines

October 2005

The Newsletter of the Office of Biological Safety, UW-Madison Safety Department  
[www.fpm.wisc.edu/biosafety](http://www.fpm.wisc.edu/biosafety)

## **Guidelines for Use of Halothane and Isoflurane**

Personnel who use anesthetic gases for animal procedures may be at risk unless proper procedures are followed. The Occupational Safety and Health Administration (OSHA) recommends that no worker be exposed to concentrations of waste anesthetic gases greater than 2 parts per million of any halogenated anesthetic agent. The following discussion is adapted from guidance being developed by the Occupational Health Program in conjunction with Research Animal Resources Center (RARC).

Halothane and isoflurane are volatile anesthetics that pose a risk to personnel who are chronically exposed to them. They are considered hazardous because they are experimental teratogens and are toxic by inhalation. They are particularly hazardous to pregnant women as these anesthetics can cause a breakdown of nerve cells in developing brains. In research, the amount of these gases used to anesthetize laboratory animals is comparatively small, but prolonged or repeated exposure may cause human liver damage. Halothane use is being phased out and is often replaced by isoflurane.

Training in the proper use of these gases is available from the RARC Training Department. Personnel who use anesthetic gases should be aware of the acute exposure symptoms associated with their handling and use. This information is available in the material safety data sheet (MSDS) provided by the supplier of the specific product. Read the MSDS and incorporate the precautions into your Chemical Hygiene Plan and standard operating procedures.

To minimize occupational exposure, adequate ventilation must be used during procedures involving these gases. If one is available, a certified chemical fume hood should be used. When inhalant anesthetics are administered on the open lab bench, scavenging equipment should be used, and even then, it is a good practice to direct the exhaust air into a fume hood since it may leak waste gas. If using a canister system, document how you monitor the continued effectiveness of the activated charcoal filter and when this filter is exchanged. The used charcoal filters can be discarded as normal trash.

Use of these anesthetic gases does not require submission of a biosafety protocol if this is the only activity that fits the criteria for submission. Please include a description of use and precautions for anesthetic gases in your biosafety protocol when submitting it for other reasons. You also are encouraged to describe what anesthetic gas you will be using and how you plan to minimize occupational exposure in the occupational health section of the animal care protocol. This will facilitate the timely processing of your protocol. Your animal care and biosafety protocols should be consistent in the description of these materials and precautions and other details.

## **Microtome and Cryostat Safety**

Histologists have a unique profession in the world of science. They are both skilled laboratorians and artists at the same time. A section of tissue is given to them and the result of their work, the processed specimen, delivers valuable information to the clinician and research scientist. If you consider the artistic aspects, the finished product shows the beauty as well as the ominous side of nature.

Histology has a dangerous side in the form of microtomes and cryostats. A microtome is an instrument for cutting sections of tissues for microscopic examination. A cryostat is a microtome that is housed in a refrigeration/freezer unit. The knives used to slice the tissue in these devices are among the sharpest blades utilized by any profession. A cut with the subsequent chance of infection is a constant threat.

Here are tips for microtome/cryostat safety:

- Wear proper personal protective equipment (PPE) such as lab coats, gloves and protective eyewear.
- Request full disclosure about the specimen. Specimens may harbor pathogens and should be considered infectious if derived from:
  - humans
  - primates, especially Old World monkeys
  - animals infected with a pathogen or containing a graft of human or primate origin.
- Offer immunization against Hepatitis B for all staff who handle human tissues.
- The risks of unfixed human tissue or animal tissue infected with a pathogen remain in frozen samples since freezing does not necessarily inactivate infectious agents.
- Consider solutions used for staining unfixed sections to be contaminated.
- Avoid using pressurized cryo-propellants for maintaining frozen sections as they may cause spattering of droplets of infectious material.
- Prions are not deactivated by standard histology preparation steps.
- The trimmings and other waste from biohazardous samples are considered contaminated and should be rendered non-infectious prior to disposal.
- Routinely decontaminate the microtome/cryostat with an appropriate disinfectant.
- Do not move a microtome or cryostat prior to decontamination. Do not transport it with knives in position.
- Always handle the knives very carefully and follow manufacturer's directions explicitly.
- Do not leave knives on countertops or unattended. Store knives in a covered container with guides to hold the blades firm and rigid.

