

# BioSide Lines

April 2007

The Newsletter of the Office of Biological Safety, UW-Madison Safety Department

[www.fpm.wisc.edu/biosafety](http://www.fpm.wisc.edu/biosafety)

## Improper Pipette Disposal Causes Injuries

Take a close look at this photo. Do you see the problem? Pipettes can be seen poking through the plastic trash bag, evidence that they were not properly discarded and posed a hazard to the individual who carried the trash bag from the lab to the waste collection site. Several injuries are reported every year because pipettes and pipette tips poked through plastic bags and cut an individual. A cut by a pipette or tip whose origin is not known must be treated as if there could be transmission of a bloodborne pathogen. The injured individual should seek medical attention and will deal with the difficult uncertainty of not knowing if they were exposed to a hazardous material.



Such incidents are easily prevented by following these steps.

- First, decontaminate the pipettes and pipette tips. Autoclaving is an acceptable way to accomplish this, but oftentimes it is easier to directly deposit the pipette or tip into a solution of chemical disinfectant such as bleach as you finish work with it.
- When working in a biological safety cabinet, the container for collecting used contaminated pipettes and tips should be placed within the cabinet, never on the floor outside the cabinet.
- After allowing these materials to soak (an hour in an effective disinfectant should be sufficient), the liquid can be discarded via the sanitary sewer.
- After decontamination, the pipettes and tips must be packaged in a way that prevents injury to individuals taking this waste to the dumpster. We recommend putting them in a plastic lined cardboard box. Boxes may be purchased from laboratory supply companies. It is acceptable to use other reasonably sized cardboard boxes. Label the box appropriately, e.g., "Fragile Glass and Plasticware." When full, seal the box with tape and arrange for it to be removed as regular trash.

If you have the misfortune of being injured while on the job by a pipette or other sharp, be sure to seek a medical evaluation as the consequences could be serious. Always file a Report of Injury or Disease with your employer. Please consult with Tom Kenney, Occupational Health Officer, if you have questions or concerns regarding this or other workplace injuries.

## Safety Advance: Transposon Gene Delivery Systems

What do the terms "*piggyBac*", "*Sleeping Beauty*", and "*Frog Prince*" have to do with biological safety? These transposons as well as others such as *To12*, *Mos1*, and *Himar1* are being developed as nonviral DNA/gene delivery systems. The relevance to safety is that nonviral delivery methods have the primary advantage over viral vectors of being noninfectious.

Transposons or transposable elements are DNA elements that can move or "transpose" from one location in a DNA molecule to another location, either on the same or a different DNA molecule. The phrase "jumping genes" coined from Nobel Prize winning scientist Barbara McClintock's research with maize may be a more recognizable expression. Transposases are the enzymes that catalyze the movement. The development of gene delivery systems, commonly referred to as vectors, is often driven by the goal of creating safer and more effective human gene therapy options.

Viral vectors have the major advantage over nonviral methods of the ability to achieve stable expression, especially with retroviral or lentiviral vectors where integration allows long-term expression of the gene of interest. Disadvantages of viral vectors include the potential for pathogenicity/infectivity, induction of immune inflammatory responses, and insertional mutagenesis. The risks of replication-deficient viruses gaining the genes necessary to become replication-competent or retroviruses/lentiviruses integrating near a crucial gene such as an oncogene and activating its expression are slight but necessitate the use of biosafety level 2 (BSL-2) precautions and containment.

The most common nonviral delivery route is via plasmids. Plasmids are extrachromosomal DNA rings that can replicate autonomously and provide a widely-used versatile tool for manipulating and moving genes from cell to cell. Plasmid vectors have the advantages of simplicity, safety (e.g., noninfectious components), and lack of immune activation. BSL-1 precautions and containment are appropriate for use of plasmid vectors. Advances have been made in nonviral delivery methods, but until recently, the major drawback of these systems has been the inability to achieve persistent expression due to loss and degradation of plasmids in living tissues.

A recent advance has been use of plasmids that include transposons and transposases. Transposon systems overcome the main drawback of simple plasmid delivery systems since the transposases integrate the genes of interest into the host chromosomes resulting in stable expression (Balciunas et al., 2006; Miskey et al., 2003; Wu et al., 2006). Transposon vectors offer a major step forward in safety because of the elimination of the infectious component of viral vectors. Insertional mutagenesis still remains a potential drawback of transposon delivery systems.

Transposases such as *Sleeping Beauty* have the advantage of near-random integration sites compared to retroviral/lentiviral vectors that tend to integrate into actively-transcribed regions of the genome. Ease of manipulation of transposon vectors allows modifications such as coupling of transposases to DNA-binding domains to achieve targeted integration (Kaminski et al., 2002). Use of such chimeric transposases could further improve safety of transposon vectors by targeting integration to “safe” regions of the genome that are not associated with oncogenesis.

