Viral Vector Standard

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SCOPE
This Standard applies to all persons requiring the use of viral vectors under the auspices of the University of Calgary.

PURPOSE
This Standard sets out the minimum requirements for those working with viral vectors in order to reduce risks to health and safety.

HAZARDS
Viral vector systems are increasingly used in research laboratories because of the attractive features of the viral vector system; although newer generations of viral vectors are less hazardous than earlier versions, these viral vector systems are still considered potentially hazardous (Table 1). As such, risk assessments must be conducted prior to working with viral vectors (Figure 1).

Viral vectors have a number of hazards including, but not limited to:
- the potential for generation of replication-competent virus from the vector components
- the potential for oncogenesis
- an increase in host range (tropism) through pseudotyping or genetic modification
- expression of a transgene encoding a biological toxin, oncogene, cell cycle regulator, or an inhibitor of a tumour suppressor
- exposure through direct injection, mucous membrane, inhalation and ingestion routes of infection

RESPONSIBILITIES
Supervisors
- Follow the requirements of this Standard
- Identify on the laboratory Hazard Assessment and Control Form that work may involve viral vectors
- Conduct a risk assessment (see Figure 1) for the work involving viral vectors and communicate with affected workers including staff in the Animal Resource Centre(s)
- Ensure worksite-specific training is received and documented
- Ensure that procedures for work involving viral vectors are followed
- Conduct Replication-Competent Lentivirus (RCL) testing for 2- or 3-plasmid systems

Workers
- Follow the requirements of this Standard and any additional requirements determined by the area supervisor with regard to work involving viral vectors

Environmental Health & Safety (EHS)

- Provide information on appropriate legislation, codes, standards and best practices regarding work involving viral vectors
- Assist affected parties with interpretation of this Standard
- Review and update this Standard

Risk assessment

A risk assessment must take into account the following parameters: nature of the viral vector, nature of the insert, generation, genetic modification, replication competency, volume to be produced, in vitro vs in vivo use, disinfection (see Figure 1).

Table 1. Summary of commonly-used viral vectors

Viral Vector	Risk Group	Containment Level[a] (CL) for Administration and Housing involving animals	Suitable Animal Resource Centre Location	Disinfectant(s)
Adenoviral vector	2	CL2	CL2[b]	0.5% sodium hypochlorite; Alcohol not effective
Adeno-Associated Viral (AAV) Vector	1	CL1 (helper virus absent; not propagated in human cell lines; transgene is RG1) e.g. CL1 lab or Mouse Half-way House[c]	Designated AAV location	0.5% sodium hypochlorite; Alcohol not effective
	2	CL2 (helper virus present; propagated in human cell lines; transgene is RG2)	CL2[b]	
Epstein-Barr Viral Vector	2	CL2	CL2[b]	0.5% sodium hypochlorite
Herpes Simplex Viral (HSV-I and HSV-II) Vector	2	CL2	CL2[b]	0.5% sodium hypochlorite
Retroviral Vectors e.g. Murine Leukaemia Viral (MLV) Vector or Lentiviral Vector (four-plasmid system)	2	CL2 (ecotropic, amphotrophic or pseudotyped replication-defective murine retroviruses)	CL2 and Designated MLV location[d]	0.5% sodium hypochlorite
	2	CL2 (non-replication defective murine retroviruses; human retroviruses)	CL2[b]	

[a]Containment levels refers to the minimum physical containment and operational practices required for handling infectious material or toxins safely in laboratory and animal work environments.

[b]It is not permitted to transfer an animal, to which this biohazard was administered, from the Animal Resource Centre CL2 location to the Mouse Half-way House (MHH[c]).

[d]It is permitted to transfer an animal, to which this biohazard was administered, from the Animal Resource Centre CL2 location to the designated MLV location, no earlier than 72 hours post administration and after performing cage changing.

Replication-incompetent lentiviral testing

Testing of lentiviral vector stocks prior to use in animals is of debatable value when using vector systems that cannot possibly result in the generation of infectious HIV-1 (such as systems in which vector lacks HIV-1 Env and uses a fully heterogeneous coat protein VSV-G to wrap the vector core i.e. four-plasmid systems). If appropriate, lentiviral stocks may be tested for replication competent virus by serial transfer and ELISA assay for p24 antigen.

