supplementary method of protection is to provide workers with personal protective equipment (PPE) and the know-how to use it properly.” There is a need to assess the probability of exposure as a result of working in a potentially hazardous environment or with a potentially hazardous work practice, and, whenever possible, a requirement to engineer out the problem (recognizing that engineering solutions never result in zero risk) or to change the practice. If neither of these options provides a solution, then PPE should be used. The OSHA guide (OSHA, 2000) also states that: “Employers should institute all feasible engineering, work practice, and administrative controls to eliminate or reduce hazards before using PPE to protect employees against hazards.” and indicates that employers should “...choose the appropriate PPE for protection against that hazard.” (Emphasis added). Understand that PPE is sometimes necessary, but that wearing PPE may result in a worker becoming fatigued, stressed, or encumbered to the point of losing concentration for the job-at-hand. Inappropriate use of PPE may, in some instances, be as dangerous as the hazard from which you are trying to protect the employee.

Finally, it comes to my attention that a lot of the things we do to ostensibly protect personnel and the environment from biological hazards are done “because they have always been done that way.” Biohazard science and the methodologies that we use in laboratories have changed since the old “Fort Detrick” days. Engineering has vastly improved. We have become much more sophisticated in our approach to containment. It is time that we think outside the box and develop appropriate controls based on current knowledge and current research in containment. Encourage practical biosafety research into the solutions for recognized problems. Don’t ever rely on what someone tells you is the best solution to a particular problem, just because they say it works. ASK FOR THE DATA. Critically review the protocols and look to yourself to determine if the research on the product or procedure really answers the questions and proves that the results demonstrate what is claimed.

So, that’s it—the final musings of an old biosafety professional. Oh, you will still hear from me as I read the ABSA e-mail group “biosafety” and find myself saying, “Okay, let’s get back to basics.” I will always remember, and encourage you to remember, the words on the UNC Class of 1981 t-shirt “Biohazard Scientists do it with Control.” Keep on controlling.

Reference


Animal Bytes

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Animal Bytes examines biosafety challenges posed when conducting work with animals and provides solutions that promote both safe and responsible research. Good safety and animal husbandry are essential for good science. Learn about best practices when working with animals and applied safety information that can be used every day. Please e-mail your comments, questions, and insights to barbara_johnson@verizon.net or to Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Lentiviral Vectors and Animal Biocontainment Levels for Studies with Mice

Introduction to Lentiviral Vectors

Since the late 1990s, retroviral vectors have gained increasing popularity as gene delivery systems. Lentiviruses or “slow viruses” are a subclass of retroviruses that are able to infect both proliferating and non-proliferating cells. Because they integrate into the host cell genome and have powerful promoters, lentiviruses are able to deliver genes to target cells with high efficiency and induce long-term, stable expression of the introduced gene of interest. While lentiviruses infect many species, those which have been used as viral vectors include Feline Immunodeficiency Virus (FIV), Simian Immunodeficiency Virus (SIV), and most prominently Human Immunodeficiency Virus (HIV). The focus of this article is animal biosafety considerations for HIV-based lentiviral vectors in mice.

Inherent occupational hazards associated with lentiviral vectors include the ability of the virus to become competent to replicate in host cells and cause oncogenesis. The following is a very brief overview of how commercially available lentiviral vector systems have evolved to reduce these hazards. HIV-based lentiviral vector systems, referred to as “first generation,” were developed to increase the viral titer by increasing the range of cells that could be infected. To
create the first generation system, the envelope genes from wild type HIV were typically replaced with envelope genes from vesicular stomatitis virus (VSV). The 3-plasmid construct allows for insertion of the gene of interest, and in theory allows for only one round of virus replication. However, during replication the virus can recombine in such a way that replication-competent retroviruses (RCRs) are generated and pose a hazard as the virus and gene of interest can now continue to replicate unchecked within cells. To further reduce the risk of forming RCR, second-generation vector systems were created by deleting more genetic elements (accessory genes) from the virus. This was an improvement but when assayed, RCR could still be detected. Third-generation systems made additional deletions to the virus genome (removed the tat gene) and newer, more improved systems made alterations such that the vector and packaging system were separated on 4 plasmids instead of 3 plasmids, to further reduce the probability of recombination.

In a recently published article, Reuter et al. (2012) measured viral vector shedding in mice following intravenous inoculation with either third-generation replication-incompetent lentiviral vector, an adenoviral serotype 5 vector, or a recombinant adeno-associated viral vector. For the purpose of this column, only information pertaining to lentiviral vectors will be summarized. Environmental stability tests on fomites showed infectious lentiviral vector was recoverable after deposition on dry plastic for 24 hours, and in vector-spiked soiled bedding for up to 72 hours. Infectious virus could also be recovered by swabbing the injection site (tail) for as long as 24 hours, though no infectious virus was detectable in the soiled corn cob bedding in the inoculated group-housed mice. Positive tail swabs were attributed to vector leakage when removing the needle from the injection site. No viral nucleic acids were detected in blood, urine, or fecal samples taken on days -1, 1, 3, and 7 (relative to inoculation) from inoculated mice or in co-housed non-inoculated mice (lower limit of detection for the assay was 200 IU). While the results of this study indicate that risks associated with shedding are low, the authors caution that increased exposure risk is possible if there is a pre-existing viral load/infection of the animal or laboratory and animal care staff.

