



Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail them to karen_byers@dfci.harvard.edu or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

The IBC Brings the Message About Lentiviral Vectors Home to Investigators

Why not involve the scientific members on your institutional biosafety committee (IBC) to help explain biosafety concerns to their peers? This proved to be an effective tool at the Dana-Farber Cancer Institute when we realized that the IBC and the research community had very different risk perceptions about lentiviral vectors. The following two lists illustrate that difference in perspective.

The Advantages of Using Lentiviral Vectors from the Scientist's Perspective

The potential of the lentiviral vector system may make it attractive for widespread use in the research community because:

- Lentiviral vector systems infect dividing and nondividing cells.
- Reported transduction rates are very high.
- Second and third generations of lentiviral vectors have multiple gene deletions so that only the gene

delivery system of the wild type is present. An additional safety feature is the use of multiple plasmids.

- The vectors are replication incompetent; only one round of infection should occur.
- Lentiviral vectors are readily available. Expression kits are available commercially and “custom services” are available from the academic research community.
- VSV-g pseudotyping greatly enhances the stability of vectors and broadens the host range of the vector.

The Potential Hazards of Working with Lentiviral Vectors from the Biosafety Perspective

- Lentiviral vectors could infect dividing and nondividing cells.
- These vectors could very efficiently transduce a wide range of cells.
- VSV-g pseudotyping greatly enhances the stability of vectors (higher titers may be harvested) and host range of vectors (a wider range of cells may be transduced). Theoretically, the risk of mucosal exposure may be increased when vectors have been pseudotyped with VSV-g.

The three points listed above are strong arguments for requiring the research staff to assess carefully the risks of an exposure incident. Hopefully, this will lead to improved compliance with biosafety practices.

- Researchers may erroneously assume that since kits are commercially available, lentiviral vector systems are inherently “safe” and strict adherence to Biosafety Level 2 is not required.
- Researchers may also erroneously assume that since kits can be ordered, there is no requirement to register the proposed use of lentiviral vectors with

the Institutional Biosafety Committee.

- Second and third generation vector systems do greatly improve safety by separating the vector functions on multiple plasmids. However, stable packaging cell lines are not available yet so every transient transfection is a “roll of the dice”—a series of random events that can result in the production of a replication-competent virus. There is some risk of nonhomologous recombination; this estimated risk varies from system to system and should be considered (Trono, 2002). Due to the multiple deletions of genes in HIV-based lentiviral vectors, the replication-competent virus is not likely to be HIV unless some cross-contamination of stocks has occurred. The theoretical risk is generation of a VSV-g pseudotyped replication-competent virus bearing the gene of interest capable of infecting dividing and nondividing cells. This is another good reason to handle these cultures with appropriate precautions.

- Researchers without extensive microbiological experience are now working with viral vectors. The fact that a given vector stock may contain replication-competent virus particles may not be a routine consideration for them. There was discussion about the potential generation of replication competent vectors at the 2002 ABSA conference when Gary Nolan presented the talk “What makes a Virus Safe?” He referred us to his web site for information on this subject, see:

<http://www.stanford.edu/group/nolan>

Our IBC requires careful review of procedures for propagation and use of lentiviral vectors. Approved protocols are conducted within the BSL-3 biohazard core laboratory or in a “Biosafety Level 2+ for lentiviral vectors” laboratory set-up according to the specific requirements of our internal policy.

I reported to the IBC that I had heard more than once the comment, “If it’s commercially available...it must be safe...so why do I have to register this protocol?” So far discussing our lentiviral vector fact sheet with groups registered to use these vectors had not altered the fact that some researchers did not believe the additional safety practices were warranted. So I asked the IBC members for help.

A meeting was organized to discuss the safe use of lentiviral vectors. The announcement listed the IBC participants and an outside speaker (an author-

ity on the use of viral vector systems) and indicated that attendance was mandatory for lentiviral vector users and open to all others. Amazingly, on short notice there was standing room only in a large conference room. The IBC Chair, Myles Brown, M.D., opened the meeting with a general statement about the importance of being aware of the potential risks associated with viral vector systems and the need for sensible work practices to minimize the risk of staff exposure. He also emphasized the requirement to register such experiments; a few investigators had overlooked that step since kits to make lentiviral vectors can be ordered so easily. He assured staff that they shouldn’t be afraid to call their Biosafety Officer, who would work with them not blocking their work but helping them to set up appropriate conditions to conduct experiments safely and responsibly.

Following the IBC Chair’s presentation, a noted AIDS researcher (Joseph Sodroski, M.D.), an IBC member known for his thoughtful reviews, explained various lentiviral vector systems. This included a thorough explanation of the potential for generating a replication-competent virus. Then the Biosafety Officer discussed the Institute’s lentiviral vector policy (Figure 1) and demonstrated that plastic aspiration pipets do not cause puncture wounds. Despite the fact that glass Pasteur pipets have caused potential exposures in some Biosafety Level 2 labs, it has been difficult to convince staff to stop using them to aspirate tissue culture fluids. Since I had a captive audience, I also distributed the excellent article “Safety Considerations for Retroviral Vectors: A Short Review,” shared through the BIOSAFETY listserv. This thoughtful review was prepared by Donald E. Mosier, TSRI Institutional Biosafety Committee Chair with the assistance of Carolyn Keierleber, TSRI Biosafety Officer and Associate Director of Environmental Health & Safety, and Richard Gulizia, TSRI BSL-3 Facility Director.