When setting-up your microtome/cryostat:

1. Align and clamp the sample block tightly prior to mounting the knife, never the other way around.
2. For added protection, stainless steel mesh gloves should be worn when changing knife blades.
3. Attach a handle before removing a knife from its storage container.
4. Do not use the "finger test" to determine blade sharpness.
5. If a blade is dropped, do not attempt to catch it, and be careful where feet are positioned because the blades can cut through shoe leather.
6. Be very careful when tightening screws used to hold disposable blades and be certain that the blade is firmly in place.
7. Make sure that the brake is tight if it is applied. A common accident occurs when the brake slips and the operator's hand makes contact with the blade.
8. Always use the knife guards on the microtome and be certain they are secure when stepping away.
9. Keep tissue blocks moist while in the microtome to reduce airborne shavings while slicing.
10. Use forceps when retrieving slices from the boat so hands have maximum distance from moving parts.
11. Use manufacturer's procedures to clean a mounted blade. A high density polystyrene rod will help keep your hands free from contact.
12. Use forceps and surgical clamps when changing or removing blades.
13. When dislodging stuck blocks use forceps or high density polystyrene rods.

Following these simple rules will lessen the chance of injury when using a microtome or cryostat.

References:

- Hoslink, ABC of Safety Practice in the Biomedical Sciences, Microtome Knives, Roy Ellis and Don Perry, 2001.
- Univ. Nebraska-Lincoln Environmental Health and Safety, Safe Operating Procedure-Microtome Safety, Revised 5/05.
- Yale University Biological Safety Manual, Microtome/Cryostat, July 2001.
- Univ. Illinois - Chicago, Biological Safety Program Manual, 5.19.4 Microtomes and Cryostats, November 2004

## Basics of Animal Biosafety Level 2

Animal facilities are specialized laboratories. Research projects involving animals have some safety issues in common with laboratory projects but many aspects are unique due to the basic nature of working with animals. Pathogens cannot be contained as neatly in an infected animal as in a test tube. They may be exhaled, secreted, and excreted, and the animals may bite or scratch, creating the potential for occupational exposures.

Categorizing appropriate precautions according to biosafety levels is a simple way to communicate the risks. Biosafety levels, however, are not uniform directives. Precautions and containment need to be tailored to each specific situation and the hazardous materials that are utilized. Precautions will vary somewhat depending on the type and characteristics of the pathogen(s), experimental procedures including route of administration, nature of the host animal, available containment equipment, etc.

Animal biosafety level 1 (ABSL-1) is suitable for work involving well-characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment. ABSL-2 involves practices for work with agents associated with human disease and those that may cause harm to other animals. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

Projects for which ABSL-2 precautions and containment are appropriate include: immunocompromised animals that have been administered human cells, animals that have been administered a human pathogen (including replication-deficient viral vectors), and animals that potentially harbor a zoonotic pathogen. Examples of animals that are potentially infectious: Old World monkeys (Herpes B virus), wild rodents (Hantavirus), pregnant sheep, goats and cattle (Q fever), reptiles (*Salmonella*). Some of the ABSL-2 precautions also are appropriate for work that involves administration of hazardous chemicals to animals, such as enhanced personal protective equipment (PPE) and use of containment for aerosol-generating procedures.



What does it mean to use ABSL-2 precautions and containment? And how does that differ from ABSL-1?

- Biohazard signs are posted on procedure rooms when BSL-2 materials are handled (e.g., inoculations) and on doors of animal housing rooms. Additional signage is placed on cages to indicate potential hazards and precautions to be taken.

Training specific to the potential biological hazards is done.

- Access to the animal room is limited to the fewest number of individuals possible. There may be recommended immunizations or limitations on entry for persons at high risk for the pathogen involved.
- Gloves should be used routinely in addition to the standard PPE worn for ABSL-1, which includes lab coats/smocks and protective eyewear. A splash shield or respiratory protection may be appropriate in some circumstances.
- Aerosol-generating activities such as inoculations (especially intranasal), cage changes, and necropsy should be done using containment equipment (e.g., biosafety cabinets, fume hoods, downdraft tables, cage change hood) whenever feasible. Respiratory protection may be a reasonable option when containment is not feasible, such as when procedures involve large animals.
- Bedding and cages from infected animals are decontaminated, typically by autoclaving. After disinfection, bedding may be discarded as normal trash. In some situations, disposal via the sanitary sewer is acceptable.
- Animal tissues and carcasses are bagged, boxed and frozen, to be picked up by the Safety Department for disposal, as is the case for ABSL-1. For ABSL-2, the identity of the pathogen is marked on the pink animal tissue disposal form and the boxes are labeled with the biohazard symbol. Boxes must weigh less than 45 lbs.
- Surfaces are treated with an effective disinfectant.
- Biological spill protocols and exposure response plans are developed for the specific hazards present.
- Use of sharps is eliminated whenever possible or needle safety devices are used.

