Scope:
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.

Background:
All work involving recombinant nucleic acids must be registered and approved by the Institutional Biosafety Committee (IBC) according to the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) and Tulane University Institutional Biosafety Committee policy.

Tulane receives funding from NIH grants for research involving recombinant nucleic acids; therefore, ALL research conducted at the University must comply with the NIH Guidelines and University policies.

The NIH Guidelines and the Recombinant DNA Advisory Committee (RAC) guidance document on lentiviral vectors explicitly state the containment level in many cases.

- 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 BSL-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 BSL-2 containment may be down-graded to ABSL-1 if and when animals are considered to be no longer shedding virus.

Suggested biosafety containment levels are provided for each vector system. 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 IBC and/or Biological Safety Officer (BSO).

Sources and Resources:
Tulane IBC: [https://www2.tulane.edu/asvpr/biosafety/committee/ibc-approval.cfm](https://www2.tulane.edu/asvpr/biosafety/committee/ibc-approval.cfm)
<table>
<thead>
<tr>
<th>Viral Vector</th>
<th>Risk Group</th>
<th>Special Requirements</th>
<th>USDS Permit Required</th>
<th>Disinfectant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 5</td>
<td>2</td>
<td>Adenoviral vector must be administered to animals under ABSL-2 containment. *Animals must be housed under ABSL-2 containment for 72-hours post administration.</td>
<td>NO</td>
<td>Freshly prepared 1:10 household bleach solution. *Alcohol not effective disinfectant against adenovirus.</td>
</tr>
<tr>
<td>Adeno-associated virus (AAV)</td>
<td>1</td>
<td>*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.</td>
<td>NO</td>
<td>Freshly prepared 1:10 household bleach solution. *Alcohol not effective disinfectant against AAV.</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>2</td>
<td>Epstein-Barr viral vectors must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.</td>
<td>NO</td>
<td>Freshly prepared 1:10 household bleach solution.</td>
</tr>
<tr>
<td>Herpes Virus HSV-I and HSV-II</td>
<td>2</td>
<td>HSV vectors must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.</td>
<td>NO</td>
<td>Freshly prepared 1:10 household bleach solution.</td>
</tr>
<tr>
<td>Pseudorabies virus</td>
<td>2</td>
<td>PRV vectors must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.</td>
<td>YES</td>
<td>Freshly prepared 1:10 household bleach solution.</td>
</tr>
<tr>
<td>Recombinant Rabies Virus</td>
<td>3</td>
<td>Wildtype virus must be administered in and animals housed under ABSL-3 containment Recombinant vectors must be administered in and animals housed under ABSL-2 containment</td>
<td>YES</td>
<td>70% Ethanol</td>
</tr>
<tr>
<td>Retroviruses / Murine Leukemia virus (MLV)</td>
<td>1/2</td>
<td>Murine specific retroviruses may be handled at BSL-1 / ABSL-1. *BSL-2 required for broad host or pseudotyped murine retroviruses **ABSL-2 for 72-hours post administration.</td>
<td>NO</td>
<td>Freshly prepared 1:10 household bleach solution.</td>
</tr>
<tr>
<td>Retroviruses / Lentivirus</td>
<td>3</td>
<td>Lentiviral vector must be administered to animals under ABSL-2 containment. Animals must be housed under ABSL-2 containment.</td>
<td>YES, for non-human lentivirus</td>
<td>Freshly prepared 1:10 household bleach solution.</td>
</tr>
<tr>
<td>Vesicular stomatitis virus (VSV)</td>
<td>2*/3</td>
<td>VSV vectors must be administered to animals and animal housed under ABSL-2 containment. **Laboratory adapted strains are RG 2 **Chandipura and Piry viruses require BSL-3/ABLS-3.</td>
<td>YES</td>
<td>Freshly prepared 1:10 household bleach solution. *Alcohol not effective disinfectant against VSV</td>
</tr>
</tbody>
</table>

Individual vector constructs may not require USDA permit depending on their composition.
Adenovirus

GENERAL

There are more than 49 immunologically distinct types of adenoviruses (medium-sized (90-100 nm), non-enveloped, icosahedral, double-stranded DNA viruses) capable of causing human infection. Recombinant adenoviruses used for biomedical research are 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.

