Working with Viral Vectors
(2013)

Introduction: Viruses and viral vectors have become a staple of the molecular biology community. As such, it is important for users to understand the origins of these tools and potential implications of their use. Expanded sections for each virus contain information on virology, clinical features, epidemiology, treatment, laboratory hazards, Personnel Protective Equipment (PPE), disinfection, and use with animals.

General comment on containment: Suggested biosafety containment levels are provided for each vector system. Use of a higher-level containment facility 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, products released into the circulation, products that may have a general effect on the host-immune system (see Viral Vector chart for more information). [1] Work with viral vectors that are classified as BSL-1 does not require Biosafety program approval – common examples are Baculovirus and AAV (when oncogenes or toxins are not cloned into the vectors) and ecotropic MMLV. Work with BSL-2 or 3 agents require Biosafety Committee (APB) review and approval prior to the start of work (see APB approval web site). Additional approval from APLAC is required for research involving BSL-2/3 viral vectors and animals (see APLAC web site).

Click on link at end of each section for additional virus specific information,

1. **Adenovirus**: Adenoviruses are infectious human viruses which often cause mild respiratory illness, pink eye or gastroenteritis. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Biosafety Level 2 **(BSL-2)** is appropriate for many constructs. Particular care should be given to vectors containing genes that make products similar to those of the deleted adenovirus genes. Additional Adenovirus information

2. **Adeno-associated virus (AAV)**: These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host chromosome. The NIH Guidelines (Appendix B) state that "adeno-associated virus (AAV) types 1 through 4, and recombinant 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)**. This level of containment is modified by other considerations (see above General Comments). Additional AAV information

3. **Epstein - Barr virus (EBV):** Epstein-Barr virus, frequently referred to as EBV, is a member of the herpesvirus family and one of the most common human viruses. The virus is found worldwide, and most people become infected with EBV sometime during their lives. In the United States, as many as 95% of adults between 35 and 40 years of age have been infected. Infants become susceptible to EBV as soon as maternal antibody protection (present at birth) disappears. Many children become infected with EBV, and these infections usually cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood. In the United States and in other developed countries, many persons are not infected with EBV in their childhood years. When infection with EBV occurs during adolescence or young adulthood, it causes infectious mononucleosis 35% to 50% of the time. EBV also establishes a lifelong dormant infection in some cells of the body's immune system. A late event in a very few carriers of this virus is the emergence of Burkitt's lymphoma and nasopharyngeal carcinoma.

EBV is a transforming virus and is often used to produce immortalized cell lines. **BSL-2** is appropriate for most experiments. Additional EBV information

4. **Herpesvirus**: Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is common in the general population, but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity. Biosafety Level 2 **(BSL-2)** is appropriate for many constructs. Additional Herpesvirus information

5. **Retrovirus**: These are infectious viruses which can integrate into transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses - most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses, when vector particles express glycoproteins (GPs) derived from other enveloped viruses (which can also infect human cells). The most common GP currently used is VSV-g, however there are newer pseudotypes being derived from viruses such as measles (Rubeola), Ebola and Marburg. Pseudotyping vectors often results in a higher Biosafety level. Containment for vectors with the ability to infect human cells (amphotropic) will usually be recommended at **BSL-2/2+**, whereas for ecotropic vectors with no ability to infect human cells, **BSL-1** containment may be appropriate.

