MU IBC policy on handling lentivirus, adenovirus, and AAV-based vectors

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Introduction and Rationale

Recombinant lentivirus, human adenovirus (HAdV), and adeno-associated virus (AAV) vectors are common tools to overexpress heterologous genes in cell culture and/or laboratory animals. AAV is an attractive vector for gene therapy purposes. Lentiviruses derived from human immuno-deficiency virus (HIV) and HAdV, if replication-competent, have the capacity to cause disease in healthy humans. By contrast, non-modified AAV have not been associated with any disease symptoms in humans. The use of adenoviral, lentiviral, and AAV based vectors has been increasing because they are commercially available, relatively easy to manipulate, and very versatile in their applications. However, research involving these viral vector systems also raises biosafety issues. This document applies to human immuno-deficiency virus (HIV)-based lentivirus vectors, (as opposed to non-human lentivirus vectors such as FIV, SIV, EIAV), recombinant HAdV, and AAV based vectors.

This document provides the MU research community with Institutional Biosafety Committee (IBC) approved guidance on the handling of lentivirus, adenovirus, and AAV vectors in the course of research.

Training guidelines

The IBC requires each Principal Investigator (PI) to complete laboratory specific training according to his/her IBC protocol that should reference these following guidelines regarding the use of lentivirus, adenoviral, or AAV-based vectors.

Primary laboratory staff and animal care staff must receive training in items listed below.

- How the agent in question can be transmitted
- Signs and symptoms of infection with the agent
- Treatment options
- How animal infections are conducted

- How infected animals should be handled
- Signs and symptoms infected animals will exhibit

Biological risks associated with lentivirus vectors

The major risks to be considered for research with HIV-1 based lentivirus vectors are:

- Potential for generation of replication-competent lentivirus (RCL)
- Potential for oncogenesis through insertional mutagenesis.

See also: *Collins DE, Reuter JD, Rush HG, Villano JS. Viral Vector Biosafety in Laboratory Animal Research. Comp Med. 2017; 67(3):215-221. PMID: 28662750.*

These risks can be mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector. Most commercially available lentivirus vectors are considered risk group (RG) 2 agents being used under biosafety level (BSL)-2 conditions. Pseudotyping lentivirus vectors with murine leukemia virus glycoproteins instead of (potentially human infecting) vesicular stomatitis virus (*Rhabdoviridae*) envelope glycoprotein increases the overall handling safety of the former. This approach should be generally considered for those experiments that are to be conducted in mice. Since the murine leukemia virus pseudotyped vector has no more human host specificity, it may be possible to use it under BSL-1 conditions.

See also: *Schambach A, Galla M, Modlich U, Will E, Chandra S, Reeves L, Colbert M, Williams DA, von Kalle C, Baum C. Lentiviral vectors pseudotyped with murine ecotropic envelope: increased biosafety and convenience in preclinical research. Exp Hematol. 2006; 34(5):588-592. PMID: 16647564.*

Work involving use of lentiviral vectors, **including those purchased in cloning kits**, constitutes recombinant DNA (rDNA) experimentation. Therefore, these activities fall under the definition of rDNA research as outlined in the NIH Recombinant DNA Guidelines. Experiments must be submitted for review and approval by the Institutional Biosafety Committee (IBC) **before** being conducted.

Biological risks associated with adenovirus and AAV based vectors

Replication-competent and -deficient adenoviruses are classified as RG2 agents. They inefficiently integrate into the (human) genome. Most adenoviral vectors are derived from adenovirus type 5 in which key elements for replications have been deleted. The packaging system, however, is retained. Adenoviruses in general are very immunogenic but often cause only minor respiratory illness.

A potential, but rather small risk could be a recombination or cross-packaging event between a wild-type adenovirus naturally acquired by the lab personnel (or lab animals) and the recombinant adenovirus, resulting in a new replication-competent virus.

AAVs do not integrate their genomes efficiently into host DNA and they need a helper virus for their propagation. There is no health risk described when working with these viruses. AAV, therefore, is considered a RG 1 agent.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines) consider all AAV serotypes and recombinant or synthetic AAV constructs at risk group 1 (RG1) as long as the transgene does not encode either a potentially tumorigenic gene product (e.g. oncogene) or a toxin molecule and are produced in the absence of helper virus. However, rAAV that are produced in human cell lines are to be handled in accordance with the OSHA Bloodborne Pathogens Standard, under BSL2 containment.

The IBC will require BSL2/ABSL-2 containment if:

1) The transgenes express an oncogenic protein or toxin, or
2) The rAAV is produced using a helper virus of human origin, or
3) The rAAV is produced in human cell lines without purification prior to use.

Transport of lentivirus, adenovirus, and AAV

- Transport all lentivirus, HAdV, and AAV material in a double-sealed leakproof container.

- Label the container with a biohazard symbol, the name of the agent, the amount, and the Principal Investigator's name and telephone number.
- If shipping and/or receiving adenoviral vectors off campus, please contact the Environmental Health & Safety Services (EHS) for guidance and training.
- Call the EHS for assistance in transporting infectious materials at 573-882-7018.

Laboratory experiments

- Recombinant lentivirus vectors (not pseudotyped with murine leukemia virus glycoprotein) and HAdV are considered RG 2 (BSL-2) pathogens, while AAV is considered a RG 1 (BSL-1) pathogen.
- Laboratory coats, gloves, and safety glasses or goggles must be worn.
- Materials containing lentivirus, or HAdV should be handled inside biological safety cabinets (BSC). If your planned laboratory procedures make the use of the viral material inside a BSC difficult, contact EHS Biosafety Staff for discussion of additional containment measures and/or personal protective equipment.
- In the event of a spill outside the BSC, contact EHS Biosafety Staff for guidance and/or assistance in spill cleanup. Note: spills involving recombinant materials are reportable to the NIH. Contact EHS Biosafety Officer for all spills involving recombinant materials so that an incident investigation can be initiated.
- When performing centrifugation procedures, use sealed centrifuge safety cups or sealed rotors.
- Protect the vacuum lines and system with disinfectant traps and HEPA filter.
- Pay special attention to the possible generation of aerosols from unwanted biological materials.