Additional guidance on the precautions and containment used for animal biosafety can be obtained from Biosafety in Microbiological and Biomedical Laboratories (for non-recombinant work) and Appendices G (lab animals) and Q (large animals) of the NIH Guidelines (for recombinant work). Health Canada provides material safety data sheets (MSDS) for a number of pathogens. These documents and other guidance documents can be accessed from our webpage ([www.fpm.wisc.edu/biosafety](http://www.fpm.wisc.edu/biosafety)).



It is the responsibility of the principal investigator to communicate about the risks of the project to anyone who may be affected. This communication must extend to animal care workers as well as laboratory staff. Since precautions will vary depending on the specific hazards present, it cannot be assumed that standard animal handler training will cover the precautions necessary for your project.

Feel free to contact the Office of Biological Safety with any questions and comments regarding ABSL-2 or other biosafety topics.

## Office of Biological Safety



Jan Klein



Darren Berger



Nancy Schensky



Margy Lambert



Terry Lawrin

### Contacts

Jan Klein	General Contact	263-2037	<a href="mailto:biosafety@fpm.wisc.edu">biosafety@fpm.wisc.edu</a>
Margy Lambert	Biological Safety Officer	263-9026	<a href="mailto:jklein@fpm.wisc.edu">jklein@fpm.wisc.edu</a>
Darren Berger	Associate Biosafety Officer	263-9013	<a href="mailto:mlambert@fpm.wisc.edu">mlambert@fpm.wisc.edu</a>
Terry Lawrin	Facilities Engineer	263-2187	<a href="mailto:dberger@fpm.wisc.edu">dberger@fpm.wisc.edu</a>
Nancy Schensky	Biosafety Specialist	262-6670	<a href="mailto:tlawrin@fpm.wisc.edu">tlawrin@fpm.wisc.edu</a>
	Administrative Support	263-2037	<a href="mailto:nschensky@fpm.wisc.edu">nschensky@fpm.wisc.edu</a>

## ***Shipping Infectious Substance and Other Biological Materials***

The Department of Transportation requires that persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials. The regulations for shipping by air state that certification of training is valid for 2 years but also that training must be updated when there are significant changes, which has been the situation recently. The Office of Biological Safety keeps records of training and sends notices to alert trainees when training needs to be done to remain in compliance.

It is critical that all persons involved in shipping hazardous materials have a valid certificate, especially if they have responsibility for making the determination as to the proper classification, selecting appropriate packaging, and signing the dangerous goods declaration. Violations with regard to training and certification can result in substantial fines.

In an effort to facilitate compliance in the face of recent changes, we developed a training “patch” for individuals who trained and certified recently (after December 15, 2004). These individuals do not need to take a class or do the online training session. Instead, they can simply review a file that summarizes the changes and return a prepared statement to OBS. Instructions were emailed to affected individuals in August. Contact us if you would like to receive this information.

For those who trained prior to December 2004, training can be done by attending a class or by taking online training. The web training modules may be accessed from the OBS homepage ([www.fpm.wisc.edu/biosafety](http://www.fpm.wisc.edu/biosafety)), click on links to HazMat Shipping Training. Check the dates given to determine whether you need to take the “Update” or “Recertification” training. Computer-based training is available only for those who attended the class for their initial certification.

The Office of Biological Safety offers a class quarterly on shipping Infectious Substance and other biological materials, with a focus on safety and regulatory compliance for research laboratories. Upon request, additional sessions will be provided for a group of individuals at a given location. The next class is scheduled to be held:

**Thursday, October 13, 2005**  
**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](mailto: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.

### ***Basic Biosafety Class Offered***

**Wednesday November 16, 2005**  
**Union South, 1:30 – 3:30 p.m.**

- Risk assessment for biological research (lab and animal areas)
- Making safety a part of your daily routine
- Prevention of lab-acquired infections
- Understanding risk groups and biosafety levels
- Using containment and personal protective equipment
- Proper disposal of potentially hazardous biological wastes
- Services provided by the Office of Biological Safety

Students and staff working in biological research, especially those new to research or new to the UW, should attend this introduction to biological safety and institutional procedures. Call 263-2037 to register or to obtain more info.

An online version of the training is available for those who cannot attend a class ([www.fpm.wisc.edu/biosafety](http://www.fpm.wisc.edu/biosafety)).