    A. MMLV: The host range of recombinant MMLV vectors 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 murine and nonmurine cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells. Biosafety Level 2 **(BSL-2)** is appropriate for many constructs, while higher levels may be required depending upon the construct. Additional MMLV information

    B. Lentivirus: Lentiviruses are a subset of retroviruses, with the ability to integrate into host chromosomes, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human/non-primate origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human viruses (HIV). The more recent generation vectors have been designed to significantly diminish the possibility for recombination to occur resulting in a wild type- potentially infectious virus. Typical lentivirus vectors are packaged using pseudotyped enveloped proteins. The most common envelope protein used for this purpose is from vesicular stomatitis virus (VSV). It is usually recommended that work with non-human lentiviruses that are incapable of establishing productive infections in humans be conducted at **BSL-2**. Work with simian or human lentiviruses (SIV, HIV) is typically conducted at a higher containment level. Additional Lentivirus information

6. **Poxvirus**: Poxvirus vectors include avian viruses (avipox vectors) such as NYVAC and ALVAC, which cannot establish productive infections in humans, as well as mammalian poxviruses, which can productively infect humans -such as vaccinia virus and modified vaccinia viruses (MVA). Poxviruses are highly stable, and vaccinia virus can cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus. The use of **BSL-2** is appropriate for many poxviruses and constructs. Additional Pox virus information

7. **Baculovirus**: Non-mammalian virus vectors that infect insects, these are very stable and may remain dormant in the environment for years before infecting insects. Work is mostly done at the **BSL-1** level.

8. **Rabies virus:** Rabies virus is a member of the Rhabdoviridae family and is a common zoonotic infection from bats and other wild mammals. Infection results in encephalitis or paralysis, and is often fatal. Due to its neuronal tropism, pseudotyped rabies virus vectors can be used to study neuronal trafficking or express endogenous genes efficiently in neurons. Biosafety Level 2 (**BSL-2**) is appropriate for many constructs. Additional Rabies virus information

9. **Sendai virus:** Sendai virus (SeV) causes respiratory disease in rodents and sometimes swine. There is limited evidence of zoonotic transmission to humans, but 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**. Additional Sendai virus information

Adenovirus [2]

Virology: Adenoviruses are medium-sized (90-100 nm), nonenveloped icosahedral viruses containing double-stranded DNA. There are more than 49 immunologically distinct types (6 subgenera: A through F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

The adenovirus infection cycle can be clearly divided into two phases, which are separated by viral DNA replication. The first or "early" phase covers the entry of the virus into the host cell and the entry of the virus genome to the nucleus. The late genes are transcribed from the major late promoter. The “late” phase is involved in making gene products that are related to production and assembly of capsid proteins.

Adenoviral Genes	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

Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors is capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

The probability of producing replication competent adenovirus (RCA), 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.

Clinical features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash-associated illnesses. 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 that can cause more systemic diseases (e.g. hepatitis).

Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (e.g., serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. ARD is most often associated with adenovirus types 4 and 7 in the United States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory hazards: Ingestion; droplet exposure of the mucous membrane.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing post injection/exposure of animals. In rodents in the absence of human cells, ABSL-2 for 48h then ABSL-1.

Adenovirus MSDS

Adeno-associated virus[3]

Virology: Adeno-associated virus gets its name because it is often found in cells that are simultaneously infected with adenovirus. AAV are Parvoviridae: icosahedral, 20-25 nm in diameter; single stranded DNA genome with a protein capsid. AAV is dependent on the presence of wild type adenovirus or herpesvirus for replication; in the absence of these helper viruses, AAV will stably integrate into the host cell genome. Co-infection with helper virus triggers a lytic cycle as do some agents which appropriately perturb host cells. Wild type AAV integrates preferentially into human chromosome 19q13.3-qter; recombinant vectors lose this specificity and appear to integrate randomly, thereby posing a theoretical risk of insertional mutagenesis

Clinical features: No known pathology for wild type AAV serotype 2.

Epidemiology: Not documented definitively. Infection apparently via mouth, esophageal, or intestinal mucosa.

Treatment: No specific treatment.

Laboratory hazards: Ingestion, droplet exposure of the mucous membrane, direct injection; insertional mutagenesis; integration and expression of oncogenes or potential oncogenes (see Viral Vector chart for more information).

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

Use in Lab: BSL-1; BSL-2 in the presence of helper virus

Use with Animals: ABSL-1 housing; ABSL-2 housing in the presence of helper virus.

