

Guidelines for Research Involving Viral Vectors



Introduction:

Viral vectors have become a fundamental tool among molecular biology researchers. It is important to understand how these tools came about and the potential implications of their use. The most common viral vectors are outlined below, including information on virology, laboratory hazards, biological safety containment procedures, appropriate Personal Protective Equipment (PPE), disinfection, and animal use.

General Containment:

Each vector system is listed with a suggested biological safety containment level. A higher-containment level may be required in specific cases, depending on the specific properties of the vector and/or insert. The biological safety containment level is ultimately determined by the Institutional Biosafety Committee (IBC) and/or Biological Safety Officer (BSO).

Viral Vectors & Recombinant Nucleic Acids:

All work involving recombinant nucleic acids must be approved and/or registered with the IBC/BSO as required by University of Kentucky policy and the National Institutes of Health (NIH). Because the University receives funding from NIH grants for research involving recombinant nucleic acids, ALL research conducted at the University must comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and University policies.

In many cases, the [NIH Guidelines](#) and the [Recombinant DNA Advisory Committee \(RAC\) guidance document](#) on lentiviral vectors explicitly state the containment level.

- NIH Guidelines, Section III-D-3: Recombinant viruses in tissue culture
- NIH Guidelines, Section III-D-4-a: Recombinant viruses in animals
- NIH Guidelines, Appendices B-II-D through B-IV-D: Risk Group Classification of Various Viruses

The default biological safety containment level for recombinant viruses is BSL 2 / ABSL 2; however, there are exceptions.

- A lower biological safety containment level may suffice for incomplete viruses cultured in vitro.
- A few animal and human viruses qualify for lower biological safety containment.
- Animals with recombinant viruses which ordinarily require BSL 2 containment may be down-graded to ABSL 1 if and when animals are considered to be no longer shedding virus.

Viral Vectors as Infectious Agents:

Viral vectors which are commercially acquired **and** 3rd generation or higher are not considered infectious agents by the University of Kentucky IBC. Viral vectors which are not commercially acquired and/or which are 1st or 2nd generation are considered to be "potentially infectious", and therefore must be registered as infectious agents.

Use of Cells Transfected by Viral Vectors in Animals:

Human or animal cells transfected with 3rd generation (or greater) viral vectors should be washed three (3) times prior to administration, after which, animals injected with these cells may be housed as ABSL-1 containment.

University of Kentucky
Department of Biological Safety



Adenovirus Vectors



University of Kentucky
Department of Biological Safety

General Description

There are more than 49 immunologically distinct types of human adenovirus that can cause infection. Recombinant adenoviruses used for biomedical research are based on Adenovirus 5. These are medium-sized (90-100 nm), non-enveloped, icosahedral, double-stranded DNA viruses.

Virus packaged via transfection of HEK 293 cells with adenoviral-based vectors are capable of infecting human cells. The probability of producing replication competent adenovirus (RCA) increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells.

Potential Health Hazards

Adenovirus is a pathogen of respiratory, gastrointestinal mucosa and mucous membranes. The symptoms of respiratory illness resulting from adenovirus 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 those with compromised immune systems.

Adenoviral vectors **do not** have to be replication competent to cause corneal and conjunctival damage.

LABORATORY HAZARDS:

- 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.

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 adenovirus, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.

ANIMAL BIOSAFETY CONTAINMENT:

- Adenoviral vector must be administered under ABSL-2 containment with the use of a BSC.
- Animals may shed/excrete adenovirus for some time post-administration. Animals must be housed under ABSL-2 conditions for 72 hours during this period, after which animals may be moved to ABSL-1 housing.

DISINFECTION:

- Adenovirus 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 (0.5% Sodium hypochlorite) recommended.
- Alcohol **NOT** effective disinfectant against adenovirus.

Additional Information:

Public Health Agency of Canada—[Adenovirus MSDS](#)



Adeno-Associated Virus



University of Kentucky
Department of Biological Safety

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, 20-25 nm in diameter, single-stranded DNA viruses with a protein capsid. Wild type adenovirus or herpesvirus must be present in order 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 cycle.

