GUIDELINES FOR USE OF ADENO-ASSOCIATED VIRAL VECTORS (AAV VECTORS)

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Abstract

All laboratories using AAV vectors must adhere to this code of practice

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Purpose:

To provide guidelines for safely working with Adeno-associated virus (AAV) and recombinant Adeno-associated virus (rAAV) at the University of Lethbridge. Note that these are guidelines and all virus/vector use requires a risk assessment and review by the Biosafety Committee. Please contact the Biosafety Officer before beginning any work with AVV/rAAV vectors.

Introduction:

Recombinant AAV vectors are derived from a non-pathogenic adeno-associated virus (AAV) belonging to the *Parvoviridae* family, and can efficiently transfer genes of interest to a broad range of mammalian cell types leading to high levels of stable and long-term gene expression after a single application.

The Canadian Biosafety Standards and Guidelines (2013) defines viral vectors as "transfer vehicles used to deliver genetic material into host cells for subsequent gene expression". Recombinant viral systems are commonly used in molecular biology as research tools and gene therapy vehicles in both human and animal studies.

In order to assess the risk of a given system, it is critical to understand the parts, including viral backbone, gene or DNA inserts, packaging systems, replication competency, and the host system. A risk assessment MUST be performed for every new modification made to an existing system.

Factors Determining Biosafety Containment:

The University of Lethbridge Biosafety Committee (BC) will look at the following criteria for determining appropriate biosafety containment and handling of AAV/rAAV when reviewing risk assessments:

• Propagation with or without helper virus, including the use of adenovirus.

• Presence of transgenes encoding oncogenes or toxins.

• Propagation (insect cell lines versus human cell lines).

• Purification techniques and quality control methods used when propagation of virus occurs in human cell lines.

Specific requirements of AAV/rAAV use at CL1/CL1-Ag

The BC will consider designating adeno-associated viruses or recombinant adeno-associated viruses for use at **CL1/CL1-Ag** if:

1. Transgene **does not** encode an oncogenic protein or a toxin.
2. AAV/rAAV is generated **without** using adenovirus or any other helper virus of human origin.
3. AAV/rAAV is propagated into non-human cell lines.

Good microbiology laboratory practices should be followed.

Specific requirements of AAV/rAAV use at CL2/CL2-Ag

Adeno-associated viruses or recombinant adeno-associated viruses ***must*** be used at CL2/CL2-Ag if:

1. Transgenes encodes an oncogenic protein or toxin.
2. Helper virus of human origin is used to generate AAV/rAAV.
3. AAV/rAAV is propagated in human cell lines ***without*** further purification before use.

Laboratory practices will follow the Canadian Biosafety Standards and Guidelines

Exceptions:

AAV/rAAV are typically propagated in HEK 293 cells, a commercially available human cell line. The Alberta Occupational Health and Safety Code (2009) specifies that as of July 1, 2010 employers must ensure that a worker's exposure to bloodborne pathogens is controlled. The University of Lethbridge requires all human-derived materials to be handled using Universal Precautions and the laboratory work to be performed in CL2 facililty.

The BC will consider reducing the biosafety level to CL1/CL1-Ag on a case-by-case basis for researchers who are generating AAV/rAAV in their own laboratories when the following criteria are met and documented in the risk assessment in addition to the oncogene/toxin expression and helper virus criteria listed above:

- AAV/rAAV generated in non-human cells, or AAV/rAAV generated in human cells by a helper virus- free plasmid transfection method with subsequent purification and appropriate quality control
    - The investigator must provide details of the methodology for purification and quality control.
    - For example, purification by cesium chloride or iodixanol gradient, and/or column chromatography followed by quality control using SDS-PAGE.
    - or similar purification and quality control methods may be used to justify application for work with AAV/rAAV at CL1/CL1-Ag
- Investigators who are not generating their own viruses but are acquiring viruses from a recognized core facility should provide the method used for generating, purification, and quality control methodology from the core facility.

Special Notes:

Inventories:

Researchers must keep an inventory of biological organisms, this includes AAV/rAAV.

Disinfection:

The most effective disinfectant against AAV is a 1% Sodium hypochlorite solution that is made fresh daily. Ensure contact time of 15 minutes. To prevent corrosion rinse disinfectant off of reusable equipment and surfaces.

Disinfectant alternatives include 2% glutaraldehyde, and 0.25% sodium dodecyl sulfate (SDS)

Decontamination:

Autoclaving for 1 hour at 121°C or 250°F (15 lbs psi of steam pressure).

This decontamination method should be used for reusable equipment, liquid waste or solid waste.

Animal Practices:

1. When animals are infected with AAV vectors, appropriate animal containment will be determined by a risk assessment. Approvals are needed from the Biosafety

Committee and the Animal Welfare Committee. Containment level required may vary depending on the presence of helper virus and/or the gene inserted.

2. All bedding, waste and animals shall be treated as biohazardous. It is recommended a certified Class II biosafety cabinet be used to change out rodent cages if this is not possible appropriate controls including PPE and procedures will be followed. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal.

3. Animal carcasses must be disposed of as biohazardous waste (incinerated).

4. All animal facility personnel should be informed of the use of AAV/rAAv and any risks associated with the protocols.

References:

1. Canadian Biosafety Standards and Guidelines First Edition. 2013. Ottawa, Canada.
2. National Institutes of Health (NIH), Recombinant DNA Advisory Committee (RAC), NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
3. University of Kentucky. (n.d.). Guidelines for research involving viral vectors. Retrieved from: http://ehs.uky.edu/docs/pdf/bio_ia_uk_viral_vector_guidance_0001.pdf
4. University of Pittsburgh IBC. (n.d.). Guidance on biosafety level assignment of adeno-associated virus. Retrieved from: http://www.ibc.pitt.edu/ViralVector/IBCGuidanceAAV.pdf