Biological Safety

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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 transfect a human cell line on a plate, it can also transfect YOUR cells if accidentally exposed.

SAFETY CONSIDERATIONS FOR ALL VIRAL VECTORS

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

1. What potential does your method of viral vector production have to generate a replication competent virus?
    a. Generation of viral vector refers to the number of recombination events required to form a replication competent virus. For example, if you're using a lentivirus that is split up between 4 plasmids (gag/pol, VSV-g, rev, transgene), 3 recombination events must take place to create a replication competent virus, therefore you are using a 3rd generation lentiviral vector.
2. What specific hazard(s) does the transgene pose?
    a. If your transgene is GFP, the hazard is relatively low. If your transgene is KRAS (an oncogene), the hazard is much greater.
3. What is the tropism of the virus?
    a. 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.

USE OF CELLS TRANSFECTED BY VIRAL VECTORS IN ANIMALS:

While testing for Replication Competent Virus (RCV) is required for viral vector use in animals, special dispensation is given to transduced cells under specific conditions. Human or animal cells transfected with 3rd generation (or greater) viral vectors must be washed three (3) times prior to administration to animals (at minimum), after which animals may be housed at ABSL1 containment. RCV testing is required prior to use of human or animal cells transfected with 1st or 2nd generation viral vectors in animals. Animals administered cells transfected with 1st or 2nd generation viral vectors must be housed at ABSL2 for 72 hours post administration, after which they may be moved to ABSL1.

ADENOVIRUS

GENERAL DESCRIPTION:

There are more than 49 immunologically distinct types of adenovirus that can cause infection. Recombinant adenoviruses used for biomedical research are 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.

POTENIAL HEALTH HAZARDS:

Adenovirus is a pathogen of respiratory, gastrointestinal mucosa, and mucous membranes. Symptoms of respiratory illness resulting from adenovirus infection can range from the common cold to 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.
- Potential recovery of E1 from HEK293 cells to produce replication competent virus.

BIOSAFETY CONTAINMENT:

- BSL2+.
- 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.
- Centrifuge tubes must be sealed (i.e. plates sealed with Parafilm) or capped.

ANIMAL BIOSAFETY CONTAINMENT:

- Adenoviral vector must be administered under BSL2 containment.
- Animals may shed/excrete adenovirus for some time post-administration. Animals must be housed at ABSL2 containment for 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 121°C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach recommended.
- Alcohol is NOT an effective disinfectant against adenovirus.

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.

POTENIAL 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:

- Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.
- No specific treatment for infection with AAV.

BIOSAFETY CONTAINMENT:

- Construction of AAV with helper virus should be performed at BSL2 within BSC.
- Once constructed, AAV may be manipulated at BSL1.
- PPE required for manipulation at BSL1 or BSL2: eye protection, lab coat, disposable gloves.

ANIMAL BIOSAFETY CONTAINMENT:

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

DISINFECTION:

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

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: occlusion-derived 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).

POTENIAL 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 increased 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.
- PPE required for manipulation at BSL1 or BSL2: eye protection, lab coat, disposable gloves.

ANIMAL BIOSAFETY CONTAINMENT:

- Baculovirus 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 121°C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach recommended.

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.

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.

LABORATORY HAZARDS:

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

BIOSAFETY CONTAINMENT:

- BSL-2.
- **NO** open-bench work.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- 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 vector must be administered under BSL-2 containment.
- Animals must be housed under ABSL-2 containment.

DISINFECTION:

- EBV is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) recommended.

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.

POTENIAL HEALTH HAZARDS:

- Oral herpes – primary infection is typically mild and occurs in early 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 infecting the genital tract or oral mucosa.
- Latency and activation from latency are not well understood.

LABORATORY HAZARDS:

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

BIOSAFETY CONTAINMENT:

- BSL-2.
- **NO** open-bench work.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- 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 must be administered under BSL-2 containment.
- Animals must be housed under ABSL-2 containment.

DISINFECTION:

- HSV is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

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.

POTENIAL HEALTH HAZARDS:

Unlike other 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.
- PPE Required for BSL2 work: eye protection, disposable gloves, and laboratory coat in addition to BSC.
- BSL1 for the following strains: NYVAC (derived from Copenhagen), TROVAC (Fowlpox virus), and ALVAC (Canarypoxvirus).
- PPE required for BSL1 work: eye protection, disposable gloves, and laboratory coat.

