The following provides information on many of the recombinant viral vectors used in laboratories to express foreign genes in cultured cells and animal models. Investigators should use these guidelines as part of their risk assessment when planning experiments with these vectors and preparing applications to the Institutional Biosafety Committee (IBC). Note the listed containment levels are the minimum that should be employed with these vectors: some experiments, such as the expression of toxins or oncogenes, may require higher levels of containment. The appropriateness of the containment should be considered as part of the investigator's risk assessment and will be reviewed by the IBC.

**Biosafety Concerns Unique to Viral Vectors**

Rendering an infectious virus to be replication incompetent or otherwise attenuated lowers the risk of working with them, and later generation viral vector systems are generally safer than early generation systems. However, these improvements in safety 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. Furthermore, recombination events or contamination from wild type virus can result in the presence of replication competent virus (RCV) in a population of replication deficient viral vectors. This policy outlines the biosafety levels and containment for commonly used vector systems.

**Research Oversight**

Because viral vectors are subject to the NIH Guidelines, the University of Utah IBC must review each project involving viral vectors. The review will include a risk assessment to determine the appropriate biosafety level, PPE, and disposal methods. The biosafety levels listed below apply to replication incompetent viral vector systems only for in vitro and in vivo experiments. In all cases, additional biosafety precautions may be recommended by the IBC.

**Training**
All research personnel working with recombinant vectors where the parental virus is classified as Risk Group 2 or higher, including lentiviral, retroviral, and adenoviral vectors, as well as recombinant adeno-associated virus (AAV) vectors requiring BSL2 containment, are required to take the following:

1) Either:
    a. Bloodborne Pathogen/BSL2/ABSL2 training offered by the University of Utah Research Administration Training Series: information and registration can be found at https://education.research.utah.edu/, OR
    b. Bloodborne Pathogen training offered by the University of Utah Research Administration Training Series: information and registration can be found at https://education.research.utah.edu/, AND the vector specific online training kindly available from the University of Cincinnati Office of Research Integrity: http://researchcompliance.uc.edu/Biosafety/Training/ViralVectorWebtraining.aspx (Module 1 plus relevant vector modules), OR
    c. Bloodborne Pathogen offered by the University of Utah Research Administration Training Series: information and registration can be found at https://education.research.utah.edu/, AND the Recombinant Viral Vector training offered at the Huntsman Cancer Institute: contact the Biosafety Office for details on registration.

AND

2) Laboratory-Specific Training

Research personnel working with recombinant AAV vectors requiring BSL1 containment are requested to complete the AAV specific online training kindly available from the University of Cincinnati Office of Research Integrity: http://researchcompliance.uc.edu/Biosafety/Training/ViralVectorWebtraining.aspx (Modules 1 and 5). Please note that if you are working with human or non-human primate cell lines you are required to take Bloodborne pathogen training, as described above.

For individuals taking the University of Cincinnati Office of Research Integrity training, please complete the Online Training Assurance form (*link here*) and send to the Biosafety Office at biosafety@ehs.utah.edu or attach in the documents section of your laboratory BioRAFT registration.

**All training should be repeated annually.**

**Notes on Containment**

Suggested biosafety containment levels are provided for each vector system. Use of a higher-level containment facility or PPE may be required in some cases, depending on the specific properties of the vector and/or insert. Special care should be given to the design and handling of virus vectors containing genes that make growth-regulating products (oncogenes, growth factors, etc), products released into the circulation, or products that may have a general effect on the host- immune system or may be shed from animals (toxins).

# Lentivirus

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| **NIH Risk Group** | RG2<br>Lentiviruses are a subset of retroviruses, which are simple, enveloped single-stranded RNA viruses. |
| **Biocontainment Level** | The Recombinant DNA Advisory Committee of the NIH Office of Biotechnology Activities issued a report that reviewed biosafety issues relating to lentivirus vectors. This report advised that reduced biosafety level containment was appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that 1) separated vector and packaging functions onto multiple plasmids, 2) were produced at laboratory scale quantities, and 3) lacked expression of oncogenic transgenes. They specifically recommended that 4-plasmid systems that met specific criteria could be used at BSL-2 without the need to assay for replication competent virus (RCV).<br><br>BSL-2 enhanced:<br>*Oncogenic transgenes*<br>Lentivirus vectors that incorporate transgenes with oncogenic potential must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used.<br><br>*Scale of production*<br>Lentivirus vectors made at a level of production > 100 ml volume must be generated and used at BSL-2 enhanced containment regardless of whether second or third generation systems are used.<br><br>BSL-2 enhanced<br>Second generation or 3-plasmid lentivirus systems should be generated and used at BSL-2 enhanced. These systems generally have one packaging plasmid, which includes all the important packaging components: Gag, Pol, |

