# BioSide Lines

## July 2007

Newsletter of the Office of Biological Safety - Environment, Health & Safety, UW-Madison
www.fpm.wisc.edu/biosafety

## Differences between Biosafety Levels 1 and 2

The majority of the biological research labs on the UW-Madison campus use biosafety level 1 containment (BSL-1), but several hundred use higher, biosafety level 2 (BSL-2) precautions. Organisms that typically do not cause disease in a healthy human adult may be handled using BSL-1 practices, while BSL-2 containment is used for organisms that can cause disease and for which treatments (e.g., antibiotics) are available. Human and non-human primate cell and tissue cultures (established or primary) are also maintained in BSL-2 conditions.

Surprisingly, there are very few differences in the facility characteristics between these two containment levels. These two types of labs look very much alike. Common features are a sink for hand washing, a nearby eyewash station, impervious counters and furniture, and negative air pressure relative to the public corridor. Doors to the lab should be kept closed to maintain negative air flow, keeping airborne contaminants out of public areas.

The foundation for BSL-1 procedures is good microbiological practices and common sense, with training as an essential component. Lab workers must refrain from eating, drinking, applying make-up, or other personal care practices in the lab. Food for human consumption must not be stored in lab areas. Mouth pipetting is not allowed. Personal protective equipment (PPE) such as lab coats, eye protection, and gloves should be worn in the lab to prevent exposure to hazardous materials. PPE is removed before leaving the lab and hands are washed frequently and thoroughly. Sharps must be handled with caution and disposed of safely. Surfaces must be decontaminated after a spill and at the end of the work day. Biological waste is decontaminated before disposal by chemical disinfection or autoclaving. Access to the lab is limited when experiments are in progress. Hazard communication on potentially harmful organisms (e.g., opportunistic pathogens) in the lab is particularly important for the immuno-compromised person.

BSL-2 containment relies on all the BSL-1 practices and has additional requirements. BSL-2 risk assessments consider pathogen-specific characteristics and tailor precautions accordingly. BSL-2 precautions include use of biohazard signage, containment equipment (usually a biosafety cabinet, sometimes a fume hood) for aerosol-generating procedures and aerosol-transmitted pathogens, and additional PPE such as a respirator if containment equipment cannot be utilized. Procedures that do not generate aerosols can be done on the lab bench.

The differences between BSL-1 and BSL-2 are not tremendous but can make all the difference in preventing a lab-acquired infection. The containment standards are detailed in CDC/NIH's *Biosafety in Microbiological and Biomedical Laboratories* and the NIH *Guidelines for Activities Involving Recombinant DNA Molecules*, both available as links from the OBS website.

## Protect the Immuno-compromised

What do you think of when you hear the word immuno-suppression? Many people's thoughts focus on life-threatening chronic conditions such as severe combined immunodeficiency (SCID) and acquired immunodeficiency syndrome (AIDS). In reality, there are many factors that can cause suppression of the immune system – transiently as well as chronically.

Immuno-compromised individuals have increased susceptibility to micro-organisms. Many opportunistic pathogens that do not cause illness in a healthy individual can cause illness in people who have suppressed immunity. In addition, infections often have more serious consequences for immuno-compromised people. Some conditions that affect immunity include:

- Age: The very young and very old have weaker immunity either because the immune system is not fully developed or the level of function has declined over time.