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

SOURCE/SPECIMENS:
- Fecal samples
- Respiratory secretions
- Other tissues may contain virus depending on symptoms

PRIMARY HAZARDS:
- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation
- Ingestion

EXPOSURE CONTROL AND PERSONAL PROTECTION

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

BIOSAFETY CONTAINMENT REQUIREMENTS:
- BSL-2
- Biological Safety Cabinet (BSC) required
- When centrifuging adenovirus, aerosol-proof rotors/buckets must be used, loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC
- Eye protection, disposable gloves, laboratory coat required in addition to 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: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution)
- Freshly prepared 1:10 household bleach solution recommended
- Alcohol NOT effective disinfectant against adenovirus

SOURCES AND ADDITIONAL INFORMATION

Centers for Disease Control and Prevention – Adenoviruses
Public Health Agency of Canada - Adenovirus MSDS
Adeno-associated virus

GENERAL
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, wild-type AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle. AAV vectors are commonly used in optogenetics 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.

LABORATORY HAZARDS

SOURCE/SPECIMENS:
Depending on the serotype, AAV infects various tissues and cell types including:
- CNS
- heart
- kidney
- liver
- lung pancreas

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

EXPOSURE CONTROL AND PERSONAL PROTECTION

RISK GROUP CLASSIFICATION:
- NIH classifies AAV (all serotypes) as Risk Group 1. Germany classifies serotypes 1, 4, and 6 as Risk Group 2

BIOSAFETY CONTAINMENT REQUIREMENTS:
- Packaging of AAV should be performed at BSL-2 within a biological safety cabinet because of the use of transformed HEK293 cells for production
- Once packaged, AAV may be manipulated at BSL-1
- Work with AAV in the presence of helper virus, such as adenovirus or herpes simplex virus, must be conducted at BSL-2
- PPE required for manipulation at BSL-1 OR BSL-2: eye/face protection, lab coat, and disposable gloves

ANIMAL BIO SAFETY CONTAINMENT:
- Animal housing may be maintained at ABSL-1
- ABSL-2 is required if helper virus is present

DISINFECTION:
- Adeno-Associated virus (AAV) susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 0.25% sodium dodecyl sulfate
- Freshly prepared 1:10 household bleach solution recommended
- Alcohol NOT effective disinfectant against AAV

SOURCES AND ADDITIONAL INFORMATION
- NIH Guidelines Appendix B-I: Risk Group 1 Agents
- ADDGENE’s Adeno-associated Virus (AAV) Guide
GENERAL

Epstein-Barr virus (EBV), also known as human herpesvirus 4, has been found in the tumor cells of a heterogeneous group of malignancies and is a transforming virus and can immortalize B-cells and cause lymphoma in various animal models. The virus can infect B-cells and epithelial cells.

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.
- **Diseases in immunocompromised hosts**: In AIDS patients or other immunosuppressed patients, many EBV-associated diseases may occur, such as oral hairy leukoplasia, interstitial lymphocytic pneumonia, B-cell or T-cell lymphomas and mesenchymal lymphomas (for e.g. leiomyosarcoma). In transplant patients, early and late onset lymphoproliferative diseases are often caused by EBV.

LABORATORY HAZARDS:

**SOURCES/SPECIMENS:**
- Clinical specimens - blood, saliva and throat specimens

**PRIMARY HAZARDS:**
- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation
- Ingestion.

EXPOSURE CONTROL AND PERSONAL PROTECTION

**RISK GROUP CLASSIFICATION:**
- Risk Group 2

**BIOSAFETY CONTAINMENT REQUIREMENTS:**
- BSL-2
- Biological Safety Cabinet (BSC) required for viral packaging procedures
- Eye protection, disposable gloves, laboratory coat required in addition to BSC
- When centrifuging EBV, aerosol-proof rotors/buckets must be used, 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 ABSL-2 containment
- Animals must be housed in ABSL-2 containment

**DISINFECTION:**
- Epstein-Barr Virus is susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 70% ethanol
- Freshly prepared 1:10 household bleach solution recommended

**SOURCES AND ADDITIONAL INFORMATION**

Public Health Agency of Canada - Epstein-Barr Virus MSDS
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 is highly infectious, so HSV vectors are efficient vehicles for the delivery of exogenous genetic material to cells. 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.

Only treatment available is anti-viral drug therapy for symptoms.