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| | Rev, and Tat, an envelope plasmid and the transfer vector. In general, lentiviral transfer vectors with a wildtype 5' LTR need the 2nd generation packaging system because these vectors require TAT for activation.<br><br>The investigator may request a downgrade in biosafety level to BSL-2 following demonstration that virus preparations have no detectable RCV based on results of an accepted RCV assay as described below. A protocol modification requesting reduction in biosafety level and including data from the RCV test must be submitted to *and* approved by the IBC before any BSL-2 or ABSL-2 work can be performed.<br><br>BSL-2<br>Third generation or 4-plasmid system vectors may be generated and used at BSL-2, as may second generation lentivirus systems that use a self-inactivating vector (see below) The 4 plasmids of the third generation system include 2 packaging plasmids, an envelope plasmid, and a transfer plasmid. 3rd generation packaging system offers maximal biosafety but require the transfection of four different plasmids into the producer cells. The main differences in the 3rd generation system are as follows:<br>• The Tat gene has been eliminated from the packaging completely<br>• Rev is expressed on a separate plasmid<br>• The 5'LTR of the transfer plasmid has been modified to include a conventional promoter and the U3 region of the 3’LTR has been deleted. This is termed a self-inactivating (SIN) vector and can be packaged by both 2nd and 3rd generation packaging systems. |

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| | The potential for generation of RCV from HIV-1 based lentivirus vectors depends upon several factors, the most important of which are:<br>• The number of recombination events necessary to reassemble a replication competent virus genome<br>• The number of essential genes that have been deleted from the vector/packaging system.<br><br>Earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCV. The IBC does not require testing for RCV when 4-plasmid (third generation) systems are used or when a SIN vector is used with a 2nd generation packaging system (see IBC guidelines for RCV testing). |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | Lentiviruses are transmitted via direct exposure to infected bodily fluids, sexual contact, sharing unclean needles. Lentiviruses may persist lifelong due to their ability to integrate into the host chromosome and ability to evade host immunity. Lentiviruses replicate, mutate and undergo selection by host immune responses. |
| **Laboratory Hazards** | Risks include direct contact with skin and mucous membranes of the eye, nose and mouth, parenteral inoculation, ingestion. |
| **Disease** | The clinical manifestation of HIV infection includes non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue. Can cause severe immunologic and neurological disease in hosts. The major risks associated with lentiviral vectors are insertional mutagenesis and local inflammation. |
| **Treatment/Prophylaxis** | NRT inhibitors, Protease inhibitors |
| **Pathogenesis** | Insertional mutagenesis. Can infect non-dividing cells including immune cells. Can infect non-target cells. Can persist lifelong. High mutation rates. Inappropriate expression |

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| | of gene product. Rescue by other human pathogenic viruses |
| **Replication Competent** | Possible |
| **RCV Testing** | Can be performed by the investigator using a standard p24 ELISA kit providing the assay has a sensitivity of < 12.5 pg/ml. A positive control for virus infection is not required; the IBC does not want the investigator to work with infectious HIV-1 for this assay. However, the assay must contain a positive control for the ELISA itself in the form of p24 antigen.<br><br>Virus should be tested for RCV by serial passage of tissue culture supernatant on 293T cells for 3 passages with subsequent testing of supernatant from each passage for p24 antigen by ELISA.<br><br>Investigators who are not generating their own viruses from 2 or 3-plasmid system but are acquiring already constructed virus stocks from a commercial source that has documentation filed with the IBC of acceptable RCV testing will not be required to test for RCV. |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-2: When animals are infected with lentiviral vectors, the Animal Biosafety Level of the project will be generally assigned to ABSL-2: the use of vectors where RCV may be generated requires ABSL-2-enhanced. Animals must be injected in a Biological Safety Cabinet.<br><br>Infected animals can excrete lentivirus, so cages and bedding are considered |

