

BioSide Lines

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The Newsletter of the UW Office of Biological Safety

Laboratory Security

For most staff and students at UW-Madison, the word "security" means safeguarding electronic equipment and personal belongings. The recent terrorist attacks and anthrax mail scares, however, have brought increased attention to laboratory security. These appalling events have forced us to look at security in terms of preventing theft of materials that have the potential to harm our community.

The UW-Madison Police Department recommends four basic precautions:

- Don't prop doors open; lock doors when no one is present.
- Wear visible identification.
- Remove sensitive data from the Web.
- Adhere to basic lab security precautions.

The degree to which laboratory security is implemented should be commensurate with risk. All laboratories, including those handling only low-risk biological materials under biosafety level 1 (BL1) containment practices, must maintain a basic level of security. The Centers for Disease Control and Prevention's guidance for BL1 microbiological and biomedical laboratories states, "Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress." Translated into common practice, this statement means that everyone entering a laboratory should have approval to be there. Furthermore, you should make an effort to know all the people who work in your area, to greet unknown persons who enter labs, and to ask their purpose.

Security concerns also extend to the storage of hazardous material. Unauthorized persons should not be able to access it. For materials handled or stored in the laboratory, this means that the laboratory door should be locked when the room is unoccupied. For hazardous materials stored outside of the laboratory, such as a freezer located in a hallway or a common service room, the equipment must be locked at all times.

Additional guidance on laboratory security issues can be found in Appendix F of CDC's Biosafety in Microbiological and Biomedical Laboratories. This document can be obtained electronically at: <http://www.cdc.gov/od/ohs/biosfty/bmbl4/b4af.htm>.

Viral Vectors and Biological Safety

Viral vectors are often designed to enter mammalian cells and deliver genes of interest. Viral vectors are usually replication-deficient. Genes necessary for replication of the virus are removed from the vector and supplied separately – through plasmids, helper viruses, or packaging cell lines. Accidental infection due to inappropriate handling precautions can result in delivery of the foreign gene although disease is unlikely to result.

There are several biosafety concerns that arise with the use of viral vectors including:

- 1) Tropism (host range) – viral vectors that can enter (infect) human cells are often used.
- 2) Replication-deficient viral vectors can gain back the deleted genes required for replication (become replication-competent) through recombination - referred to as replication-competent virus (RCV) breakthroughs.

- 3) Genes may be expressed in tissues and/or organisms where they are normally not expressed. In the case of some genes such as oncogenes this could have far-reaching negative consequences.

When evaluating safety for use of viral vectors, there are a number of factors that need to be considered including: risk group (RG) of the organism; tropism (organism and tissue); route of transmission; whether the virus integrates into the host genome; and the specific gene(s) being introduced. Please contact the Office of Biological Safety (OBS) for more information on physical barriers and safety practices to use with specific viral vectors. This article concentrates on biological barriers that can be employed to improve safety when using viral vectors.

Viral vectors frequently used are retrovirus/lentivirus, adenovirus, adeno-associated virus, poxvirus, herpes virus, and baculovirus. Amphotropic Moloney murine leukemia virus (MMLV) and adenovirus are common viral vectors used to introduce genes into human cells. Amphotropic means the virus is able to broadly infect mammalian cells, whereas an ecotropic MMLV strain can only infect murine cells.

NIH classifies both amphotropic MMLV and adenovirus as RG2 pathogens. Both these viral vectors infect human cells and both have potential for RCV breakthroughs. Amphotropic MMLV integrates into the host's genome – this translates into stable expression of introduced genes and the potential for insertional mutagenesis of host genes. Adenoviruses are cold germs that can be transmitted by aerosol and can cause eye damage. Adenoviral vectors are known to have a relatively high rate of RCV breakthroughs compared to other viral vectors.

There are a number of ways to improve safety when working with viral vectors. Briefly these include:

- 1) Consider the alternative of nonviral vectors. Advances in nonviral vector technology may mean that a nonviral vector will serve your purpose.
- 2) Limit tropism – narrow host range and/or tissue infected. Even if you need your vector to infect human cells, you may be able to engineer your viral vector so that it only infects specific tissue(s).
- 3) Use strategies to decrease chances of RCV breakthroughs.
 - a) Split genomes of viral replication genes. Having replication genes on different constructs means that more recombination events would need to occur in order to get a RCV breakthrough.
 - b) Remove viral regulatory regions. This decreases the chance of homologous recombination occurring.
 - c) Produce virus as a transient single batch (simultaneous transfection of plasmids) rather than as continuous culture (use of a packaging cell line with replication genes integrated into the genome of the cell line). There is an increased risk of RCV breakthroughs with the use of packaging cell lines – especially with large-scale production.
- 4) For viruses with complex genomes like adenovirus, delete as much of the viral coding sequence in the vector as possible (e.g., “gutless” adenoviral vectors). This helps to eliminate expression of viral genes in transduced cells.
- 5) Use ecotropic MMLV with methods that allow the virus to enter human cells in a limited manner. This strategy would be especially relevant for introduction of genes such as oncogenes, mutant tumor suppressor genes, mutant repair genes, and some signal transduction pathway genes.

Method 1: First transfect the cell line with the ecotropic MMLV receptor, and then infect that cell line with ecotropic MMLV. The virus would not be able to infect any human cells that had not already been transfected with the ecotropic MMLV receptor.

Method 2: Use ecotropic MMLV linked to poly-lysine (retrovirus molecular conjugate). The ecotropic virus can enter human cells in this form but any RCV breakthroughs that arise would not be linked to poly-lysine and therefore would not be able to infect human cells.
- 6) Use controlled recombination (site-specific recombination mechanisms) to activate/inactivate viral replication genes.

When using viral vectors, ways to optimize safety must be considered. Training staff to safely handle viral vectors and animals infected with viral vectors is the responsibility of the Principal Investigator. Please contact Margy Lambert (3-9013; mlambert@fpm.wisc.edu) for more information and if you have any questions or suggestions.

Shipping Infectious Substances and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping infectious substances 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, January 22, 2002

Union South 1 to 3 p.m.

Refreshments will be served.

Registration is required.

Contact Margy Lambert at 3-9013 or mlambert@fpm.wisc.edu.

Staff approaching their two-year expiration for certification will receive a notice in advance of that date. You are welcome to attend the class. Computer-based training is now available as an alternative, but only for those who have attended the class for their original certification.