- Pregnancy: Immunity is suppressed during pregnancy so that the mother's body does not reject the fetus as foreign. Some pathogens are especially hazardous to pregnant women (e.g., *Listeria monocytogenes*, *Toxoplasma gondii*, and lymphocytic choriomeningitis virus) because they can cause miscarriage and/or birth defects.
- Malignancy: Immune function is diminished during cancer and the cancer treatment (e.g., surgery, radiation, chemotherapy) itself can dampen immunity.
- Genetic immunodeficiency: Disorders such as SCID suppress immunity.
- Nutritional status: Poverty, anorexia, alcoholism, and other conditions that cause poor nutrition reduce immunity.
- Infection: An existing infection (even a minor illness such as a cold) can make individuals more susceptible to infection with other agents. More severe infections (e.g., human immunodeficiency virus [HIV] in AIDS) cause increased susceptibility on a long-term basis.
- Medications: Some drugs suppress the immune system (e.g., corticosteroids, antihistamines, immuno-suppressants such as cyclosporine A used to prevent rejection of transplanted organs).
- Hazardous chemicals: Occupational or environmental exposure to some chemicals (e.g., halogenated aromatic hydrocarbons such as dioxin, organic solvents such as benzene, polychlorinated and polybrominated biphenyls [PCBs and PBBs], heavy metals such as lead and mercury) can inhibit immunity.
- Radiation: Exposure to radiation as a medical treatment or through environmental or occupational sources can lower immune response. Ultraviolet (UV) exposure, in particular, is associated with immuno-suppression.

Following are some precautions to protect workers and the community that are especially important in protecting the immuno-compromised:

- Encourage staff to consult their doctors on the impact of their jobs on current health status.
- Train all staff on the increased risks for the immuno-compromised and how to reduce those risks. Training all staff is important because supervisors are often unaware of the immune status of their employees and because we are all immuno-compromised at some point in our lives. Part of human nature is to deny that we are in a weakened condition or to "tough it out".
- Stay at home during the infectious period of illnesses that can be transmitted such as colds and flu, for the sake of others and yourself.
- Minimize or prohibit exposure to hazardous materials and performance of hazardous procedures by immuno-compromised individuals.
- Increase precautions including enhanced use of personal protective equipment (PPE), containment equipment (e.g., biosafety cabinets, fume hoods), and preventive measures such as vaccination for people at higher risk.
- Inform staff (e.g., training, signage) of risks and necessary precautions including any additional procedures in place for immuno-compromised individuals.
- Ensure appropriate disinfection and disposal of hazardous materials to protect people at the workplace and in the community.
- Use good hygiene so that you won't bring contaminants home to your family or disperse contaminants into the environment.
- Obtain medical evaluations post-exposure to hazardous materials. Prompt medical follow up is of heightened importance for the immuno-compromised. Information on emergency preparedness including exposure procedures can be accessed from our website (www.fpm.wisc.edu/biosafety).
- Re-assign duties if necessary. Medical advice to the immuno-compromised individual may be to avoid certain materials or tasks on a short-term or long-term basis.

Confidential occupational health consultations are available through the Occupational Health Program (263-2177). There are increasing numbers of immuno-compromised people in our communities and in our workplaces. Risk assessments for work with potentially-hazardous materials must include a consideration of the impacts on immuno-compromised people.

## Lentiviral Vector Guidance

More and more researchers are using viral vectors, and lentiviral vectors, in particular, have become a common choice for gene delivery. Lentivirus is a category of retroviruses that includes human immunodeficiency virus (HIV). Lentiviral vectors have modifications that make the virus replication-deficient and much safer to handle than the parent virus.

UW-Madison's Institutional Biosafety Committee (IBC) reviews all biosafety protocols that involve viral vectors such as lentivirus that can infect humans. The IBC's risk assessment and independent review must be done prior to initiation of the project.

The National Institutes of Health Office of Biotechnology Activities (NIH OBA) recently released a guidance document for risk assessments of lentiviral vector projects which is summarized here.

The major risks of lentiviral vectors are the potential for the generation of replication-competent lentivirus (RCL) and the potential for oncogenesis. Both of these events are unlikely but must be considered because of the severe consequences that could result. Recombination events that reassemble the virus genome can convert replication-deficient lentivirus into RCL. Oncogenesis can result from delivery and expression of an oncogene or from insertional mutagenesis that alters the regulation of genes associated with cancer. Risks can be mitigated by enhanced safety features of the vector system and exacerbated by the nature of the transgene. The following table summarizes the factors affecting the risks of lentiviral vector use:

| Biosafety Considerations and Risk Levels | | |
|---|---|---|
| Biosafety Consideration | Higher risk | Lower risk |
| | ⟷ | |
| Vector Design | Vector packaging functions on two plasmids | Vector and packaging functions separated onto multiple plasmids |
| Transgene | Oncogene | Non-oncogene |
| Vector Generation | Large scale | Laboratory scale |
| Animal Hosts | Permissive host<br>Animals engrafted with human cells | Non-permissive host |
| Animal Manipulations | Vector administration (e.g., use of sharps during injection) | Housing and husbandry (no use of sharps) |