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| | biohazardous for a minimum of 72 hours post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>On the fourth day following infection, animals injected with replication incompetent vectors can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be handled as with other ABSL-1 animals. However, for rodents that contain any human cells or tissues, step down to BSL1 will generally not be allowed: determined by IBC.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

# Adenovirus

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| **NIH Risk Group** | RG2<br>Adenoviruses are non-enveloped icosahedral viruses containing double-stranded DNA. |
| **Biocontainment Level** | BSL-2.<br>1st Generation: Deletion of regions E1, E3 genes (less safe)<br><br>2nd Generation: Deletion of regions E1, E2, E3, E4 genes (more safe)<br><br>Expression of oncogenes or toxins may raise BSL containment requirements |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | Wild-type adenoviruses are spread directly by oral contact and droplets. They are indirectly spread by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person. It is possible for a person who is infected, but asymptomatic, to shed virus for many months or years. |
| **Laboratory Hazards** | Inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion.<br><br>Adenovirus is unusually stable in the environment. Adenovirus can still be infective after having been extracted with ether and/or chloroform. |
| **Disease** | Apart from respiratory involvement, illnesses and presentations of adenovirus include gastroenteritis, conjunctivitis, cystitis, and rash illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection.<br><br>*Pharyngoconjunctival fever* is a specific presentation of adenovirus infection:<br>• high fever that lasts 4–5 days |

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| | • pharyngitis (sore throat)<br>• conjunctivitis (inflamed eyes, usually without pus formation like pink eye)<br>• enlargement of the lymph nodes of the neck<br>• headache, malaise, and weakness<br>• Incubation period of 5–9 days<br><br>Replication-defective recombinant adenoviral vectors have caused corneal and conjunctival damage. |
| **Treatment/Prophylaxis** | Most infections are mild and require no therapy or only symptomatic<br><br>Treatment/Prophylaxis. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection. |
| **Pathogenesis** | Can infect a variety of non-dividing cells. Stays episomal (does not integrate) |
| **Replication Competent** | Possible |
| **RCV Testing** | The probability of producing replication competent virus (RCV), although low, increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells. It is suggested to use early amplification stocks when needed to produce additional quantities of adenovirus. RCV testing is recommended for 1$^{st}$ generation vectors. PCR for E1 prior to use or plate on non-susceptible cell types |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br><br>Note: Alcohol is NOT an effective disinfectant against non-enveloped viruses such as adenovirus. |

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| **Animals** | ABSL-2: When animals are infected with adenoviruses/adenoviral vectors, the Animal Biosafety Level of the project will be generally assigned to ABSL-2.<br><br>Animals must be injected in a Biological Safety Cabinet. Infected animals can excrete adenovirus, so cages and bedding are considered biohazardous for a minimum of 5 days post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material: adenovirus is excreted by animals. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>After 5 days animals can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>For first generation vectors or infection of animals containing human cells or tissues, ABSL-2 containment may be required for longer periods. This will be determined by the IBC.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

# Adeno-Associated Virus

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| **NIH Risk Group** | RG1 (AAV 1-4)<br>AAV are non-enveloped icosahedral viruses with a single stranded DNA genome. |
| **Biocontainment Level** | BSL-1; unless it encodes oncogene/toxin or helper virus present (BSL-2) |
| **Infectious to Humans/Animals** | Yes (Humans/Primates) |
| **Route of Transmission** | • AAV may be transmitted through direct contact with an infected individual or through indirect contact with the contaminated environment.<br>• Transmission routes include respiratory, gastrointestinal and possibly sexual transmission.<br>• A concern for vertical transmission from mother to fetus also exists.<br>Most adults (85-90% in the US) are seropositive for AAV and about 30% have neutralizing antibodies. |
| **Laboratory Hazards** | Inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion. |
| **Disease** | • AAV is not associated with any human disease; however, there is evidence of AAV infection in the human embryo and an association of AAV with male infertility.<br>• A significant correlation was found between the presence of AAV DNA in amnion fluids and premature amniorrhexis (rupture of the amnion) and premature labor.<br>• Recombinant AAV vectors lose site specific integration into chromosome 19, thereby raising the theoretical concern of insertional mutagenesis. |
| **Treatment/Prophylaxis** | Supportive care. No specific Treatment/Prophylaxis |
| **Pathogenesis** | Infects multiple cell types. May be associated with insertional mutagenesis and cancer. Inserts itself on human chromosome 19 and remains latent. Can be potentially reactivated later in the presence of a helper virus and |