Epstein - Barr virus [4]

Virology: Double-stranded linear DNA, 120-150 nm diameter, enveloped, icosahedral; types A and B; Herpesviridae (Gammaherpesvirinae). Epstein-Barr virus (EBV), a ubiquitous B-lymphotropic herpesvirus, has been found in the tumor cells of a heterogeneous group of malignancies (Burkitt's lymphoma, lymphomas associated with immunosuppression, other non-Hodgkin's lymphomas, Hodgkin's disease, nasopharyngeal carcinoma (NPC), gastric adenocarcinoma, lymphoepithelioma-like carcinomas, and immunodeficiency-related leiomyosarcoma). EBV is a transforming virus and can immortalize B-cells and cause lymphoma in various animal models.

Clinical Features: Infectious mononucleosis - acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy; one to several weeks, rarely fatal/ Burkitt's lymphoma - monoclonal tumor of B cells, usually involving children, jaw involvement is common; AIDS patients(25% -30% are EBV related) / Nasopharyngeal carcinoma - malignant tumor of epithelial cells of the nasopharynx involving adults between 20 and 40 years

Epidemiology: EBV infects 80 - 90% of all adults worldwide; mononucleosis is common in early childhood worldwide, typical disease occurs in developed countries mainly in young adults; Burkitt's tumor is found worldwide but hyperendemic in highly malarial areas such as tropical Africa; carcinoma is worldwide but highest in Southeast Asia and China.

Transmission: Mononucleosis - person-to-person by oropharyngeal route via saliva, possible spread via blood transfusion (not important route); Burkitt's lymphoma - primary infection occurs early in life or involves immunosuppression and reactivation of EBV later, malaria an important co-factor; NPC is associated with EBV infection in early life and reactivation later with epithelial invasion.

Treatment: No specific treatment

Laboratory hazards: Ingestion, accidental parenteral injection, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials. Note that cell lines are often immortalized by transformation with EBV.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to disinfectants - 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing.

Epstein-Barr virus MSDS

Herpesvirus

Virology: *Herpesviridae*, *Alphavirinae*, genus *Simplexvirus*; double-stranded linear DNA virus, icosahedral, lipid envelope, 110 - 200 nm diameter, HSV types 1 and 2 can be differentiated immunologically. Vectors derived from Herpes simplex virus (HSV) have some unique features. The vectors have a wide host range and cell tropism, infecting almost every cell type in most vertebrates that have been examined. In addition, the natural property of the virus to infect and establish latent infection indefinitely in post-mitotic neurons has generated substantial interest in using it to deliver therapeutic genes to the nervous system.

Clinical Features: Classic presentation of primary HSV-1 is herpes gingivostomatitis - oral mucosa, HSV 1 - primary infection is usually mild (10% of cases can be severe) and 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, may be CNS involvement (meningoencephalitis), 70% mortality rate if left untreated; causes about 2% of acute pharyngotonsillitis; Classic presentation of a primary HSV-2 infection is herpes genitalis, HSV 2 - genital herpes, sexually transmitted, associated with aseptic meningitis, vaginal delivery can cause risk to newborn, encephalitis and death; either HSV-1 and HSV-2 may infect the genital tract or oral mucosa.

Epidemiology: Type 1 - contact with saliva of carriers, infection of hands of health care personnel; Type 2 - usually by sexual contact; infected secretions from symptomatic or asymptomatic individuals. Virus may be secreted in saliva for up to 7 weeks after recovery and from genital lesions for 7-12 days: asymptomatic oral and genital infections, with transient viral shedding, are common; reactivation can be precipitated by over-exposure to sunlight, febrile, physical or emotional stress or foods and drugs, especially chemotherapy; HSV may be shed intermittently from mucosal sites for years, possibly lifelong.

HSV is spread by direct contact with epithelial or mucosal surfaces. 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 as 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 produce an environment where replication defective vectors could replicate.