LABORATORY HAZARDS

SOURCES/SPECIMENS:
- Virus is shed from saliva, cervix, and urethra

PRIMARY HAZARDS:
- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation
- Ingestion

EXPOSURE CONTROL AND PERSONAL PROTECTION

RISK GROUP:
- Risk Group 2

BIOSAFETY CONTAINMENT:
- BSL-2
- Biological Safety Cabinet (BSC) required for viral packaging procedures
- Eye protection, disposable gloves, laboratory coat required in addition to BSC
- When centrifuging HSV, aerosol-proof rotors/buckets must be used, 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 ABSL-2 containment
- Animals must be housed in ABSL-2 containment

DISINFECTION:
- HSV is susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 70% ethanol, Lysol, quaternary ammonium disinfectants
- Freshly prepared 1:10 household bleach solution recommended

SOURCES AND ADDITIONAL INFORMATION

Public Health Agency of Canada - Herpes Simplex Virus (HSV) MSDS
Gene Therapy Net – Herpesvirus Viral Vectors
Pseudorabies Virus

GENERAL

Pseudorabies virus (PRV) is a herpesvirus of swine and the etiological agent of Aujeszky’s disease. PRV is a double-stranded DNA, enveloped virus with icosahedral symmetry. It is classified as a member of the subfamily Alphaherpesvirinae within the family Herpesviridae. Characteristics of this subfamily include a wide host range, the ability to establish latent infections in sensory neurons, and a relatively short replication time and lysis of cells in culture. The virus can be propagated in a wide variety of cell lines, most commonly of kidney origin such as PK15, Vero, and MDBC cells derived from the pig, monkey, and bovine. The virus also replicates in chick embryo fibroblasts.

PRV can also cause fatal encephalitis in a wide variety of animal species except its natural host. PRV is widely studied by those interested in disease control in swine agriculture as well as neuroscience as it is useful as a “live” tracer of neuronal pathways due to its propensity to infect synaptically connected neurons.

Pseudorabies is not related to rabies. The term pseudorabies was coined because the disease may resemble rabies.

POTENTIAL HEALTH HAZARDS

PRV does not cause disease in humans.

LABORATORY HAZARDS

SOURCES/SPECIMENS:
- Tissues or body fluids from infected animals

PRIMARY HAZARDS:
- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation
- Ingestion

EXPOSURE CONTROL AND PERSONAL PROTECTION

RISK GROUP:
- Risk Group 2 (Germany)

BIOSAFETY CONTAINMENT:
- BSL-2
- Biological Safety Cabinet (BSC) optional
- Eye protection, disposable gloves, laboratory coat required

ANIMAL BIOSAFETY CONTAINMENT:
- PRV vector must be administered under ABSL-2 containment
- Animals must be housed in ABSL-2 containment

DISINFECTION:
- Susceptible to: 1:10 dilution household bleach (10% Bleach), Lysol, quaternary ammonium disinfectants
- Freshly prepared 1:10 household bleach solution recommended

SOURCES AND ADDITIONAL INFORMATION

ISU Center for Food Security and Public Health – Pseudorabies Virus
USDA – Pseudorabies (Aujeszky’s Disease) and Its Eradication Review
CDFA – Pseudorabies Fact Sheet
UCSD – Pseudorabies Information Sheet
Recombinant Rabies Virus

GENERAL

Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Rabies is an enveloped, single-stranded, negative sense RNA virus. Due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons.

SAD-B19 (ΔG) is a modified rabies virus with the viral envelope B19 glycoprotein deleted, thereby rendering the virus unable to produce competent or infectious particles in transduced cells. As a result, the mutant virus cannot spread to any other surrounding cells from the originally infected cells. If the B19-glycoprotein is (intentionally) over-expressed as a transgene in a defined group of infected cells, the virus can trans-synaptically transport to adjacent cells only (singlestep) and never go beyond. The tropism of the viral vector may also be changed so that it cannot infect any mammalian cells except those that express a genetically-specified neuronal population transgene that encodes the envelope receptor (TVA) of this pseudotyped virus. Since mammalian neurons do not express TVA, the injected virus cannot infect wild-type human neurons. Alternatively, rabies vectors may be pseudotyped with a number of different envelope genes that increase its tropism, including EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env.

Since the rabies virus is a negative-strand RNA virus, it does not integrate into the cell genome and has no chance to produce a G protein RNA template. Therefore, there is essentially no risk to generate replication competent rabies virus.

POTENTIAL HEALTH HAZARDS

Infection with wild-type rabies virus results in encephalitis or paralysis, and is often fatal.

LABORATORY HAZARDS

SOURCES/SPECIMENS:
- saliva
- cerebrospinal fluid
- brain tissue
- conjunctival or corneal imprints

PRIMARY HAZARDS:
- Inhalation of aerosolized droplets
- Mucous membrane contact
- Parenteral inoculation

EXPOSURE CONTROL AND PERSONAL PROTECTION

RISK GROUP:
- Risk Group 3

BIOSAFETY CONTAINMENT:
- BSL-3 for wildtype or virulent recombinant strains
- BSL-2 for recombinant molecular viral vectors (determined by risk assessment)
- Biological Safety Cabinet (BSC) required for all viral work
- Eye protection, disposable gloves, laboratory coat required

ANIMAL BIOSAFETY CONTAINMENT:
- Wildtype virus must be administered and animals housed under ABSL-3 containment
- Incompetent recombinant virus must be administered and animals housed under ABSL-2 containment

DISINFECTION:
- Susceptible to: 70% ethanol, 1:10 dilution household bleach (10% Bleach), CIDE (2.4 % glutaraldehyde solution), Lysol, quaternary ammonium disinfectants
- 70% ethanol recommended

SOURCES AND ADDITIONAL INFORMATION

Public Health Agency of Canada - Rabies Virus MSDS
Retrovirus: Murine Leukemia Virus (MLV)

GENERAL

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.

