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Viral Vectors

WHAT IS A VIRAL VECTOR?

Viral vectors work like a “nanosyringe” to deliver nucleic acid to a target. They are often more efficient than other transfection methods, are useful for whole organism studies, have a relatively low toxicity, and are a likely route for human gene transfer.

All viral vectors require a host for replication. The production of a viral vector is typically separated from the ability of the viral vector to infect cells. While viral vectors are not typically considered infectious agents, they do maintain their ability to “infect” cells. Viral vectors just don’t replicate (although there are some replicating viral vectors in use) under experimental conditions. An HIV-based lentiviral vector no longer possesses the ability to infect an individual with HIV, but it does maintain the ability to enter a cell and express genetic information. This is why viral vectors are useful, but also require caution. If a viral vector can transduce a human cell line on a plate, it can also transduce YOUR cells if accidentally exposed.

SAFETY CONSIDERATIONS FOR ALL VIRAL VECTORS

When utilizing ANY viral vector, the following questions must be addressed...

- What potential does your method of viral vector production have to generate a replication competent virus?
  - The "Generation" of a viral vector refers to the number of recombination events required to form a replication competent virus. For example, if you're producing a lentivirus that is split up between 4 plasmid (gag/pol, VSV-g, rev, transgene), a total of 3 recombination events must take place in order to create a replication competent virus, therefore you are using a 3rd generation lentiviral vector.
- What specific hazard(s) does the transgene pose?
  - If your transgene is GFP, the hazard is relatively low. If your transgene is hazardous (i.e. an oncogene, toxin, immunogen, allergen, gene drive, etc.), the hazard is much greater. Consider what your transgene would do to YOU should you be exposed. Does your viral vector overexpress an oncogene? Knock down a tumor suppressor? What does your transgene DO?
- What is the tropism of the virus?
  - Ecotropic viral vectors have a narrow host range and can only infect one or a small group of species or cell lines. Amphotropic viral vectors have a wide host range and are capable of infecting numerous species or cell lines.

Replication Competent Virus (RCV) Testing

Most viral vectors used today are disabled such that replication competent viruses are not readily formed by any biological process that might occur in normal hosts. The Department of Biological Safety encourages the use of such vectors in all relevant applications. In particularly sensitive applications, demonstrating that the viral stock used has no apparent contamination with replication competent vectors is essential. The issue is not whether replication competent virus (RCV) is present, but how much replication competent virus is present. Of course, assays for replication competence will never be perfect or absolute, so the Institutional Biosafety Committee (IBC) asks that one use a current procedure of demonstrated sensitivity and specificity. Even more rigorous testing may be required in some instances, such as a vector bearing a pathogenic gene, in human gene transfer, or in any materials that could be released to the environment.

WHEN IS RCV TESTING REQUIRED*?

- Use of ANY viral vector (regardless of generation) in Human gene transfer/therapy where replication-defective viral vector or products containing replication-defective viral vector is administered to human study participants
- Use of 1st or 2nd generation viral vectors in animals (*in vivo*)
- Use of cells transduced with 1st or 2nd generation viral vectors in animals (*in vivo*)

WHEN IS RCV TESTING NOT REQUIRED*?

- Experiments conducted entirely in cell or tissue culture (*in vitro*)
- Use of 3rd generation (or higher) viral vectors in animals (*in vivo*)
- Use of cells transduced with 3rd generation (or higher) viral vectors in animals (*in vivo*)

CONSIDERATIONS FOR AN APPROPRIATE RCV TEST:

- The test is an experiment
  - Any experiment should have adequate controls. A positive control is necessary to demonstrate that you would have detected replication competent virus if it were present.
- The test must be quantitative
  - What is the level of detection for replication competent virus? Typically, this requires running a dilution curve. This should should also be reported with results of your test.
- What is an appropriate cut-off for declaring it is afe to use this virus in animals?

SETTING AN APPROPRIATE CUT-OFF:

- FDA requirements for human use of recombinant adenovirus is <1 replication competent virus per 10^13 viruses
  - Very strict requirement because quantity of virus used is high, containment is very difficult, and accidental release is potentially disastrous.
- When working with small, easily sequestered animals (i.e. rats, mice), the cut-off can be considerably less
  - One replication competent virus per 10^6 viruses is common, but is it appropriate?
    - How many virus particiles are being introduced into animals?
    - Best estimate for number of replication competent virus in bolus?
    - How many animals are likely to receive replication competent virus?
    - What is an acceptable risk?