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| | produce infection. Shown to cause insertional mutagenesis in murine cell lines. |
| **Replication Competent** | Only in presence of helper virus (CMV, adenovirus, herpesvirus, vaccinia) |
| **RCV Testing** | If helper virus is adenovirus, test for presence of RCV after heat inactivation (56°C for 15min) |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• Alkaline solutions at pH >9.<br>• 5% phenol.<br><br>Note: Alcohol is NOT an effective disinfectant against non-enveloped viruses, such as AAV. |
| **Animals** | ABSL-1: If helper virus is used follow rules for that virus. In general, ABSL-2 will be required if a helper virus used or if host animal could house helper virus: animals must be injected in a Biological Safety Cabinet. 72 hours following infection, animals can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>Special handling of bedding and cages for 48 hours post injection. Bedding disposed in biohazardous waste.<br><br>Animal cages at ABSL-1 need not be labeled with a biohazard sign. |

| **Murine Retroviruses, such as Moloney Murine Leukemia Virus: (MoMuLV/MMLV) or Mouse Mammary Tumor Virus (MMTV)** | |
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| **NIH Risk Group** | RG1 (ecotropic)<br>RG2 (Others: amphotropic or pseudotyped)<br>MMLV is a member of the gammaretroviruses and MMTV is a beta retroviruses genera. Both are enveloped, icosahedral, diploid viruses with a single-stranded, linear RNA genome. MMLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection. |
| **Biocontainment Level** | BSL-1 (ecotropic)<br>BSL-2 (Others: amphotropic or pseudotyped) |
| **Infectious to Humans/Animals** | Possible if amphotropic or pseudotyped |
| **Route of Transmission** | Bloodborne |
| **Laboratory Hazards** | In mice, virus is transmitted via blood from infected mother to offspring; may also occur via germline infection.<br><br>In vivo infection in humans appears to require direct parenteral injection with amphotropic or pseudotyped MLV. However, contact with feces or urine from transduced animals for 72 hours post infection or with tissues and body fluids of transduced animals should be avoided. |
| **Disease** | Cell transformation and tumor formation |
| **Treatment/Prophylaxis** | None |
| **Pathogenesis** | Insertional mutagenesis possible, leading to cell transformation/tumor formation. Amphotropic Env gene or pseudotyped viruses can infect non-murine cells including human cells |
| **Replication Competent** | Yes |
| **RCV Testing** | Use permissive cell line (*Mus dunni*); screen by marker rescue assay (PG-4S+L-). In general no RCV testing for 3rd generation or later vector systems: determined by IBC. |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following: |

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| | • RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-1: Ecotropic replication incompetent murine retroviruses<br><br>ABSL-2: Amphotrophic or pseudotyped murine retroviruses must be handled at ABSL-2 for at least 72-hours post administration. Animals must be injected in a Biological Safety Cabinet. Infected animals can excrete retrovirus, so cages and bedding are considered biohazardous for a minimum of 72 hours post-exposure (replication incompetent vectors). Take precautions to avoid creating aerosols when emptying animal waste material. Soiled cages are disinfected prior to washing.<br><br>Animal cages must be labeled with a biohazard sign.<br><br>For rodents that do not or will not contain any human cells or tissues, on the fourth day following infection, animals injected with replication incompetent vectors can be transferred to ABSL-1 standard conditions. The animals will be transferred to a clean cage, and the ABSL-2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used handled as with other ABSL-1 animals.<br><br>IBC may require RCV testing for viruses to be administered at ABSL1 or studies where containment is reduced after administration.<br><br>ABSL-2 or ABSL-1 for xenografts of transduced human/animal cells. Determined by IBC. |