LABORATORY HAZARDS:

SOURCES/SPECIMENS:
• laboratory cultures
• feces or urine (within 72 hours post infection)
• tissues or body fluids from infected animals

PRIMARY HAZARDS:
• parenteral injection

EXPOSURE CONTROL AND PERSONAL PROTECTION

RISK GROUP:
• Risk Group 1: ecotropic MLV demonstrated to be replication incompetent
• Risk Group 2: amphotropic or pseudotyped MLV

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, aerosol-proof rotors/buckets must be used, loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC

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 72-hours post ad-ministration, after which animals may be moved to ABSL-1 housing

DISINFECTION:
• MLV is susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), quaternary ammonium disinfectants, 70% ethanol
• Freshly prepared 1:10 household bleach solution recommended

SOURCES AND ADDITIONAL INFORMATION

NIH Guidelines Appendix B-V: Animal Viral Etiologic Agents in Common Use
UCSF VIRACORE - Moloney Murine Leukemia Virus (MMLV) MSDS
Retrovirus: Lentivirus

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 non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue.

LABORATORY HAZARDS:

**SOURCES/SPECIMENS:**
- Blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, pleural fluid, pericardial fluid, amniotic fluid, other specimens containing visible blood, breast milk, unscreened or inadequately treated blood products, and infected human tissues

**PRIMARY HAZARDS:**
- Mucous membrane contact
- Parenteral inoculation
- Ingestion

EXPOSURE CONTROL AND PERSONAL PROTECTION

**RISK GROUP:**
- Risk Group 2: Bovine lentiviruses (Germany/Switzerland), Equine lentiviruses (Germany/Switzerland), Feline lentiviruses (Germany), Ovine/caprine lentiviruses (Germany/Switzerland)
- Risk Group 3: Primate lentiviruses (NIH)

**BIOSAFETY CONTAINMENT:**
- BSL-2
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required in addition to BSC
- When centrifuging lentivirus, aerosol-proof rotors/buckets must be used, 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 ABSL-2 containment
- Animals must be house under ABSL-2 conditions

**DISINFECTION:**
- Susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 70% ethanol
- Freshly prepared 1:10 household bleach solution recommended

SOURCES AND ADDITIONAL INFORMATION

NIH Biosafety Considerations for Research with Lentiviral Vectors
Vesicular Stomatitis Virus (VSV)

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.

LABORATORY HAZARDS

**SOURCES/SPECIMENS:**
- Blood
- Throat secretions
- Saliva
- Exudates and skin from open sores

**PRIMARY HAZARDS:**
- Exposure of skin and mucous membranes to VSV by direct contact or bite by an infected sand fly.

EXPOSURE CONTROL AND PERSONAL PROTECTION

**RISK GROUP:**
- Risk Group 2: laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
- Risk Group 3: All others

**BIOSAFETY CONTAINMENT:**
- BSL-2 for laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
- BSL-3 for Chandipura and Piry viruses
- **NO** open-bench work
- Biological Safety Cabinet (BSC) required
- Eye protection, disposable gloves, laboratory coat required in addition to BSC
- When centrifuging VSV, rotors/buckets must be loaded/unloaded within the BSC and wiped down with appropriate disinfectant prior to being removed from BSC

**ANIMAL BIOSAFETY CONTAINMENT:**
- VSV vector must be administered under ABSL-2 containment. (Chandipura and Piry viruses require ABSL-3)
- Animals must be housed in ABSL-2 containment. (Chandipura and Piry viruses require ABSL-3)

**DISINFECTION:**
- Susceptible to: 1:10 dilution household bleach (10% Bleach), CIDEX (2.4 % glutaraldehyde solution), 70% ethanol
- Freshly prepared 1:10 household bleach solution recommended

**SOURCES AND ADDITIONAL INFORMATION**

Public Health Agency of Canada - Vesicular Stomatitis Virus


http://www.cfsph.iastate.edu/Factsheets/pdfs/vesicular_stomatitis.pdf