*The UK IBC will typically require RCV testing according to the guidelines listed above. Requirements may change depending on the specific genetic constructs in use.

Replication Competent Virus (RCV) Testing

CONSIDERATIONS WHEN DESIGNING THE TEST:

- What does a recombinant virus need to regain replication competency?
  - Adenovirus needs E1
  - Lentivirus needs gag, pol, env
- Where can recombinant virus pick up the sequences needed to regain replication competency?
  - Adenovirus has easy access to E1 in HEK293 cells
  - Retroviruses may pick up "assets" from endogenous retroviruses
- How does a replication competent virus present itself?

METHODOLOGICAL APPROACHES:

- Plaque assays (for lytic viruses, i.e adenovirus*)
  - Must screen for more viruses than the cut-off limit
    - If ,1 in 10^6 is the cut-off, must screen >10^6 viruses
- ELISA for production of viral protein essential for replication
  - p24 assay for HIV (lentivirus)
  - Sensitivity poor
  - Attempt amplification in a competent host
- Quantitative PCR for an essential viral gene
  - Very sensitive
  - Problem with background

THINGS TO REMEMBER:

- Confirmation of absence of RCV must be documented by researcher PRIOR to use
  - Documentation of methodology and results must be made available to Department of Biological Safety staff on request
- Procedure must be of demonstrated sensitivity and specificity
- Must use a positive control

USE OF CELLS TRANSDUCED BY VIRAL VECTORS IN ANIMALS:

- Cells transduced with 3rd generation (or higher) viral vectors must be washed a minimum of three (3) times prior to administration to animals.
  - Animals may be housed at ABSL1 containment
- RCV testing is required prior to the use of cells transduced with 1st or 2nd generation viral vectors in animals.
  - Cells must be washed a minimum of three (3) times prior to administration to animals
  - Animals must be housed at ABSL2 containment for 72 hours post administration
  - Animals may be moved to ABSL1 containment after 72 hours post administration

Replication Competent Virus (RCV) Testing

Virus	Method	Reference
Adenovirus	Test for RCV by PCR for E1a	Dion LD, Fang J, Garver RI Jr. Supernatant rescue assay vs. polymerase chain reaction for detection of wild type adenovirus-contaminating recombinant adenovirus stocks.*J Virol Methods*. 1996;56(1):99-107. doi:10.1016/0166-0934(95)01973-1
Adeno-Associated Virus (w/ Adenovirus Helper)	Test for RCV by PCR for E1a	Hehir KM, Armentano D, Cardoza LM, et al. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence.*J Virol*. 1996;70(12):8459-8467. doi:10.1128/JVI.70.12.8459-8467.1996
Retrovirus (Amphotrophic & Ecotrophic)	Test for RCV by amplification in permissive cell line followed by screening by appropriate detection assay	Wilson CA, Ng TH, Miller AE. Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors.*Hum Gene Ther*. 1997;8(7):869-874. doi:10.1089/hum.1997.8.7-869 Uchida E, Sato K, Iwata A, et al. An improved method for detection of replication-competent retrovirus in retrovirus vector products.*Biologicals*. 2004;32(3):139-146. doi:10.1016/j.biologicals.2004.08.002 U.S. Food and Drug Administration. Center for Biologics Evaluation and Research. Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. January 2020. Accessed February 28, 2022. https://www.fda.gov/media/113790/download
Lentivirus	Test for RCV by PCR for psi-gag and VSV-G sequences	Sastry L, Xu Y, Johnson T, et al. Certification assays for HIV-1-based vectors: frequent passage of gag sequences without evidence of replication-competent viruses.*Mol Ther*. 2003;8(5):830-839. doi:10.1016/j.ymthe.2003.08.003

Adenovirus

GENERAL DESCRIPTION:

There are more than 49 immunologically distinct types of adenovirus that can cause infection. Recombinant adenoviruses used for biomedical research are typically based on Adenovirus 5. These are linear, non-enveloped, icosahedral, double-stranded DNA viruses of approximately 36kb with a lytic infection cycle.

Virus packaged via transfection of HEK293 cells are capable of transfecting human cells. Deletion of E1 renders the virus replication incompetent. Deletion of E3 allows for larger inserts. Because recovery of E1 is the only recombination event required to create a replication competent virus, all adenoviral vectors are 1st generation.