Later generation lentivirus vector systems are highly recommended because of enhanced safety features. Two major safety features that decrease the chances that RCL will occur are: 1) separating vector and packaging functions onto four or more plasmids; and 2) deleting genes and sequence (e.g., Tat gene) that are not necessary for the vector function. Testing for RCL is required or recommended in some cases (e.g., human gene therapy trials, large scale vector production, and higher hazard transgenes).

NIH OBA recommends either biosafety level two (BSL-2) or enhanced BSL-2 precautions and containment for HIV lentiviral projects. Enhanced BSL-2 conditions might include wearing personal protective equipment (PPE) that prevents mucosal exposures (e.g., a face shield in addition to protective eyewear), avoiding sharps or using heightened sharps precautions, using biosafety cabinets for all work with vector (including loading and unloading of centrifuge rotors), and consulting in-person with the Office of Biological Safety (OBS) prior to initiation of the experiments.

Another topic discussed in the guidance document is animal studies involving lentiviral vectors. Permissive animals support replication of the type of virus that has been administered while nonpermissive animals do not. Therefore, handling non-permissive animals that have been administered lentiviral vectors is a lower risk than similar research with permissive animals. NIH OBA suggests that it may be acceptable for the IBC to lower the containment level from BSL-2 to BSL-1 at some point post-inoculation (1-7 days) for projects involving nonpermissive animal hosts. Use of lentiviral vectors in all animals that have received human grafts requires minimally a BSL-2 level of containment.

Nonhuman lentiviral vectors (e.g., feline immunodeficiency virus [FIV], simian immunodeficiency virus [SIV], and equine infectious anemia virus [EIAV]) are briefly discussed. BSL-1 conditions are generally appropriate for agents not associated with human disease while BSL-2 precautions and containment are suitable for nonhuman lentiviral vectors that have been modified to allow transduction of human cells. The envelope (env gene) of lentiviruses or retroviruses can be replaced to alter tropism (ability to infect different species and

tissues). Insertional mutagenesis remains a potential risk even for nonhuman lentiviral and retroviral vectors when tropism has been broadened to include humans.

Lentivirus is a more complex type of retrovirus while simple retroviruses, such as Moloney murine leukemia virus, have only 3 genes (env, pol, gag). When the envelope of simple murine retroviruses is replaced with an envelope that enables infection of humans, the virus becomes amphotropic and BSL-2 rather than BSL-1 conditions are merited. From a safety perspective, nonhuman lentiviral vectors are comparable to simple retroviruses in that risks are heightened if the virus has been engineered to infect human cells with the biosafety level going from BSL-1 to BSL-2 in both cases.

The NIH OBA guidance document concludes with some scenarios demonstrating risk assessments of lentiviral projects. A comprehensive risk assessment that evaluates the risks of lentiviral projects and the mitigating aspects of the experimental design is essential. Please contact OBS with any biosafety questions including those regarding safe handling of lentiviral vectors.

## Working with Toxins of Biological Origin

Many researchers on campus find it necessary to work with toxins of biological origin. A biological toxin is any toxic substance produced by an animal, plant or microbe. Toxins can be very hazardous – some are the most lethal substances found in nature. Given the potential for acute toxicity, working with toxins can present unique challenges.

