NEW YORK MEDICAL COLLEGE
A MEMBER OF THE TOURO COLLEGE AND UNIVERSITY SYSTEM

Guidance for Working with Viral Vectors

NYMC Department of Energy, Environment, Health & Safety
Vosburgh Pavilion, 914-594-4078 NYMC_EHS@NYMC.edu

INTRODUCTION

Viral vectors are a fundamental tool in research. Production of viral vectors usually entail rendering an infectious virus to be replication incompetent or attenuated. This reduces the risks of working with these agents. Later generation viral vector systems are generally safer than early generation systems. However, the improvements in safety of later generations and the increased commercial availability of viral vectors have resulted in a culture around their use that includes a false sense of security and a decrease in practicing safe science. The purpose of this guidance document is to provide investigators with sufficient information to conduct informed risk assessments when working with viral vectors in the laboratory including suggested biosafety containment level, appropriate Personal Protective Equipment (PPE) selection, required containment procedures, appropriate disinfection practices, virology of the vector and the risks associated with its use in animal models. The most common viral vectors are outlined in the pages below.

REGISTRATION REQUIREMENTS

All work involving recombinant nucleic acids must be registered and approved by the NYMC Institutional Biosafety Committee (IBC) following the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules Guidelines (NIH Guidelines) and New York Medical College (NYMC) policy. Registration and approval includes work that is considered to be exempt

For many cases, the NIH Guidelines and the Recombinant DNA Advisory Committee (RAC) guidance document on lentiviral vectors explicitly state the containment level (see below).

- 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

While, the default biological safety containment level for recombinant viruses is Biosafety Level (BSL-2) and Animal Biosafety Level 2 (ABSL-2), there are exceptions.

- A lower biosafety containment level may suffice for incomplete viruses cultured in vitro.
- A few animal and human viruses qualify for lower biosafety containment.
- Animals with recombinant viruses which ordinarily require ABSL-2 containment may be down-graded to ABSL-1 if and when animals are considered to be no longer shedding virus. In these cases the NYMC IBC approves downgrading after 7 days unless data is available to justify a shorter time period.

Suggested biosafety containment levels and practices are provided in this document. A higher-containment level may be required in specific cases, depending on the specific properties of the vector and/or insert, or procedures to be performed. The biological safety containment level and any additional biosafety measures implemented are ultimately determined by the NYMC IBC.

GENERAL BIOSAFETY MEASURES

In general, viral vectors are used following biosafety level 2 (BSL-2) practices and procedures outlined below:

- Biological Safety Cabinet (BSC) required for procedures that produce aerosols, splashes or splatter
- Eye Protection, disposable gloves, laboratory coat required in addition to BSC
- If work cannot be performed in a BSC, additional PPE may be required to compensate

- When centrifuging, aerosol-proof rotors/buckets must be used, loaded and unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC
- Routine disinfection of work surfaces must be conducted with appropriate disinfectant
- Liquid waste must be disinfected before disposal into the sanitary sewar
- Procedures for safe handling of sharps must be written into protocols

GENERAL ANIMAL BIOSAFETY MEASURES:

- Most viral vector must be administered under ABSL-2 containment
- Agent should be administered to animals inside a biosafety cabinet
- Animals should be chemically or physically restrained for agent administration
- Animal contact items (bedding, cages, water bottles, etc) must be decontaminated before going to the cage wash
- Animal handling and cage changes should occur inside a biosafety cabinet