POTENTIAL HEALTH HAZARDS:

Adenovirus is a pathogen of respiratory, gastrointestinal mucosa, and mucous membranes. Symptoms of respiratory illness resulting from adenovirus infection can range from asymptomatic disease, common cold, pneumonia, croup, and bronchitis. Additional clinical symptoms include conjunctivitis (pink eye), cystitis, gastroenteritis (stomach flu), tonsillitis, rash-associated illness, and rare cases of severe disease (especially in immune compromised individuals). Adenoviral vectors DO NOT have to be replication competent to cause corneal and conjunctival damage.

LABORATORY HAZARDS:

- Routes of exposure include inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.
- Adenovirus is unusually stable in the environment. Adenovirus can still be infective after having been extracted with ether and/or chloroform. Adenovirus can persist for 7 days to 3 months on dry inanimate surfaces.
- Potential recovery of E1 from HEK293 cells to produce replication competent virus.
- No specific treatment for adenovirus infection.

BIOSAFETY CONTAINMENT:

- BSL2+ containment
- NO open bench work
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required
- When centrifuging adenovirus, rotors/buckets must be loaded/unloaded in the BSC and wiped down with appropriate disinfectant prior to removal from BSC
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped
- Limit use of needles, syringes, and other sharp objects

ANIMAL BIOSAFETY CONTAINMENT:

- Adenoviral vector must be administered under BSL2/ABSL2 containment.
- Adenoviral vector stocks must be tested for RCV prior to use directly in animals or in transduced cells administered to animals.
- Animals may shed/excrete adenovirus for some time post-administration. Animals must be housed at ABSL2 containment for a minimum of 72 hours during this period, after which animals may be moved to ABSL1 housing.

DISINFECTION:

- Susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure
- Freshly prepared 10% household bleach recommended
- Alcohol is NOT an effective disinfectant against adenovirus

REFERENCES:

Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy.*Curr Gene Ther*. 2013;13(6):421-433. doi:10.2174/15665232133666131125095046

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/adenovirus-types-1-2-3-4-5-7-pathogen-safety-data-sheet.html

Adeno-Associated Virus

GENERAL DESCRIPTION:

Adeno-Associated Virus (AAV) is coined as such because it is most often found in cells that are simultaneously infected with adenovirus. AAV are parvoviridae, icosahedral, single-stranded DNA viruses with a protein capsid. Wild typed adenovirus or herpesvirus must be present for AAV to replicate. If these helper viruses are not present, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic infection cycle. AAV has a broad host range and produces little to no immune response. At only 22nm in diameter, it is one of the smallest viruses known. There are at least 11 natural serotypes of AAV. AAV2 is the basis for most recombinant AAV vectors, but it is usually pseudotyped.

POTENTIAL HEALTH HAZARDS:

There are no known health hazards associated with AAV. It is not known to cause direct disease in humans; however, AAV may be associated with insertional mutagenesis and cancer, thereby making AAV possibly not as safe as previously thought. The low immunogenicity of AAV leads to long-term gene expression, the effects of which are not entirely understood.

LABORATORY HAZARDS:

- Routes of exposure include inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.
- No specific treatment for infection with AAV.

BIOSAFETY CONTAINMENT:

- Construction of AAV with helper virus (Adenovirus or Herpesvirus) must be performed at BSL2 within a BSC.
- Once constructed, AAV may be manipulated at BSL1.
- Eye protection, disposable gloves, laboratory coat required.

ANIMAL BIOSAFETY CONTAINMENT:

- Animal housing may be maintained at ABSL1.
- ABSL2 containment is required if helper virus is present

DISINFECTION:

- Susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 5% Phenol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure
- Freshly prepared 10% household bleach recommended
- Alcohol is NOT an effective disinfectant against AAV

REFERENCES:

Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy.*BioDrugs*. 2017;31(4):317-334. doi:10.1007/s40259-017-0234-5

BACULOVIRUS

GENERAL DESCRIPTION:

Baculoviruses are lytic DNA viruses that are primarily pathogenic for insects. The nucleocapsids of Baculoviruses are rod-shaped and enveloped, with circular genomes of double-stranded DNA, ranging in size from 80-180kbp. Baculoviruses produce two distinct types of virions: occlusionderived virions (ODV), embedded in large protein crystals called occlusion bodies, and budded virions (BV). ODV are responsible for horizontal transmission between insects, whereas BV help spread infection from cell to cell. There have been more than 500 baculovirus isolates identified based on hosts of origin. Apart from their utility as gene expression vectors, they are also useful as biological pesticides. The two most common isolates used for gene expression are Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) and Bombyx mori (silkworm) nuclear polyhedrosis virus (BmNPV).