Potential Health Hazards

There are no known health hazards associated with AAV. AAV 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.

LABORATORY HAZARDS:

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

BIOSAFETY CONTAINMENT:

- Construction of AAV should be performed at BSL-2 within BSC.
- Once constructed, AAV may be manipulated at BSL-1.
- PPE required for manipulation at BSL-1 OR BSL-2: eye protection, lab coat, disposable gloves.

ANIMAL BIOSAFETY CONTAINMENT:

- Animal housing must be maintained at ABSL 1.
- ABSL 2 if helper virus is present.

DISINFECTION:

- Adeno-Associated virus (AAV) susceptible to: 0.5% Sodium hypochlorite, 2% Glutaraldehyde, 0.25% sodium dodecyl sulfate, or Autoclave for 30 minutes at 121°C under 15 lbs per square inch of steam pressure.
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) recommended.
- Alcohol **NOT** effective disinfectant against AAV.

Additional Information:

[NIH Guidelines Appendix B-I: Risk Group 1 Agents](#)

Epstein-Barr Virus



University of Kentucky
Department of Biological Safety

General Description

Epstein-Barr virus (EBV) is a ubiquitous B-lymphotropic herpesvirus. EBV causes the common childhood disease mononucleosis. It is an icosahedral, lipid envelope double-stranded linear DNA virus sized 120-150nm in diameter. EBV has been found in the tumor cells of a heterogeneous group of malignancies (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 tumor 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 inoculation, or ingestion.
- Cell lines are often immortalized by transformation with EBV.

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 EBV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.

ANIMAL BIOSAFETY CONTAINMENT:

- EBV vector must be administered under BSL-2 containment.
- Animals must be housed as ABSL-2 containment.

DISINFECTION:

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

Additional Information:

Public Health Agency of Canada—[Epstein-Barr Virus MSDS](#)



Herpesvirus



University of Kentucky
Department of Biological Safety

General Description

Herpes Simplex 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, able to infect almost every cell type in most vertebrates that have been examined.

Potential 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 pharyngotonsillitis.

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.

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:

- BSL-2
- **NO** open-bench work
- Biological Safety Cabinet (BSC) required
- 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.

ANIMAL BIOSAFETY CONTAINMENT:

- HSV vector must be administered under BSL-2 containment.
- Animals must be housed as 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) recommended.

Additional Information:

Public Health Agency of Canada—[Herpes Simplex Virus \(HSV\) MSDS](#)

American Biological Safety Association—[Risk Group Classification for Herpesvirus](#)

Retroviruses / Murine Leukemia Virus (MLV)



University of Kentucky
Department of Biological Safety

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 allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of the transgene or additional introduced genetic element (s) may pose additional risk.

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 amphotropic or pseudotyped MLV.
- No recommended treatment.

BIOSAFETY CONTAINMENT:

- BSL-1 containment for ecotropic MLV demonstrated to be replication incompetent.
- PPE required for BSL-1 work: eye protection, lab coat, disposable gloves.
- BSL-2 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 the BSC and wiped down with appropriate disinfectant prior to being removed from BSC.

ANIMAL BIOSAFETY CONTAINMENT:

- MLV vector must be administered under BSL-2 containment.
- Animals administered ecotropic MLV may be housed under ABSL-1 conditions .
- Animals administered amphotropic/pseudotyped MLV must be housed under ABSL-2 conditions for 72-hours post administration, after which animals may be moved to ABSL-1 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) recommended.

Additional Information:

[NIH Guidelines Appendix B-V: Animal Viral Etiologic Agents in Common Use](#)

Retroviruses / Lentivirus



University of Kentucky
Department of Biological Safety

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 lentiviruses: Bovine immunodeficiency virus, Jembrana disease virus

Equine lentiviruses: Equine infectious anemia virus

Feline lentiviruses: Feline immunodeficiency virus

Ovine/caprine lentiviruses: Caprine arthritis-encephalitis virus, Ovine lentivirus, Visna virus

Primate lentivirus group: Human immunodeficiency virus (HIV) types 1-3, Simian AIDS retrovirus (SRV-1), Human T-cell lymphotropic virus type I and II, Simian immunodeficiency virus

The majority of 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.