Viral Vector Biosafety Reference

Vector	Risk Group	Containment Level		Additional Requirements	Disinfectant/s
		Lab work	Animal Work		
Adenovirus	2	BSL-2	ABSL-2*	Adenoviral vector must be administered to animals under ABSL-2 containment. *Animals must be housed under ABSL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment in less than 7 days.	Freshly prepared 1:10 household bleach solution. **Alcohol not effective disinfectant against adenovirus**.
Adeno-associated virus (AAV)	1	BSL-1/BSL-2*	ABSL-1 / ABSL-2**	*AAV must be packaged under BSL-2 due to use of HEK293 cells; once packaged, AAV may be handled at BSL-1. **Animals are housed under ABSL-1 containment; if helper virus is present, ABSL-2 containment is required	Freshly prepared 1:10 household bleach solution. **Alcohol not effective disinfectant against AAV.**
Retroviruses / Murine Leukemia virus (MLV)	2	BSL-2	ABSL-2*	Retrovirus vector must be administered to animals under ABSL-2 containment. *Animals must be housed under ABSL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment in less than 7 days.	Freshly prepared 1:10 household bleach solution. 70% ethanol Quaternary ammonium disinfectants.
Lentivirus	2	BSL-2	ABSL-2*	Lentivirus vector must be administered to animals under ABSL-2 containment. *Animals must be housed under ABSL-2 containment for at least 7 days unless data is provided to justify a downgrade of containment less than 7 days. Containment procedures can be made more stringent if the transgene is an oncogene.	Freshly prepared 1:10 household bleach solution. 70% ethanol
Baculovirus	1	BSL-1/BSL2*	ABSL-1	*Baculoviral vectors modified for mammalian cells must be handled at BSL-2	Freshly prepared 1:10 household bleach solution or 70% ethanol
Vesicular stomatitis virus (VSV)	2	BSL-2	ABSL-2	VSV vectors must be administered to animals and animals must be housed under ABSL-2 containment.	Freshly prepared 1:10 household bleach solution. Alcohol not effective disinfectant against VSV

Adenovirus Fact Sheet

GENERAL: Adenoviruses are medium-sized (90-100 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types of adenoviruses capable of causing human infection. Recombinant adenoviruses used for biomedical research are usually based on Adenovirus 5. Adenoviruses are unusually resistant to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

Viruses 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; therefore, it is suggested to use early amplification stocks when needed to produce additional quantities of adenovirus. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells.

ADENOVIRAL GENE FUNCTION

Early genes (E): E1A, E1B, E2, E3, E4 Adenoviral gene transcription, replication, host immune suppression, inhibition of host cell apoptosis
Delayed early genes: IX, IVa2 Packaging
Major late Unit (L) Assembly

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.

ROUTES OF ESPOSURE:

- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation
- Ingestion

RISK GROUP CLASSIFICATION:

- Adenovirus is globally classified as Risk Group 2, but may not apply to all serotypes

SPECIFIC ANIMAL BIOSAFETY CONTAINMENT:

- Animals may shed/excrete adenovirus for some time post-administration. Animals must be house under ABSL-2 conditions for 7 days hours during this period, after which animals may be moved to ABSL-1 housing. To downgrade containment before 7 days data must be available for justification.

SPECIFIC DISINFECTION:

- Susceptible to: 1:10 bleach dilution (10% Bleach), CIDEX (2.4 % glutaraldehyde solution)
- Alcohol NOT effective disinfectant against adenovirus

Adeno-associated virus Fact Sheet

GENERAL: Adeno-Associated virus (AAV) gets its name 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, wild-type AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle. For certain experiments, these vectors are preferred over lentiviral vectors because they remain primarily episomal while lentiviral vectors integrate into the genome. Eleven serotypes of AAV have thus far been identified, with the best characterized and most commonly used being AAV2. These serotypes differ in their tropism, or the types of cells they infect, making AAV a very useful system for preferentially transducing specific cell types.

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.

ROUTES OF EXPOSURE:

- Exposure of mucous membranes (eyes, nose, mouth)
- Parenteral injection
- Ingestion
- Inhalation of aerosolized droplets
- Direct contact with skin

RISK GROUP CLASSIFICATION:

- The NIH Guidelines (Appendix B) state that " adeno- associated virus (AAV – all serotypes); and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at biosafety level 1 (BSL-1). However, AAV vectors are typically grown in human cell lines and therefore an infectious risk potential from the human cell lines exists.