Example scenarios and associated risk assessments (adapted from the National Institutes for Health)

In vitro

Study A: Use of a 4-plasmid derived lentiviral vector encoding siRNA against Lck in primary human T cells.

Considerations

1. What is the amount of vector to be produced? Answer = Low (100 ml)
2. What is the nature of the vector? Answer = 4-Plasmid System
3. What is the nature of the insert? Answer = Non-oncogenic

Tentative Risk Assessment = CL2

(Note that the use of primary human cells would require CL2 independent of the vector)

In vitro

Study B: Use of a 2-plasmid derived lentiviral vector encoding luciferase in a human cell line (A549 cells).

Considerations

1. What is the amount of vector to be produced? Answer = Low (100 ml)
2. What is the nature of the vector? Answer = 2-Plasmid System (non-commercial)
3. What is the nature of the insert? Answer = Non-oncogenic

Tentative Risk Assessment = CL2 with Additional Operational Practices from Public Health Agency of Canada (see below)

In vivo

Study A: Use of a 4-plasmid derived lentiviral vector encoding brain-derived neurotrophic factor (BDNF) in mouse brain.

Considerations

1. What is the amount of vector to be produced? Answer = Low (100 ml)
2. What is the nature of the vector? Answer = 4-Plasmid System
3. What is the nature of the insert? Answer = Non-oncogenic (*: see below)
4. What is the nature of the animal host? Answer = Non-permissive for HIV-1

Tentative Risk Assessment = CL2 for lab work and injection of mice (which would probably be done using a stereotactic frame);

In vivo

Study B: Use of adeno-associated viral (AAV) vector encoding fluorescent tag in mouse brain.

Considerations

1. What is the amount of vector to be produced? Answer = Low (100 ml)
2. What is the nature of the vector? Answer = Derived from Risk Group 1 biohazard
3. What is the nature of the insert? Answer = Non-oncogenic (*: see below)
4. What is the nature of the animal host? Answer = Permissive for AAV

Tentative Risk Assessment = CL1 for lab work and initial injection of mice (which would probably be done using a stereotactic frame). Animals housed in designated AAV location.

Added explanation: Stereotactic injection frames cannot easily be placed into a Biosafety Cabinet (BSC), and may use a syringe or pulled glass pipette for inoculation; they may also use a pump to ensure a slow rate of delivery of the agent. CL2 does not require the use of a BSC, and is therefore compatible with the use of a stereotactic frame, even if that frame is not contained within a BSC.

ADDITIONAL OPERATIONAL PRACTICES FROM PUBLIC HEALTH AGENCY OF CANADA:

- All activities should be conducted in a BSC.
- A solid-front gown with tight-fitting wrists must be worn when infectious materials are directly handled and must be removed after completion of work and kept by the dedicated work area.
- Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC. If it is not possible to use a BSC, sufficient time must be allowed for aerosols to settle before safety cups or rotors are opened.

SHARPS USE AND DISPOSAL, PPE, STORAGE, EMERGENCY RESPONSE PROCEDURES AND TRAINING

As per the Laboratory Safety Rules, Laboratory Safety Program

RELATED DOCUMENTS

Animal Care and Use Policy

REFERENCES

Assessment of Hazard Risk Associated with the Intravenous Use of Viral Vectors in Rodents. Comparative Medicine. Reuter et al., 2012

Risks Associated with Lentiviral Vector Exposures and Prevention Strategies. Journal of Occupational and Environmental Medicine. Schlimgen et al., 2016

Guidelines for Research Involving Viral Vectors, Environmental Health and Safety, University of Kentucky

Minutes of the Recombinant DNA Advisory Committee, 2006, United States Department of Health and Human Services, Public Health Service, National Institutes of Health

Pathogen Safety Data Sheets and Risk Assessment, Public Health Agency of Canada

Working with Viral Vectors, Stanford University, 2013

Figure 1. Biosafety considerations when conducting a Risk Assessment