POTENTIAL HEALTH HAZARDS:

Non-genetically modified, wild-type baculoviruses are typically not capable of replicating in vertebrate cells, and therefore do not pose much risk to laboratory personnel. Baculoviruses for gene expression which utilize polyhedrin or p10 promoters will only transfect insect cells. Baculoviruses that have been engineered with mammalian specific promoters do achieve expression of foreign genes in mammalian cell lines and primary cell cultures.

LABORATORY HAZARDS:

- The budded virions (BV) are not infectious to insect hosts, minimizing potential spread to the environment.
- Baculovirus is very sensitive to human complement. Should exposure occur, rapid inactivation of the virus is expected.
- Pseudotyping with VSV-G may confer resistance to complement inactivation.

BIOSAFETY CONTAINMENT:

- Baculoviruses with insect specific promoters (i.e. polyhedrin or p10) may be handled at BSL1.
- Baculoviruses with mammalian specific promoters must be handled at BSL2.
- Eye protection, disposable gloves, laboratory coat required.

ANIMAL BIOSAFETY CONTAINMENT:

- Baculoviruses with mammalian specific promoters must be administered under BSL2 containment.
- Animals may be housed at ABSL1 containment.

DISINFECTION:

- Susceptible to: 70% Ethanol, 0.5% Sodium hypochlorite, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach recommended

REFERENCES:

Ono C, Okamoto T, Abe T, Matsuura Y. Baculovirus as a Tool for Gene Delivery and Gene Therapy.*Viruses*. 2018;10(9):510. Published 2018 Sep 19. doi:10.3390/v10090510

EPSTEIN-BARR VIRUS

GENERAL DESCRIPTION:

Epstein-Barr virus (EBV) is a ubiquitous B-lymphotrophic herpesvirus. EBV causes the common childhood disease mononucleosis. It is an icosahedral, lipid enveloped, double-stranded DNA virus sized 120-150 nm in diameter. EBV has been found in the tumor cells of a heterogeneous group of malagnancies (i.e. Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's Disease, nasopharyngeal carcinoma, gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma). 80-90% of adults worldwide are infected with EBV. Most wild-type EBV infections are asymptomatic and acquired during childhood, with symptoms indistinguishable from other childhood acute viral syndromes.

POTENTIAL HEALTH HAZARDS:

- Infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy; lasting one to several weeks; rarely fatal.
- Burkitt's lymphoma - monoclonal tumors of B cells; typically involving children; jaw involvement also common; hyperdemic in highly malarial areas.
- Nasopharyngeal carcinoma - malignant tumor of epithelial cells of the nasopharynx; usually involving adults between 20 and 40 years of age.
- In immunosuppressed individuals, oral hairy leukoplasia, interstitial lymphocytic pneumonia, B-cell or T-cell lymphomas, and mesenchymal lymphomas may occur.

LABORATORY HAZARDS:

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.
- Cell lines are often immortalized by transformation with EBV.

BIOSAFETY CONTAINMENT:

- BSL2
- NO open bench work
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required
- When centrifuging EBV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

ANIMAL BIOSAFETY CONTAINMENT:

- EBV vectors must be administered under BSL2/ABSL2 containment.
- Animals must be housed under ABSL2 containment.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended

REFERENCES:

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/epstein-barr-virus.html

Kazuyuki Kiyosue, Yoshihiro Miwa, Epstein-Barr virus-derived vector suitable for long-term expression in neurons, Heliyon, Volume 6, Issue 3, 2020, e03504, ISSN 2405-8440, https://doi.org/10.1016/j.heliyon.2020.e03504.

HERPESVIRUS

GENERAL DESCRIPTION:

Herpes Simple Virus (Types I and II) are icosahedral, lipid enveloped, double-stranded linear DNA viruses approximately 110-200nm in diameter. HSV types I and II can be differentiated immunologically. HSV-I is herpes gingivostomatitis; whereas HSV-II is herpes genitalis, or genital herpes. HSV-derived vectors are unique in that the vectors have a wide host range and cell tropism in dividing and non-dividing cells, and are able to infect almost every cell type in most vertebrates. HSV has a dual life cycle – a lytic growth cycle in epithelial cells and latent infection of neuronal cells. This latency in neuronal cells leads to persistent, long-term expression.