| Herpes Simplex Virus | |
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| **NIH Risk Group** | RG2<br>Herpesviruses are enveloped, icosahedral, double-stranded linear DNA viruses. |
| **Biocontainment Level** | BSL-2 |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | HSV-1 is typically transmitted by saliva or by the infection on hands of healthcare personnel. HSV-2 is typically transmitted through sexual contact. HSV can be transmitted by direct contact with epithelial or mucosal surfaces. |
| **Laboratory Hazards** | In the laboratory, HSV can be transmitted by ingestion, parenteral injection, droplet exposure of the mucous membranes (eyes, nose or mouth), and inhalation of aerosolized materials. |
| **Disease** | Depends on type:<br>• Oral Herpes<br>• Genital Warts<br>• Herpes esophagitis<br>• Herpes encephalitis or meningitis |
| **Treatment/Prophylaxis** | Antivirals may reduce shedding |
| **Pathogenesis** | After infection, the viruses are transported along sensory nerves to the nerve cell bodies, where they reside lifelong. Causes of recurrence may include: decreased immune function, stress, and sunlight exposure. The first episode is often more severe and may be associated with fever, muscle pains, swollen lymph nodes and headaches. Over time, episodes of active disease decrease in frequency and severity |
| **Replication Competent** | All versions of HSV vectors are prone to recombination. Additionally, approximately 50% - 90% of adults possess antibodies to HSV type 1; 20% - 30% of adults possess antibodies to HSV type 2. This is a concern since reactivation from latency is not well understood. Infection by HSV vectors into latently infected cells could potentially reactivate the wild-type virus, or spontaneous reactivation of a latent infection could |

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| | produce an environment where replication defective vectors could replicate. |
| **RCV Testing** | Viral preparations used for *in vitro* studies should be tested every 6 months for replication competent viruses by plaque assay. These assays should be tested at a sensitivity limit of 1 infectious unit per mL. |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-2: Animals will be maintained at ABSL-2 for the duration of the study. Animals must be injected in a Biological Safety Cabinet. All bedding, waste and animals infected with HSV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.<br><br>Animal cages must be labeled with a biohazard sign. |

| Sendai Virus | |
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| **NIH Risk Group** | RG2 for human paramyxoviruses. |
| **Biocontainment Level** | BSL-2<br>Sendai virus (SeV) causes respiratory disease in rodents and sometimes swine. There is limited evidence of zoonotic transmission to humans. However, the virus is capable of infecting human cell lines and is similar to human parainfluenza virus type 1. For these reasons, SeV work is usually classified as BSL-2.<br><br>Recombinant constructs expressing oncogenes or toxins should be handled at BSL-2 enhanced |
| **Infectious to Humans/Animals** | Mice |
| **Route of Transmission** | SeV is responsible for a highly transmissible respiratory tract infection in mice, hamsters, guinea pigs, rats, and occasionally pigs, with infection passing through both air and direct contact routes. |
| **Laboratory Hazards** | No reported cases of laboratory acquired disease but inhalation of aerosolized droplets, mucous membrane contact, parenteral inoculation, or ingestion are possible routes of infection. |
| **Disease** | Respiratory disease. Infections of mice are usually associated with a high mortality rate although latent infections can occur. |
| **Treatment/Prophylaxis** | Antivirals may reduce shedding |
| **Pathogenesis** | The respiratory infection of Sendai virus in mice is acute. Virus may first be detected in the lungs 48 to 72 hours following exposure. As the virus replicates in the respiratory tract of an infected mouse, the concentration of the virus grows most quickly during the third day of infection. After that, the growth of the virus is slower but consistent. Typically, the peak concentration of the virus is on the sixth or seventh day, and rapid decline follows that by the ninth day. A fairly vigorous immune response mounted against the virus is the cause of this decline. |

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| **Replication Competent** | Yes |
| **RCV Testing** | No |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-3: Animal cages must be labeled with a biohazard sign. Note there are currently no ABSL-3 suites at the University of Utah. |