Prior to working with a toxin you should have a clear understanding of its properties. The lethal dose can vary significantly based on the specific toxin and the form it is in. Any risk assessment should take into account the potential route of exposure, the concentration of toxins being used, and the potential dose to which one may be exposed. Containment, personal protective equipment (PPE), and overall safety practices should be thoroughly reviewed. For many toxins BSL-2 facilities, practices, and procedures are an appropriate starting point when considering ways to mitigate the risk. Here are some other biosafety aspects you should consider before beginning your work:

- Require training on the specific toxin of all lab personnel before starting work; additional training should also be provided at appropriate intervals. As with pathogens, this training should also include a thorough review of symptoms of exposure.
- PPE can protect you from exposures but take care to correctly dispose of or decontaminate it. Lab coat, gloves, and safety glasses are usually a minimum requirement. Make sure the gloves you choose are not only impervious to the toxin, but also to any solvent used along with the toxin. Avoid using gloves, such as latex gloves, that generate static electricity when working with electrostatic powders.
- Inactivate all toxins prior to disposal. This can be done via autoclaving or chemical destruction (such as bleach). Autoclaving, however, is not universally effective on all toxins. It is important to know how to deactivate a toxin prior to working with it.
- Routinely decontaminate equipment and work surfaces using methods known to be effective for the specific toxin. The interior of biological safety cabinets (BSC) and fume hoods should also be periodically decontaminated.
- Protect vacuum pumps from contamination by inserting a HEPA filter into the vacuum line.

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- Use special precautions when handling dry powders or preparing stock solutions. This work should be performed in a certified BSC, fume hood, or glove box. Additional containment, such as glove bag within the BSC, may be necessary for some circumstances.
- Two knowledgeable individuals should be present during any high risk procedure, especially whenever there is the possible exposure to more than an estimated human lethal dose.
- Be prepared for emergencies. Spills and accidents can and do happen. As when working with pathogens such as viruses and bacteria, you should have a spill kit available in areas where you work with toxins. Carefully consider emergency scenarios concerning toxin use and plan for these.
- Post signage at the lab entrance to indicate that toxins are in use and state any special entry requirements.
- If using the toxin-producing organism in addition to the toxin, safety precautions that protect against both biological and chemical hazards have to be implemented.
- Finally, remember that a biosafety protocol must be submitted to the Biosafety office if the toxin is being used to elicit a biological effect or, in all instances, if a select agent is being used (see below).

Security is an important consideration when working with toxins. Implement an inventory system and track usage of the toxin. Store these materials in locked storage rooms, cabinets, or freezers with access restricted to those requiring use of the toxins. Additionally, specific toxins classified by the federal government as a Select Agent are subject to very stringent regulations. Luckily, quantities below a published threshold are exempt from the Select Agent regulations. These toxins and the exempt quantities are listed below:

| Toxin | Exempt Quantity |
|---|---|
| Botulinum neurotoxins | 0.5 mg |
| Staphylococcal enterotoxins | 5.0 mg |
| *Clostridium perfringens* epsilon toxin, Shigatoxin, Abrin, Conotoxin, Ricin, Saxitoxin, Tetrodotoxins, Shiga-like ribosome inactivating proteins, | 100 mg |
| T-2 Toxin, Diacetoxyscirpenol | 1000 mg |

Should you feel that you will need to work with greater quantities than these exempt amounts, contact Jeff Zebrowski (890-0993) and he will do his best to dissuade you or, if unsuccessful, he will work with you to fulfill the required registration process. You may never legally exceed the threshold limit without prior government approval.

Another consideration is that toxins are generally considered to be hazardous chemicals and are therefore subject to the OSHA laboratory standard. This standard emphasizes hazard communication, training, and safe practices. A Chemical Hygiene Plan must be implemented, which includes necessary work practices, procedures and policies to ensure that employees are protected from all potentially hazardous chemicals in their work area. Contact John Wendt
(265-5517) if you have any questions concerning CHPs.

*Biosafety in Microbiological and Biomedical Laboratories* is a good source for more information about practices for handling specific toxins of biological origin. Section VIII-G has information about specific toxins and Appendix I provides guidelines for working with toxins.

## UPS: Limited Carrier for Some Dangerous Goods

United Parcel Service (UPS) is a preferred shipping vendor for UW-Madison. This commercial carrier will accept certain biological materials and dry ice. The costs to use UPS may be more favorable under the campus contract than other carriers, but some dangerous goods will not be accepted by UPS. The UPS carrier requirements are in addition to the federal (DOT) and international (IATA) regulations. The following discussion provides important information for shipping biological materials via UPS, and assumes the reader is familiar with these regulations and has attended training on hazardous materials shipping provided by OBS.