POTENTIAL HEALTH HAZARDS:

- Oral herpes - primary infection is typically mild and occurs early in childhood; reactivation of latent infection results in fever blisters or cold sores, usually on the face and lips, which crust and heal within a few days; possible CNS involvement (meningoencephalitis), 70% mortality rate if left untreated; causes approximately 2% of acute pharyngotonsilitis.
- Genital herpes - sexually transmitted, associated with aseptic meningitis; vaginal delivery may pose risk to newborn (encephalitis and death).
- Both HSV-I and HSV-II are capable of infected the genital tract or oral mucosa.
- Latency and reactivation from latency are not well understood.

LABORATORY HAZARDS:

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.
- Only treatment available is anti-viral drug therapy for symptoms.

BIOSAFETY CONTAINMENT:

- BSL2
- NO open bench work
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required
- When centrifuging HSV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

ANIMAL BIOSAFETY CONTAINMENT:

- HSV vectors must be administered under BSL2/ABSL2 containment.
- Animals must be housed under ABSL2 containment.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended

REFERENCES:

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/herpes-simplex-virus.html

Burton EA, Fink DJ, Glorioso JC. Gene delivery using herpes simplex virus vectors.*DNA Cell Biol*. 2002;21(12):915-936. doi:10.1089/104454902762053864

POXVIRUSES

GENERAL DESCRIPTION:

The Poxviridae family is divided into two subfamilies: Chordopoxviridae, with a vertebrate host range, and Entomopoxviridae, with an insect host range. Chordopoxviridae is further broken down into eight genera: Avipoxvirus, Capripoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus, Suipoxvirus, and Yatapoxvirus.

Poxviruses are enveloped, with a double-stranded DNA genome with hairpin loops at each end and a lytic infection cycle. Poxviruses do not integrate into the hosts' genome because they remain in the cytoplasm and utilize virally encoded polymerases to carry out replication and transcription. Members of the Orthopoxvirus genus have both narrow and broad host range. Variola, the agent of smallpox, only infects humans. The absence of other host species has made the eradication of smallpox possible. On the other hand, Vaccinia virus has a very broad host range. Vaccinia is used as a live vaccine for protection against smallpox. Vaccinia's large genome (approximately 190kb) allows for the stable insertion of DNA as large as 25kb.

POTENTIAL HEALTH HAZARDS:

Unlike many viral vectors utilized, vaccinia is a replication competent vector. Vaccinia virus presents varying levels of health risk to laboratory personnel, depending on the strain utilized. Highly attenuated strains are typically unable to replicate or replicate poorly in human cells. Non-highly attenuated strains can replicate in human cells and pose a health risk. The classical symptom of poxvirus infection is a vesicular or pustular lesion on the skin at the inoculation site. Vaccinia can cause severe disease in people with active skin disorders (i.e. eczema, psoriasis), pregnant women, and immune compromised individuals.

LABORATORY HAZARDS:

- Ingestion, parenteral inoculation, droplet or aerosol exposure to mucous membranes or exposure to broken skin.
- Vaccinia and other poxviruses are stable at ambient temperatures when dried and can remain infectious for long periods of time.

BIOSAFETY CONTAINMENT:

- BSL2 for the following strains: MVA (Modified Vaccinia Ankara), WR (Western Reserve), and NYCBOH (used in vaccinia vaccine), Copenhagen, Temple of Heaven, Lister, Cowpox, Monkeypox.
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required
- BSL1 for the following strains: NYVAC (derived from Copenhagen), TROVAC (Fowlpox virus), and ALVAC (Canarypoxvirus)
- Eye protection, disposable gloves, laboratory coat required

ANIMAL BIOSAFETY CONTAINMENT:

- Animals must be manipulated and housed under BSL2/ABSL2 containment.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended

REFERENCES:

https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/vaccinia-virus.html

GuoZS,LuB,GuoZ, *et al*Vaccinia virus-mediated cancer immunotherapy: cancer vaccines and oncolytics*Journal for ImmunoTherapy of Cancer*2019;**7:**6.doi:10.1186/s40425-018-0495-7

RETROVIRUSES/MURINE LEUKEMIA VIRUS

GENERAL DESCRIPTION:

Murine Leukemia Virus (MLV) is an enveloped, icosahedral, single-stranded virus with a linear RNA genome, approximately 100nm in diameter. MLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection.