SPECIFIC BIOSAFETY CONTAINMENT REQUIREMENTS:

- AAV that is packaged in human cell lines (HEK 293) can be used at BSL-1 if the cells are helper virus free and there is subsequent purification of the vector. The method and assessment of purification needs to be documented in the IBC registration.
- Work with AAV in the presence of helper virus, such as adenovirus or herpes simplex virus, must be conducted at BSL-2
- AAV generated in insect cell lines can be handled at BSL-1
- Work with AAV in which the transgene encodes an oncogene or toxin must be conducted at BSL-2

SPECIFIC ANIMAL BIOSAFETY CONTAINMENT:

- Animal housing may be maintained at ABSL-1 if the requirements for biosafety level one above are met
- ABSL-2 is required if helper virus is present or the transgene encodes a toxin or oncogene

SPECIFIC DISINFECTION:

- Susceptible to: 1:10 bleach dilution (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 0.25% sodium dodecyl sulfate
- Alcohol NOT an effective disinfectant against AAV

Retrovirus: Murine Leukemia Virus (MLV) Fact Sheet

GENERAL: These are infectious viruses which can integrate into transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Murine leukemia virus (MLV) is named for its ability to cause cancer in murine (mouse) hosts. 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.

ROUTES OF EXPOSURE:

- Parenteral injection
- Contact with tissues and body fluids of infected animals

RISK GROUP CLASSIFICATION:

- Risk Group 1: Ecotropic MLV demonstrated to be replication incompetent
- Risk Group 2: Amphotropic or pseudotyped MLV

SPECIFIC BIOSAFETY CONTAINMENT:

- BSL-1 containment for ecotropic MLV demonstrated to be replication incompetent
- BSL-2 containment for amphotropic or pseudotyped MLV

SPECIFIC ANIMAL BIOSAFETY CONTAINMENT:

- Animals administered ecotropic, murine specific, MLV may be housed under ABSL-1 conditions
- Animals administered amphotropic, broader host range, or pseudotyped MLV must be housed under ABSL-2 conditions for 7 days post ad-ministration, after which animals may be moved to ABSL-1 housing.

SPECIFIC DISINFECTION:

- Susceptible to: 1:10 bleach dilution (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), quaternary ammonium disinfectants, and 70% ethanol.

Retrovirus: Lentivirus Fact Sheet

GENERAL: 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**: Crapine 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 lymphotrophic virus type I and II, Simian immunodeficiency virus

The majority of lentiviral vectors in use today are HIV-derived vectors with the major risks being the potential for generation of replication-competent lentivirus (RCL), and potential for oncogenesis. To increase the safety of lentiviral vector systems, the components necessary for virus production are split across multiple plasmids (3 for 2nd-generation systems, 4 for 3rd-generation systems). Information on lentiviral vector generations can be found in Addgene's Lentiviral Guide. 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 nonspecific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue.

ROUTES OF EXPOSURE:

- Mucous membrane contact
- Parenteral inoculation
- Ingestion

SPECIFIC BIOSAFETY CONTAINMENT:

- BSL-2. Containment could be raised to BSL-2 enhanced depending on the properties of the transgene.

SPECIFIC ANIMAL BIOSAFETY CONTAINMENT:

- Animals must be house under ABSL-2 conditions for at least 7 days.

SPECIFIC DISINFECTION:

- Susceptible to: 1:10 bleach dilution (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), and 70% ethanol.

Baculovirus Fact Sheet

GENERAL

Baculoviruses are lytic viruses, primarily pathogenic for insects. Baculovirus vector systems are often used to obtain a high level of expression of a desired protein in insect cells (Sf9 cells). In the natural environment, wild-type baculovirus can pose a threat to certain insect species; however, commonly used baculovirus based vectors have been modified to reduce the pathogenicity to insects.