ANIMAL BIOSAFETY CONTAINMENT:

- Animals must be manipulated and housed under BSL2 containment.

DISINFECTION:

- Poxviruses are susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

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.

POTENIAL 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 appear to require direct parenteral injection with amphotropic or pseudotyped MLV.
- Exposures associated with a hazardous transgene (i.e. an oncogene or toxin) should consider the use of an antiretroviral agent (reverse transcriptase and integrase inhibitors, not protease inhibitors).

BIOSAFETY CONTAINMENT:

- BSL1 containment for ecotropic MLV demonstrated replication incompetent.
- PPE required for BSL1 work: eye protection, lab coat, disposable gloves.
- BSL2 containment for amphotropic or pseudotyped MLV.
- Biological Safety Cabinet (BSC) required.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging MLV, rotors/buckets must be loaded/unloaded within 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:

- MLV vector must be administered under BSL2 containment.
- Animals administered ecotropic MLV may be housed under ABSL1 conditions.
- Animals administered amphotropic/pseudotyped MLV must be housed under ABSL2 conditions for 72 hours post administration, after which animals may be moved to ABSL1 housing.

DISINFECTION:

- MLV is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

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.

POTENIAL 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 injection, 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:

- BSL-2+.
- **NO** open-bench work.
- Biological Safety Cabinet (BSC) required.
- Eye protection, disposable gloves, laboratory coat required in addition to BSC.
- When centrifuging Lentivirus, 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:

- Lentivirus must be administered under BSL-2 containment.
- Animals must be housed under ABSL-2 containment for 72 hours post administration, after which animals may be moved to ABSL1 containment.

DISINFECTION:

- Lentivirus is susceptible to: 0.5% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, iodine solutions containing ethanol.
- Freshly prepared 10% household bleach (0.5% sodium hypochlorite) is recommended.

REPLICATION COMPETENCY 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 Biosafety 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.

In general, the IBC will require the use of such an assay whenever viruses or virus-infected cells are used in whole animals. 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. Replication competency testing is not generally required if experiments are conducted entirely in tissue culture.

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 also be reported with the results of your test.
- What is an appropriate cut-off for declaring it is safe to use this virus in animals?

SETTING AN APPROPRIATE CUT-OFF…

- FDA requirements for human use of recombinant adenovirus are less than 1 replication competent virus per 10^{13} viruses
  - This is a very strict requirement because the quantity of virus used is high, containment is very difficult, and accidental release 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 this appropriate?
    - How many virus particles are being introduced into an animal?
    - Best estimate for number of replication competent virus in that bolus?
    - How many animals are likely to receive replication competent virus?
    - What is an acceptable risk?

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?

REPLICATION COMPETENCY TESTING

METHODOLOGICAL APPROACHES

- Plaque Assays (for lytic viruses, i.e. adenovirus)*
  - Must screen 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

*Don't use a permissive cell line (i.e. HEK293 cells contain the E1a region necessary for adenovirus to replicate)

THINGS TO REMEMBER…

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

VIRUS	METHOD	REFERENCE
ADENOVIRUS	Test for RCV by PCR for E1a	Dion DL, Fang J, Garver RI. 1996 Supernatant rescue assay vs. polymerase chain reaction for detection of wild type adenovirus-contaminating recombinant adenovirus stocks. J Virol Methods 56:99-107
ADENO-ASSOCIATED VIRUS (W/ ADENOVIRUS HELPER)	Test for RCV by PCR for E1a	Hehir KM, Armentano D, Cardoza LM, et al. 1996. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. J Virol 70:8459-8467.
RETROVIRUS (ECOTROPHIC & AMPHOTROPHIC)	Test for RCV by amplification in permissive cell line followed by screening by appropriate detection assay	Wilson, C.A., Ng, T. H., and Miller, A. E., 1997. Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors. Human Gene Therapy, 8(7): 869-874. Uchida E, Sato K, Ishii-Watabe A, et al. 2004. An improved method for detection of replication-competent retrovirus in retrovirus vector products. Biologicals. 32(3): 139-46.
LENTIVIRUS	Test for RCV by PCR for psi-gag and VSV-G sequences	Sastry, L, Xu, Y, Johnson, T, et al. 2003. Certification Assays for HIV-1-Based Vectors: Frequent Passage of Gag Sequences without Evidence of Replication-Competent Viruses. Molecular Therapy 8(5): 830-839.