Tissue culture experiments

- All tissue culture work should be conducted in a BSC with both product and personnel protection.
- A biological hazard sign indicating the use of lentivirus should be placed outside tissue culture room and on the BSC.

- Laboratory coats used inside the tissue culture room should not be worn outside the tissue culture room.
- Materials should not be stored inside the BSC. Take only what is needed to perform the procedure(s) and place it in the BSC upon initiation of the procedure. Upon conclusion of the procedure(s), decontaminate with virucide and remove materials from the BSC.
- Serological pipettes and pipette tips should be decontaminated in a virucide, such as a 1:10 dilution of household bleach for at least 30 minutes prior to discarding in solid biohazard unwanted material containers. For this purpose, a beaker containing a virucide may be kept inside the BSC while experimental procedures are being performed, or the vacuum flask setup maybe installed outside the cabinet and placed in secondary containers. If pipettes and pipette tips are to be re-used following decontamination, they should be rinsed in water prior to autoclaving.
- All plasticware placed inside the hood while working with the virus must be decontaminated with a virucide prior to disposal. This can be done by spraying all plasticware with a 1:10 dilution of household bleach (final concentration 0.525%).
- Upon conclusion of procedures in the BSC:

  For liquid unwanted biological materials contact the EHS to discuss and obtain initial approval for decontamination and disposal procedures.
  If aspirated liquid waste is 2/3 full, aspirate a fresh solution of virucide through the suction tube so that the final concentration is appropriate and allow to soak for at least 30 minutes, and empty entire contents down the drain. Refer to the MU Biological Safety Manual for additional guidance.
  Spray all BSC surfaces with a virucide, wipe down, then spray all surfaces with 70% ethanol, and wipe down. Allow the ethanol to air dry. NOTE: Spray the full interior of the BSC (work surface, sides and back of cabinet, as well as the back of the viewing glass).

If you will dispose of unwanted biohazardous material by autoclaving: When the solid biohazard unwanted material bag is full, seal it with autoclave tape, and take the bag (double bagged and in secondary containment) to the autoclave room for sterilization. After the autoclave cycle is complete and you note the change in the autoclave tape, place the bag inside a black trash bag. The autoclaved unwanted biological material may then be disposed of within a regular sanitation pickup container in accordance with the Autoclave Procedure in the Biological Safety Manual.

When disposing of unwanted biohazardous material without autoclaving, you must be registered at EHS as an "unwanted biohazardous material generator". All unwanted biohazardous material needs to be placed inside a designated cardboard box labeled with a yellow sticker (providing the waste generator's information), and containing a red biohazard plastic bag. To collect your unwanted biohazardous material, submit a Waste Pickup Request for your material through Environmental Health & Safety Assistant. This will also be the method used to order replacement containers and labels. If you have questions about EHSA, call (573) 882-3736, or email hazmat@missouri.edu.

NOTES:

- Do not overfill biohazard unwanted material bags.
- Two-third's (2/3) is considered full.
- Biohazard bins or boxes MUST NOT weigh more than 40 pounds.
- Biohazard unwanted material boxes are NOT SHARPS CONTAINERS.
- Sharps must be disposed of within a closed/sealed hard plastic sharps containers.
- Sealed sharps containers may be placed inside a biohazard box.
- For additional information on sharps disposal, refer to the MU Biological Safety Manual.

Small animal experiments

(During shedding period of a lentivirus vector, ~ 72 hours post-infection)

Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of recombinant lentivirus from such animals is very low (even if replication competent lentivirus were present in the original vector inoculum). In general, the initial delivery of viral vector should be performed under Animal Biosafety Level 2 conditions (ABSL-2) or under enhanced ABSL-2 containment so as to minimize the risk of autoinoculation by the investigator. However, it may be permissible to reduce the containment level at some point following viral vector delivery. This applies to lentivirus vectors pseudotyped with murine leukemia virus glycoprotein. Otherwise, if there is no expectation of infection (see below), the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing containment from ABSL-2 to ABSL-1 within a few days (the specific time period can be specified by the local IBC and may vary anywhere from 1-7 days depending on local and experimental considerations). The University of Missouri IBC has recommended 72 hours of ABSL-2 containment post-injection based on this information. Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a higher level of containment.

Assume the shedding period for lentivirus to be 72 hours minimum unless it can be demonstrated on permissive cell lines to be different for an application. Assume the same shedding conditions when using recombinant HAdV (which should be handled under BSL-2 conditions). It is the responsibility of the investigator to submit any viral shedding results to the EHS Biosafety Office.

- A biohazard sign indicating lentivirus vector usage must be placed on animal rooms when in use and on cages with date the animals were inoculated.
- Incorporate the above laboratory experiment precautions.
- Perform all administrations and manipulations of animals inside of BSC (Class I, IIA, IIB1 or IIB2).

- Use microisolators or containment type animal housing at ABSL-2 during the initial 72 hour shedding phase of the lentivirus or HAdV.
- Perform cage change-outs in BSC (Class I or II).
- Autoclave the animal cage with bedding intact before dumping and then discard bedding in a conventional manner. NOTE: When animal cages are transported outside the ABSL2 animal room for autoclaving they must be placed within a biohazard bag and sprayed with a virucidal solution prior to removal from the ABSL2 animal room.
- Spray cage racks with a virucidal solution before removal from a BSL-2 facility.
- Allow to air dry.