**Factors to Consider in Assessing Risk When Using HIV-based Lentiviral Vectors in Mice**

The simplest approach is to identify and characterize the elements that pose risks such as:

- The type of vector system used (i.e., increased risk with a higher potential for replication competence)
- The gene of interest that has been inserted (i.e., increased risk if an oncogene)
- The host animal (i.e., increased risk if a permissive host, one that supports virus replication)
- The vector quantity and titer (i.e., increased risk with higher titer/quantity)

**Replication Competent Lentivirus (RCL) Testing**

RCL testing is required by the FDA for all lentiviral stocks used in human clinical trials. RCL testing is also desirable for safety data in laboratories not involved in human clinical trials. However, RCL testing requires specialized expertise and assays that may not be available in every lab. The NIH recognizes that handling positive controls during RCL assays may put staff at increased risk of exposure in labs that do not routinely work with lentiviral vectors (NIH, 2011). In these cases, the IBC can conduct a risk assessment of the vector system used and the data generated from past work with the system to assign an appropriate biocontainment level and identify appropriate practices and PPE.

**Containment Levels Associated with Wild Type Mice and Mice with Human Xenograft Tissue**

The IBC and IACUC should review and approve procedures and equipment plans for the initial containment of animals post-inoculation, decontamination of inoculation sites and soiled caging, and the transfer of animals to a lower containment level. Primary containment PPE is commensurate with ABSL-1 or -2 recommendations and the risk assessment for the work being conducted (U.S. Department of Health and Human Services, 2009).

Questions often arise regarding the appropriate animal biocontainment level for conducting work with established 3- and 4-plasmid third-generation HIV-based lentiviral vectors in mice. Replication of HIV is not supported in wild type mice (i.e., mice that do not express a genetic mutation or manipulation such as transgenic or knock out that could theoretically support virus replication). Hence, the risk of shedding is greatly reduced and associated primarily with the inoculum itself. In wild type mice, current best practices recommend that the initial delivery of the vector be conducted under BSL-2 containment using safe-needle systems (i.e., retracted sharps or other engineered devices) as this can potentially infect humans. Mice containing human xenograft tissue and mice injected with human cells infected with lentiviral vectors are a permissive host for the virus, allowing the replication and shedding of HIV-lentiviral vectors. Commonly, these mice are maintained at ABSL-2 or ABSL-2 enhanced.

Enhancements may include use of primary containment such as conducting cage changes in a BSC, use of filter-top cages or negatively ventilated IVC racks, minimizing the use of sharps wherever possible, and conducting other rodent manipulations in the BSC. If a risk of splash or aerosolization of liquids exists, a face shield and mask are used to prevent mucosal or ocular exposure.

In March 2006, the NIH RAC convened a meeting on biosafety considerations with lentiviral vectors and recommended that animals be initially housed in ABSL-2 containment for 1-7 days, then moved to standard ABSL-1 containment provided the inoculation site has been cleaned and the bedding changed (NIH, 2011). An Internet search was conducted on best practices among a variety of institu-
tions to identify alternative recommendations. Three institutions (Emory University, Oregon Health & Science University, and Stanford University) were found to have different approaches for housing wild type mice following inoculation. Each method provides flexibility for the type of containment space available and employs various safety procedures and/or the use of primary containment equipment for housing animals post-inoculation.

1. Emory University allows inoculated animals to be held in filter-top cages and IVC racks in demarcated areas of an ABSL-1 room. Specific signage/labelling is needed on each cage stating “ABSL-2 Biohazard Containment—Quarantine for Lentiviral Vector Research.” Seventy-two hours post-inoculation, animals can be transferred to a clean cage and housed in ABSL-1 containment.

2. Oregon Health & Science University permits animals following inoculation to be housed in standard ABSL-1 containment. The animals’ inoculation sites are disinfected immediately with 70% ethanol. Animals are temporarily placed in a holding container devoid of bedding until the sites are completely dry. The animals can then be moved to a standard ABSL-1 containment cage, and the holding container is decontaminated with 70% ethanol.

3. Stanford University requires animals to be initially housed in ABSL-2 containment. If the inoculation site has been cleaned and the bedding changed, the animals may be moved to standard ABSL-1 containment after 48 hours.

The Need for Biosafety Data: Looking at a Path Forward

The literature contains a paucity of data regarding viral vector shedding and migration in laboratory animals. However, this information is essential to assess exposure hazards and make informed decisions on appropriate biocontainment in mice inoculated with lentiviral vectors, or for that matter, other commonly used viral vectors. At risk is the health and safety of animal care personnel and research staff, and the potential for accidental exposure to viral vectors of other animals in the vivarium. The lack of scientific data on viral vector shedding and many other topics related to animal biosafety and biosafety in general hampers the ability to accurately assess and take appropriate steps to mitigate risks. The lack of research data is a reflection of the extremely limited funding available in the form of grants and other resource streams for applied and basic biosafety/animal biosafety.

There is some good news. The American College of Laboratory Animal Medicine (ACLAM) has a grant program where researchers can submit a letter of intent and apply for a grant to address knowledge gaps across a variety of topics including animal biosafety. As this column is going to print, ACLAM has issued a Request For Proposals to challenge the need for 10-15 air changes per hour in animal housing rooms and to develop rational HVAC standards for modern facilities and those using individually ventilated cages. The impact this type of research may have on safety, program sustainability through reduced operating cost, and development of animal housing environmental standards could be significant. The American Association for Laboratory Animal Science (AALAS) sponsors Grants for Laboratory Animal Science (GLAS) which provide competitive short-term research funding in the laboratory animal science field. The Elizabeth R. Griffin Research Foundation provides grants for research and training through its partner organizations, one of which is the American Biological Safety Association (ABSA). The ABSA web site posts a Request For Proposals in the June-July timeframe and makes resources developed through the grant available free of charge on its web site. While considerably more funding is needed, these organizations provide a good starting place for those investigators seeking to compete for grants and conduct research in animal biosafety.

References