UPS will not accept high hazard infectious substances (Category A). Biological materials are accepted by UPS only if they do not fit the definition of Category A including lower risk infectious substances (Biological substance, Category B), biological products, and non-hazardous genetically modified organisms and micro-organisms. Discretion is required in assigning materials to Category A versus Category B. The IATA table of indicative examples of infectious substances to be included in Category A is incomplete and the definition must also be considered.

The hazardous materials transport regulations make it clear that biological materials must be shipped as Category A if: 1) The pathogen is not on the Category A indicative examples list but fits the definition of Category A; or 2) The shipper is uncertain whether the material falls under Category A or Category B. If in doubt, the shipper must err on the conservative side and send the substance as Category A. OBS can assist with this determination.

Shipping dry ice via UPS follows the DOT regulations for domestic shipments and the IATA regulations for international shipments. Use the “Regulatory Requirements for Shipping Dry Ice” table from your HazMat Shipping training packet to determine requirements. Instructions for preparing dry ice shipments are provided at the UPS website.

Current training and certification is required by DOT and IATA, and this training requirement applies to shipping all hazardous materials including dry ice and infectious substances. During recent visits to our campus, UPS account representatives have required verification of training via hazardous material training certificates. Shipments may be refused if there is no proof of this training, especially for international

shipments. The next HazMat Shipping class for biological materials will be given on July 26th; on-line training is available for re-certification only.

Contact UPS directly (800-554-9964) for specific information about their shipping requirements.

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## BSC Certification and Conditional Waivers

Biological safety cabinets (BSCs) are the primary means of containment for working safely with infectious micro-organisms. When certified and used correctly in conjunction with good microbiological techniques, they control infectious aerosols and provide personal, environmental, and product protection.

Certification of the BSC must be completed when initially purchased, after a move, and at least annually for work in BSL-2 or higher containment. To function properly, the cabinet airflow must be closely regulated and the integrity of the HEPA filters must be checked. Experience has shown that basic maintenance of the BSC is essential. Poor performance can result from filter leaks and improper airflow balance; motor failures and electrical problems can cause fires. The Environmental Health Program (262-1809) provides service for BSCs (certification, maintenance and decontamination).

A conditional waiver may be granted by OBS on a case-by-case basis for low-risk activities for a maximum of three years. Cabinets are labeled to indicate the certification status. If not certified, the BSC must not be used with infectious or potentially-infectious material,l and users must be informed that personal, product and environmental protection cannot be assured.

To request a conditional waiver contact OBS prior to the annual re-certification date. If future work with biohazardous material is planned, contact EHP to schedule the certification of your BSC before beginning such work.

## *Classes Offered by OBS*

### *Shipping Infectious Substance and Other Biological Materials; Packaging Workshop*

- **Class: Thursday July 26, 2007** 8:30 – 11:00 a.m. at 1360 Genetics-Biotechnology Center
  **Workshop: Thursday July 26, 2007** 11:00 a.m. – noon at 1360 Genetics-Biotech. Center
- **Class: Wednesday October 17, 2007** 1:00 – 3:30 p.m. at Union South
  **Workshop: Wednesday October 17, 2007** 3:30 – 4:30 p.m. at Union South
- **Coming soon: A web training module for shipping dry ice as the only dangerous good.**

### *Basic Biosafety*

- **Tuesday July 24, 2007** 1:30 – 3:45 p.m. at 1335 Health Sciences Learning Center
- **Tuesday September 18, 2007** 1:30 – 4:00 p.m. at Union South

### Advanced Biosafety

- **Thursday August 2, 2007** 9:00 - 11:30 a.m. at 1360 Genetics-Biotechnology Center
- **Thursday September 27, 2007** 1:00 – 3:30 p.m. at Union South

**Registration is required for these courses.**

Contact OBS at 263-2037 or biosafety@fpm.wisc.edu for more detailed information.