# Vaccinia Virus

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| **NIH Risk Group** | RG2<br>The poxviruses are the largest known DNA viruses and are distinguished from other viruses by their ability to replicate entirely in the cytoplasm of infected cells. Vaccinia is an enveloped double-stranded DNA virus that is highly stable and can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. |
| **Biocontainment Level** | The biocontainment level of the vector is based on CDC criteria for the parental virus strain.<br><br>BSL-1<br>Vectors derived from highly attenuated strains including TROVAC (fowlpox) and ALVAC (canarypox) strains that do not replicate in human cells, and NYVAC (derived from the Copenhagen strain) that replicates poorly in human cells.<br><br>BSL-2<br>MVA (Ankara) is listed as a highly attenuated strain; however, the containment recommended by CDC for it is BSL2.<br><br>Non-attenuated vaccinia strains, such as NYCBOH (the strain used in the vaccinia vaccine), Western Reserve (WR), Copenhagen, Temple of Heaven, Lister or Cowpox. |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | Vaccinia virus may be transmitted via surface contact with contaminated object(s) and subsequently spread to mucus membranes (eyes, nose, and mouth) and/or to open sores on skin. |
| **Laboratory Hazards** | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of |

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| | transmission. If working with infectious animal models, then bite wounds could transmit vaccinia virus infection. |
| **Disease** | Infection of the skin can cause a localized lesion that then scabs over and heals in about 10-14 days. |
| **Treatment/Prophylaxis** | Vaccination is recommended when working with the non-attenuated strains but not recommended for working with the highly attenuated strains, including MVA, as long as no other orthopox viruses are in use.<br><br>The CDC recommends vaccination every 10 years for laboratory workers in the United States who have any contact with non-highly attenuated vaccinia strains. However, individuals who are pregnant; breastfeeding; have skin conditions such as eczema or atopic dermatitis; those with heart disease; or those with altered immune systems, are at increased risk from the vaccine, and should not be vaccinated and should not work with the virus.<br><br>The vaccination can be accompanied by fever, rash, lymphadenopathy, fatigue, myalgia and headaches. Serious complications such as ocular vaccinia, myopericarditis, eczema vaccinatum (a papular, vesicular and pustular rash that is very infectious), progressive vaccinia (progressive necrosis at the vaccination site), postvaccinial CNS disease (headache, lethargy, seizures and coma), fetus malformations and abortion (very rare) sometimes occur after vaccination. Complications are more serious in immunosuppressed individuals and the smallpox vaccine usually causes one death for every million doses. |
| **Replication Competent** | Yes |
| **RCV Testing** | NA |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following: |

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| | • RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-2: Animals must be injected in a Biological Safety Cabinet. Animals will be maintained at ABSL-2 for the duration of the study. All bedding, waste and animals infected with vaccinia shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.<br><br>Animal cages must be labeled with a biohazard sign. |

# Rabies Virus

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| **NIH Risk Group** | RG2<br>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.<br><br>Replication-deficient rabies vectors can be useful tools for investigation into neuronal trafficking or targeted expression in neurons. SADΔG-mCherry/EnvASADΔG is an example of a modified rabies virus. This modified version of the rabies virus forces neurons it infects to produce a red fluorescent protein called mCherry. mCherry makes the infected cells glow red so they are visible under a microscope. The benefit is the ability to trace a neural circuit on the cellular level as only connected/attached neurons are affected. Initial deletion: This modification deletes a gene which encodes the rabies virus envelope B19- glycoprotein (RG) and which is required for the production of competent or infectious viral particles from the virus genome 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 over-expressed as a transgene in a defined group of infected cells, the virus can trans-synaptically transport to adjacent cells only (single-step) and never go beyond.<br><br>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. Examples of this include EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env. EnvA pseudotyped virus can only infect cells expressing the complementary receptor TVA. Since mammalian neurons do not |

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| | express TVA, the injected virus cannot infect wild-type human neurons. If the virus is able to infect a TVA-positive neuron (for example, in transgenic mice), it can replicate and strongly label the first-order (initially infected) neurons, but since its genome lacks the B19 glycoprotein, it cannot infect other neurons by itself. In short, the risk for infection is specified by transgene expression and retrograde transport is limited to a single synapse. Thus the resultant virus becomes a "mono-synaptic" transneuronal tracer and significantly reduces the biohazardous risk because the virus has no potential to infect or trans-synaptically transport to any mammalian cells, including human and mice.<br><br>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 with this vector. |
| **Biocontainment Level** | BSL-2 |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | Percutaneous injury, such as animal bites. Potential non-bite modes of transmission include contamination of a pre-existing wound, contact of mucous membrane or respiratory tract with the saliva of an infected animal, exposure to aerosolised rabies virus in the laboratory (or from bats), or via organ transplantation from an infected donor, or inhalation of droplets |
| **Laboratory Hazards** | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. If working with infectious animal models, then bite wounds could transmit rabies virus infection. |
| **Disease** | Rabies virus can cause an acute infection, marked by progressive encephalomyelitis, and is usually fatal. The initial symptoms of rabies |