*Sleeping Beauty* (a synthetic transposon derived from fish) was constructed and demonstrated to be capable of transposing DNA from a plasmid to a human chromosome 10 years ago (Ivics et al., 1997). Since then, a number of transposon systems have been developed and used successfully to obtain stable gene expression in mammalian cells. Transposon vectors have been used effectively in primary and established mammalian cells from various lineages (Huang et al., 2006) as well as in animal models of gene therapy (Liu et al., 2006). Recently, a new strategy has been devised that uses the *piggyBac* transposon (derived from a moth) for production of transgenic animals (Shinohara et al., 2007).

There are still many unknowns in the development of transposon vectors including remaining questions on technical as well as safety issues. The use of such vectors, however, offers a great opportunity to maximize the advantages and minimize the drawbacks of existing delivery systems. The potential safety benefits alone merit consideration of this method. Please contact Margy Lambert of OBS with feedback on your experiences with transposon delivery systems.

#### References:

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- Miskey, C., Z. Izsvak, R. Plasterk, and Z. Ivics. 2003. The *Frog Prince*: a reconstructed transposon from *Rana Pipiens* with high transpositional activity in vertebrate cells. *Nucl Acids Res.* 31(24):6873-6881.
- Shinohara, E., J. Kaminski, D. Segal, P. Pelczar, R. Kolhe, T. Ryan, C. Coates, M. Fraser, A. Handler, R. Yanagimachi, and S. Moisyadi. 2007. Active integration: new strategies for transgenesis. *Transgenic Res.* [Epub ahead of print].
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## Centrifuge Safety

Centrifuges are essential equipment for many labs. The extreme forces that the centrifuge has to endure require due respect. During rotation an ultracentrifuge rotor may experience 1,000,000 times the force of gravity (G's). A tiny flaw, corrosion, or a scratch in a critical area may generate stresses greater than the rotor was designed to withstand. The metal fatigue of the rotor can shatter it, destroying the centrifuge, and the surrounding lab. The vast majority of centrifuge failures are the result of user error. Proper maintenance, rotor de-rating, and operation are crucial in preventing malfunctions.

Centrifuges come in three general classes; low speed (i.e., up to about 5000 rpm), high speed machines (i.e., up to about 25,000 rpm), and ultracentrifuges (i.e., up to 100,000 rpm or higher). Rotors can be made from aluminum, titanium, or carbon fiber composite. To prevent rotor failure, centrifuges should be operated only in the specified speed range.

Keep the operating manual near the centrifuge for easy reference. "De-rating", a process that determines the maximum rotation speed allowed, is based on the usage of the rotor (speed and run times). De-rating is necessary to avoid catastrophic failure for many types of rotors. Eventually, the rotor must be retired (taken out of service) and discarded. Always follow the manufacturer's recommendations regarding when a rotor should be de-rated or retired.

Three factors govern a safe life for a rotor:

- Proper design and manufacture.
- Proper care, handling during use, and maintaining a rotor use log.
- De-rating and retiring a rotor when damaged, fatigued, or at the end of manufacturer's determined life.

While operating a centrifuge may seem to be a simple process, there are numerous precautions that must be followed to avoid accidents, which warrants training of users on the following work practices and rotor maintenance:

1. Check the rotor for evidence of damage before you use it. If you find evidence of damage, do not use the rotor and report it to a manufacturer's representative for inspection.
2. Check O-ring seals on containers and rotor for deterioration (cracks, nicks, chemical degradation, pitting or corrosion). Rubber O-rings must be lubricated periodically with material recommended by the makers.
3. Before placing a rotor in the centrifuge drive, make sure the bowl is dry and that the drive spindle is clean.
4. Check containers for evidence of wear, such as cracks, etching, scratches, and chipped rims. Never overfill containers.
5. Use only the correct containers for the rotor. You should not have to modify them to make them fit.
6. If you have de-rated the rotor, ensure that you are using the correct overspeed disk.
7. Check that the rotor is seated on the drive hub correctly.
8. Ensure that the load is balanced. Tolerances for balancing are often very restricted. Remember that a 0.5 gram difference at 1,000,000 G's is equivalent to a 500 kg difference.
9. Stay at the centrifuge until it is running smoothly. Shut the machine down immediately if there is any unusual noise or vibration.

10. Do not open the lid until the rotor stops spinning.
11. Always check for spills. If you find one, keep the lid closed until the aerosols have settled.
12. Record details about the run in the rotor log.
13. Clean the centrifuge and rotor thoroughly using a non-corrosive cleanser. Clean the equipment immediately after runs containing salts or corrosives. Avoid bottle brushes with sharp metal ends and harsh detergents when cleaning aluminum rotor heads. After proper cleaning, rinse the rotor with de-ionized water and store it upside down in a warm, dry place.
14. All rotors must eventually be retired because of the high stresses to which they are exposed and as a result have a limited life span.
15. Never use a rotor that has not been approved for the centrifuge. Check the classification decal on the centrifuge and make sure it matches the classification decal on the rotor.

These recommendations have focused on maintaining the centrifuge. Always be mindful, further, of containment recommendations (see January 2004 article in BioSide Lines). Taking care of your centrifuge and rotors will help maintain a safe, uneventful day in your lab.