Next, Richard Mulligan, Ph.D., our invited viral vector expert, spoke. He agreed with the Institute’s required procedures and previous comments, and reminded the audience that if there is an error to be made, someone out there...somewhere...will make it. In his own laboratory, Dr. Mulligan does not allow staff to bring in plasmids containing the envelope gene of HIV. He reminded us that it is very easy for

Figure 1

DFCI Policy: Biosafety Level 2+ for Use of Lentiviral Vectors

Containment Level:

Consistent Biosafety Level 2 practices are adopted and rigorously followed.

Facility:

The Principal Investigator must designate a laboratory that contains a biosafety cabinet, incubator, and centrifuge. This laboratory cannot contain a desk, since it is our experience that personal desks lead to casual activities incompatible with continuous adherence to Biosafety Level 2 practices.

Signage:

A sign with the following information is posted in these areas. Staff should be aware that there is some potential for recombinant virus generation. Since such recombinants may have the enhanced host range afforded by the VSV-g pseudotype, all experimental materials must be handled with great care. Sharps must be eliminated from experimental procedures; in particular, the procedures involved in pelleting virus should be reviewed carefully.

PPE:

Gloves and lab coat.

Work Practices:

- Close laboratory door.
- Use biosafety cabinet for all manipulations.
- Load ultracentrifuge buckets in biosafety cabinet; screw on cover, spray outside with alcohol. Do not use "sharps" to harvest virus pellet.
- No "sharps (needles, glass Pasteur pipets) may be used with these cultures. Plastic aspiration pipets are a replacement for glass Pasteur pipets.
- Cells must be placed in a **dedicated** incubator, labeled "Lentivirus experiments ONLY."
- Cells exposed to lentiviral vectors are not removed from [lab room #] for experimental purposes unless inactivated by approved procedures.
- Undiluted bleach should be pulled into the aspirator flask using suction to decontaminate tubing. Final concentration in the aspirator bottle: 10% bleach.
- The aspirator flask should be disconnected, the openings covered with aluminum foil, and the flask transported in a dishpan on a cart to the autoclave room.
- The double biohazard bag containing solid plastic waste and pipets must be closed and transported to the autoclave room.
- Biosafety cabinet surfaces must be thoroughly wiped with disinfectant.
- Spill procedures are also posted with the following note: Occupational Health Services has been asked to evaluate the use of antiretroviral drugs for postexposure prophylaxis in the event of a staff exposure incident involving HIV-based lentiviral vectors. No data are available on the efficacy of this treatment

someone to grab and use the wrong eppendorf tube, and he says, "It happens more often than you might think." He recommended reviewing the inventory of plasmids in laboratories working with lentiviral vectors to minimize the risk that an error could occur, resulting in the inadvertent addition of a plasmid which supplied the deleted envelope gene.

Finally, there was an opportunity to ask questions. The most interesting question was whether lentiviral vectors based on an animal lentivirus, rather than HIV, were considered safer. According to Sodroski, there is not enough experience to answer that question. A question regarding occupational exposure to lentiviral vectors was asked. It was noted that in the case of an exposure to a multigene, deleted, HIV-based vector, only a viral load assay should be considered. In response to the question regarding what tests could be used for serological monitoring after an exposure incident involving vectors based on bovine, feline, or other lentiviruses, the occupational health options discussed included documentation of the incident and serum collec-

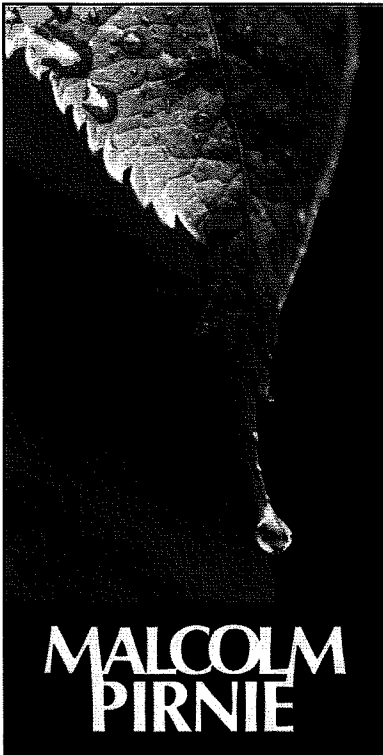
tion. With respect to testing the preparation involved in an exposure incident for replication-competent virus, participants also agreed that negative results could not be interpreted as a guarantee that it did not contain replication-competent virus.

The meeting was over in an hour, but as staff left they were still discussing the issues. I would encourage a similar approach for any situation in your institution where peer-to-peer communication would be helpful.

References

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