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| | resemble those of other systemic viral infections, including fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders. This prodromal phase typically lasts about 4 days, but can last as long as 10 days before specific symptoms develop. |
| **Treatment/Prophylaxis** | Consultation is available to determine if vaccination is appropriate for personnel working with recombinant rabies vectors. Vaccination is not needed for working with SAD B19 vaccine strain.<br><br>Post-exposure rabies prophylaxis with vaccines together with the administration of rabies immunoglobulin (RIG) is highly effective but is a medical urgency. There is no established treatment for wild-type rabies once symptoms have begun, but supportive therapy may include intubation, sedation, mechanical ventilation, fluid and electrolyte management, and nutrition. |
| **Replication Competent** | Usually no but depends on pseudotyping and expression of envelope protein |
| **RCV Testing** | No effective methods for RCV testing |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-2. |

# Epstein Barr Virus

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| **NIH Risk Group** | RG2<br>Epstein-Barr virus, frequently referred to as EBV, is a member of the herpesvirus family and one of the most common human viruses. EBV are enveloped, icosahedral viruses with a double stranded linear DNA genome. |
| **Biocontainment Level** | BSL-2 |
| **Infectious to Humans/Animals** | Yes |
| **Route of Transmission** | Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. |
| **Laboratory Hazards** | Accidental needlestick is a mode of transmission within research laboratories. Accidental ingestion of viral contaminated materials and inhalation are other routs of transmission. Note that cell lines are often immortalized by transformation with EBV. |
| **Disease** | The virus is found worldwide, and most people become infected with EBV sometime during their lives, most commonly causing infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy. A few carriers of this virus may develop Burkitt's lymphoma or nasopharyngeal carcinoma. EBV is a transforming virus and is often used to produce immortalized cell lines and cause lymphoma in various animal models. |
| **Treatment/Prophylaxis** | No specific treatment |
| **Replication Competent** | Usually no but there is the potential for recombination with a latent viral infection. |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (0.5%: use 1:10 dilution of fresh bleach)<br>• 5% Phenol<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL-2: Animals must be injected in a Biological Safety Cabinet. Animals will be |

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| | maintained at ABSL-2 for the duration of the study. All bedding, waste and animals infected with EBV shall be treated as biohazardous. After all animals are removed from their primary enclosure immediately autoclave or treat with chemical disinfectant. After disinfection, dump the cage contents and begin cleaning the cage for re-use. All waste must be decontaminated by autoclaving or chemical disinfection prior to disposal. Animal carcasses must be placed in autoclave bags and be designated for infectious waste disposal. All necropsies must be performed in a designated room using animal BSL-2 practices and procedures.<br><br>Animal cages must be labeled with a biohazard sign. |

| Baculovirus | |
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| **NIH Risk Group** | NA<br>Baculoviruses are non-mammalian enveloped, circular DNA viruses that infect insects. |
| **Biocontainment Level** | BSL1<br><br>Containment levels may be raised per IBC review if the vector is amphotropic and can infect human cells or can achieve expression of an oncogene or biological toxin in mammalian cells. |
| **Infectious to Humans/Animals** | Generally, non-genetically modified wild type baculoviruses are not capable of replicating in vertebrate cells |
| **Route of Transmission** | NA |
| **Laboratory Hazards** | Direct contact, droplet exposure of the mucous membrane, direct injection<br><br>Since they are not capable of replicating in vertebrate cells they 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. |
| **Disinfection** | Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:<br>• RECOMMENDED: Sodium hypochlorite (1.0%: use 1:5 dilution of fresh bleach)<br>• 70% Ethanol or Isopropanol |
| **Animals** | ABSL1 |

Sources:
http://web.stanford.edu/dept/EHS/prod/researchlab/bio/docs/Working_with_Viral_Vectors.pdf
http://www.dartmouth.edu/~ehs/biological/biosafety_docs/110_1_ibc_viral_vector_policy.pdf