#### References

Laboratory Centrifuge Safety Factsheet, University of Medicine and Dentistry of New Jersey  
Centrifuge and Rotor Safety Guide, Stony Brook University  
Warranty and Care Rotor Safety Guide, Beckman Coulter

## Updated Federal Biosafety Guidance

Indispensable guidance for biocontainment is found in the CDC/NIH document, *Biosafety in Microbiological and Biomedical Laboratories*. The long-awaited 5<sup>th</sup> edition was recently released (February 2007). Everyone working with biological materials should be familiar with the pertinent recommendations. This document is the standard of care for conducting research and is used by the OBS in evaluating facilities and biosafety protocols.

The 5<sup>th</sup> edition emphasizes training of personnel with regard to the hazards to which they may be exposed. The following is excerpted from BSL-1 (low risk) practices: "The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance." The assurance that this requirement is met involves documentation of the training given to staff.

A laboratory-specific biosafety manual must be prepared by labs working with agents that pose moderate (BSL-2) or higher (BSL-3) hazards to personnel and the environment. This document identifies hazards that will or may be encountered, and specifies practices and procedures designed to minimize or eliminate exposure to these hazards. This document must be available and accessible. The approved biosafety protocol will suffice to meet this requirement.

Of general interest, the 5<sup>th</sup> edition includes enhanced information on principles and practices of biosafety and on risk assessment. The agent summary statements were expanded. Information also was added on the following topics:

- Occupational medicine and immunization
- Decontamination and sterilization
- Laboratory biosecurity
- Biosafety Level 3 Ag laboratories
- Agent summary statements for some agricultural pathogens
- Biological toxins

The 5<sup>th</sup> edition is not yet available from the government printing office in hard copy. The electronic version is available as a link from the OBS website and from CDC [[www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm)]. Once anticipated corrections are provided, OBS will develop self-audit forms based on the biosafety recommendations for your use. These self-audit forms will be posted on our website, replacing those based on the prior edition. [[www.fpm.wisc.edu/biosafety/guidance/lab\\_surveys.htm](http://www.fpm.wisc.edu/biosafety/guidance/lab_surveys.htm)]

## Avoid BSC Problems

In the course of maintaining biological safety cabinets (BSCs), personnel from the Environmental Health Program periodically observe problematic practices, such as illustrated in the adjacent photo. It shows lab gear mounted permanently on the rear wall of a BSC and excessive material and equipment left in the cabinet between work sessions.



Proper function of a BSC depends on laminar air flow to maintain containment. Mounting any equipment on the wall causes a disruption in the air current and makes thorough cleaning of the interior surfaces impossible. Never hang equipment from the interior ceiling grid since this may damage the integrity of the filter system.

Some BSCs are equipped with ultraviolet (UV) light bulbs for surface disinfection although this process has limited utility and generally is not recommended. Materials left in a BSC, such as wall-mounted equipment, create a footprint and shadow that the UV light cannot reach. Newer models have a timer switch that automatically turns off the UV lamp, avoiding premature replacement of this expensive bulb.

## Safety Products/Gadgets Corner

Disinfecting surfaces is a critical part of biosafety. Bleach and alcohol solutions are among the most commonly used disinfectants. Depending on your application, you may want to consider the following two products.

LopHene® (Decon Labs) is a phenolic-based disinfectant solution that has broad efficacy against gram positive and gram negative pathogens as well as viruses and fungi. This product is recommended for hard, non-porous surfaces. The recommended contact time is 10 minutes and no rinsing is required. As the name suggests, this product is acidic so PPE such as gloves, lab coat, and eyewear should be used.

Spor-Klenz® (Steris) is a peroxyacetic acid/hydrogen peroxide-based disinfectant solution that provides a potentially higher level of disinfection as it is also effective in destroying spores. Under specific conditions, this product can be used as a sterilant. As with LopHene®, it is recommended for use on hard non-porous surfaces. In addition to the dangers of contact exposure due to its corrosiveness, the vapors can be harmful, so this product should only be used in a well-ventilated area while wearing proper personal protective equipment.

Both products are sold as concentrated products that require dilution. As with any disinfectant, it may be necessary to test the efficacy of your methods for the specific pathogen that you are using. This information is provided as a service to UW staff and students and is not an endorsement of particular products or vendors.

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## 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.

**Tuesday, April 17, 2007**

Union South 1:00 – 3:30 p.m.

Refreshments will be served.

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

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.

**Tuesday, April 17, 2007**

Union South 3:30 - 4:30 p.m.

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## Basic Biosafety Training

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. Everyone is welcome to attend.

**Tuesday, April 24, 2007**

Union South 1:30 – 3:30 p.m.

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## Advanced Biosafety Training

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 a Basic Biosafety class or by reviewing the training available at the OBS website.

**Thursday, April 26, 2007**

212 Biochemistry Bldg. 1:00 - 3:30 p.m.

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### Registration is required for these courses.

Contact OBS at 263-2037 or [biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu)

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