The host range of MLV is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles that infect only rodent cells. Amphotropic env gene allows infection of both murine and non-murine cells, including human. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

POTENTIAL HEALTH HAZARDS:

Recent data suggests a pathogenic mechanism by which chronic productive retroviral infection allows insertional mutagenesis leading to cell transformation and tumor formation. The nature of the transgene or additional introduced genetic element(s) may pose additional risk. The provirus integrates randomly into the genome which can lead to inactivation of genes for protein expression. The 5' and 3' LTRs have promoter functions that can deregulate the expression of genes.

LABORATORY HAZARDS:

- In mice, virus is transmitted via blood from infected mother to offspring; may also occur via germline infection.
- In vivo infection in humans appears to require direct parenteral injection with amphotrophic or pseudotyped MLV.
- Exposures associated with a hazardous transgene (i.e. an oncogene, toxin, etc.) should consider the use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

BIOSAFETY CONTAINMENT:

- BSL1 containment for ecotropic MLV demonstrated to be replication incompetent.
- Eye protection, disposable gloves, laboratory coat required.
- BSL2 containment for amphotrophic or pseudotyped MLV.
- Biological Safety Cabinet (BSC) required.
- Eye protection, disposable gloves, laboratory coat required.
- When centrifuging MLV, rotors/buckets must be loaded/unloaded within BSC and wiped down with appropriate disinfectant prior to being from from BSC
- Centrifuge tubes must be sealed (i.e. plates sealed with parafilm) or capped.

ANIMAL BIOSAFETY CONTAINMENT:

- MLV vector must be administered under BSL2/ABSL2 containment.
- Animals administered ecotropic MLV demonstrated to be replication incompetent by acceptable RCV testing may be housed under ABSL1 containment.
- Animals administered amphotrophic/pseudotyped MLV must be housed under ABSL2 conditions for 72 hours post administration, after which animals may be moved to ABSL1 housing.*See page 3 for RCV testing guidelines.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended

REFERENCES:

Armin Blesch, Lentiviral and MLV based retroviral vectors for ex vivo and in vivo gene transfer, Methods, Volume 33, Issue 2, 2004, Pages 164-172, ISSN 1046-2023, https://doi.org/10.1016/j.ymeth.2003.11.005

LENTIVIRUS

GENERAL DESCRIPTION:

The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. There are five (5) serotypes recognized, based upon the mammalian hosts with which they are associated: Bovine, Equine, Feline, Ovine/Caprine, and Primate.

Most lentiviral vectors in use today are HIV-derived vectors. The cis- and trans- acting factors of the lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. The vector constructs contain the viral cis elements, packaging sequences, the Rev response element (RRE), and a transgene. Lentiviral vectors can transfect dividing and non-dividing cells. Replacing the HIV envelope glycoprotein with VSV-G allows a broad host-range for the vectors, allows the viral particles to be concentrated via centrifugation, and alters the mode of transmission.

POTENTIAL HEALTH HAZARDS:

Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, and sharing unclean needles. Lentiviruses persist lifelong – being both a function of their ability to integrate into the host chromosome and ability to evade host immunity. Lentiviruses replicate, mutate, and undergo selection by host immune responses. The clinical manifestations of infection include non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue. The use of lentiviruses also present the risk of insertional mutagenesis, potentially leading to cancer. The nature of the transgene may pose additional risk.

LABORATORY HAZARDS:

- Direct contact with skin and mucous membranes, parenteral inoculation, or ingestion.
- Exposures associated with a hazardous transgene (i.e. an oncogene or toxin) should consider use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

BIOSAFETY CONTAINMENT:

- BSL2+ containment
- NO open bench work
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required
- When centrifuging lentivirus, rotors/buckets must be loaded/unloaded in the BSC and wiped down with appropriate disinfectant prior to removal from BSC
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped
- Limit use of needles, syringes, and other sharp objects

ANIMAL BIOSAFETY CONTAINMENT:

- Lentivirus must be administered under BSL2/ABSL2 containment.
- Animals must be housed under ABSL2 containment for 72 hours post administration, after which animals may be moved to ABSL1 containment.
- 1st or 2nd generation Lentiviral vectors stocks must be tested for RCV prior to use directly in animals or in transduced cells administered to animals.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol, or Autoclave for 30 minutes at 121C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended

REFERENCES:

Milone, M.C., O'Doherty, U. Clinical use of lentiviral vectors.*Leukemia* 32,1529–1541 (2018). https://doi.org/10.1038/s41375-018-0106-0