Replacing the HIV envelope glycoprotein with VSV-G allows a broad host-range for the vector and allows the viral particles to be concentrated via centrifugation.

Potential Health Hazards

Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, 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 manifestation of infection includes non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue.

LABORATORY HAZARDS:

- Direct contact with skin and mucous membranes, parenteral inoculation, ingestion.

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.

ANIMAL BIOSAFETY CONTAINMENT:

- Lentiviral vector(s) must be administered under BSL-2+ containment.
- Animals must be housed under ABSL-2 conditions for 72 hours during this period, after which animals may be moved to ABSL-1 housing.

DISINFECTION:

- Susceptible to: 0.5% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol
- Freshly prepared 10% household bleach (0.5% Sodium hypochlorite) recommended

Additional Information:

[NIH Biosafety Considerations for Research with Lentiviral Vectors](#)

Replication Competency Testing



University of Kentucky
Department of Biological Safety

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. 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. Below is a summary guide of the current IBC recommendations for common classes of vectors. If another procedure or reference method is used to accomplish the same conclusion, researchers are asked to submit that procedure and published article with their IBC registration.

In general, the IBC will require 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 or in human gene therapy, or in any materials that could be released in the environment. Viral testing is not generally required if experiments are conducted entirely in tissue culture.

Virus	Method	Reference
Adenovirus	Test for RCV by PCR for E1a prior to use. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	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 (with adenovirus helper)	Test for RCV by PCR prior to use. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	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.
Adeno-associated virus (Adenovirus-free)	Testing not required.	
Lentivirus	Test for RCV by ELISA assay for p24 antigen. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. 1998 A third-generation lentivirus vector with a conditional packaging system. J Virol 72:8463-8471.
Retrovirus (ecotrophic and amphotrophic)	Test for RCV by amplification in a permissive cell line followed by screening by appropriate detection assay. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	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. Forestell, S.P., Nando, J. S., Bohnlein, E., and Rigg, R. J. 1996. Improved detection of replication-competent retrovirus. J Virol Methods 60: 171-178.
Vaccinia	Not applicable due to use as a replicating vector.	



Viral Vector	Risk Group	Biosafety Level		Special Requirements	Disinfectant
		<i>In vitro</i>	<i>In vivo</i>		
Adenovirus	2	BSL-2+	ABSL-2*	Adenoviral vector must be administered to animals under ABSL-2 containment. *Animals housed under ABSL-2 containment for 72-hours post administration.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant. Alcohol not effective disinfectant against adenovirus.
Adeno-Associated virus (AAV)	2	BSL-1 / BSL-2*	ABSL-1 / ABSL-2*	*AAV must be constructed under BSL-2 containment; once constructed, AAV may be handled at BSL-1 containment. *Animals must be housed under ABSL-1 containment; if helper virus is present, ABSL-2 containment.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant. Alcohol not effective disinfectant against AAV.
Epstein-Barr virus	2	BSL-2	ABSL-2	Epstein-Barr viral vectors must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant.
Herpes Virus HSV-I and HSV-II	2	BSL-2	ABSL-2	HSV vectors must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant.
Retroviruses / Murine Leukemia virus (MLV)	1/2	BSL-1 / BSL-2*	ABSL-1 / ABSL-2*	*Ecotropic murine retroviruses may be handled at BSL-1 / ABSL-1. *Amphotrophic or pseudotyped murine retroviruses must be handled at BSL-2 / ABSL-2 for 72-hours post administration.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant.
Retroviruses / Lentivirus	2	BSL-2+	ABSL-2*	Lentiviral vector must be administered to animals under ABSL-2 containment. *Animals housed under ABSL-2 containment for 72-hours post administration.	Freshly prepared 0.5% sodium hypochlorite recommended disinfectant.