POTENTIAL HEALTH HAZARDS

Generally, non-genetically modified wild type baculoviruses are not capable of infecting vertebrate cells and thus do not pose any inherent hazards to laboratory workers. However, more recent studies with the use of mammalian specific promoters have achieved expression of foreign genes in a wide variety of mammalian cell lines and primary cell cultures

ROUTES OF EXPOSURE:

Transmission of baculovirus is through direct contact with the infective virus/vector. Baculovirus is highly sensitive to human complement and therefore, should an exposure occur, rapid inactivation of the virus is anticipated.

The budded form of the virus routinely used in research is noninfectious for the insect host, decreasing the risk of recombinant viral release into the environment.

SPECIFIC BIOSAFETY CONTAINMENT:

Biosafety level 1 practices and facilities are appropriate for activities involving baculovirus/viral vectors in insect cells, as determined by the Institutional Biosafety Committee (IBC, rDNA Committee).

Biosafety level 2 practices and facilities must be used for activities involving modified baculoviral vectors in mammalian cell lines

SPECIFIC DISINFECTION:

- Susceptible to 70% Ethanol

Vesicular Stomatitis Virus (VSV) Fact Sheet

GENERAL

Vesicular Stomatitis Virus (VSV) is a member of the Vesiculovirus genus, in the family Rhabdoviridae. VSV is a bullet-shaped, enveloped virus, approximately 70 nm in diameter and 170 nm in length, and has a single-stranded, negative-sense RNA genome. VSV has eight main serotypes Indiana, New Jersey, Cocal, Alagoas, Isfahan, Chandipura, Maraba, and Piry as well as laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow. The virus is zoonotic and leads to a flu-like illness in infected humans. Rare symptoms can include vesicle formation on the oral mucosa, lips, and nose. In children, the Chandipura virus has been reported to result in more serious symptoms that include fever, sensory disorders, convulsions, vomiting, diarrhea, and encephalitis leading to coma and death.

POTENTIAL HEALTH HAZARDS

VSV is an arbovirus that is transmitted naturally via the bite of an infected sand fly, by direct contact with abrasions on the skin, by contact with infected domestic animals, or by inhaling aerosols via the nasopharyngeal route. The virus has also been transmitted via accidental autoinoculation or inhalation of aerosols in a laboratory setting.

ROUTES OF EXPOSURE:

- Exposure of skin and mucous membranes to VSV by direct contact.

RISK GROUP CLASSIFICATION:

- Risk Group 2: laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

SPECIFIC BIOSAFETY CONTAINMENT:

- BSL-2 for laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

SPECIFIFC ANIMAL BIOSAFETY CONTAINMENT:

- Animals must be housed in ABSL-2 containment.

SPECIFIC DISINFECTION:

- Susceptible to 70% ethanol

References

- Biosafety in Microbiological and Biomedical Laboratories (2009 December). https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf
- National Institute of Health Guidelines (July 2017) https://osp.od.nih.gov/biotechnology/nih-guidelines/
- ABSA Risk Group Database (2016) https://my.absa.org/tiki-index.php?page=Riskgroups
- Adenoviruses for Health Care Professionals(2018, April 26) https://www.cdc.gov/adenovirus/hcp/index.html
- Adeno-associated Virus (AAV) Guide https://www.addgene.org/viral-vectors/aav/aav-guide/
- Biosafety Considerations for Research with Lentiviral Vectors https://osp.od.nih.gov/wp-content/uploads/Lenti_Containment_Guidance.pdf
- NIH Guideline FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (2016, April) https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Toc446948380
- University of Iowa. Environmental Health & Safety. “Baculovirus and Baculoviral Vectors” https://ehs.research.uiowa.edu/baculovirus-and-baculoviral-vectors
- Tulane University. “Guidance for working with Viral Vectors”. (2016). https://www2.tulane.edu/asvpr/biosafety/upload/TU-OBS-Viral-Vector-Guidance_10212016.pdf