Treatment: anti-viral drug therapy for symptoms.

Laboratory Hazards: Ingestion; accidental parenteral injection; droplet exposure of the mucous membranes of the eyes, nose, or mouth; inhalation of concentrated aerosolized materials

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to common disinfectants - 1% sodium hypochlorite, iodine solutions containing ethanol, 70% ethanol, glutaraldehyde, formaldehyde

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing. Amplicon-only is ABSL-1.

Herpes simplex virus MSDS

Lentivirus[5]

Virology: The genus of the family Retroviridae consists of non-oncogenic retroviruses that produce multi-organ diseases characterized by long incubation periods and persistent infection. Five serogroups are recognized, reflecting the mammalian hosts with which they are associated. HIV-1 is the type species.

- **Bovine lentiviruses** (e.g. Bovine immunodeficiency virus, Jembrana disease virus)
- **Equine lentiviruses** (e.g. Equine infectious anemia virus)
- **Feline lentiviruses** (e.g. Feline immunodeficiency virus)
- **Ovine/caprine lentivirus** (e.g. Caprine arthritis-encephalitis virus, Ovine lentivirus, Visna virus)
- **Primate lentivirus group**
  - Human immunodeficiency virus (HIV) types 1 - 3
  - Simian AIDS retrovirus SRV-1
  - Human T-cell lymphotropic virus type I and II
  - Simian immunodeficiency virus

Most of the lentiviral vectors presently in use are HIV-derived vectors. The *cis*- and *trans*-acting factors of lentiviruses are often on separate plasmid vectors, with packaging being provided in trans. The vector constructs contain the viral *cis* elements, packaging sequences, the Rev response element (RRE), and a transgene [6].

Lentiviral Pseudotyping
Replacement of the HIV envelope glycoprotein with VSV-G provides a broad host-range for the vector and allows the viral particles to be concentrated by centrifugation. Lentiviruses can also be pseudotyped with other envelope proteins, such as the envelope of rabies virus.

Clinical Features[4,7] : In terms of the pathogenesis of lentivirus, some key properties are:

1. **Lentiviruses persist lifelong.** This is a function both of their ability to integrate into the host chromosome and of their ability to evade host immunity. This ability to evade host immunity may be related both to the high mutation rates of these viruses, and to their ability to infect immune cells (macrophages, and in the case of HIV, T-cells).
2. **Lentiviruses have high mutation rates**. Lentiviruses replicate, mutate and undergo selection by host immune responses.
3. **Infection proceeds through at least three stages**.
   (A) Initial (acute) lentivirus infection is associated with rapid viral replication and dissemination, which is often accompanied by a transient period of disease.
   (B) This is followed by a latent period, during which the virus is brought under immune control and no disease occurs.
   (C) High levels of viral replication then resume at some later time, leading to disease.

Acute infection with human lentiviruses can appear as non-specific “flu-like” and “mononucleosis-like” symptoms, including myalgia, arthralgia, diarrhea, nausea, vomiting, headache, hepatosplenomegaly, weight loss and neurological symptoms.

Epidemiology: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

Treatment: Specific measures for the opportunistic diseases that result from AIDS; multidrug treatment for HIV

Laboratory Hazards: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral injection; ingestion; hazard of aerosols exposure unknown; insertional mutagenesis; integration and expression of oncogenes or potential oncogenes (see Viral Vector chart for more information).

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

Use in Lab: BSL-2, BSL-2+ (with amphotropic or VSV-g envelope), BSL-3 (large volumes)

BSL-2+: Defined as working with BSL-2 agents using BSL-3 practices, including, but not limited to: no bench-top work allowed—all work done in a Biosafety Cabinet; decontamination with appropriate disinfectant to be done immediately following any work with biohazardous materials; required use of lab coats and other appropriate PPE, including gloves and eye protection; use of physical containment devices (such as sealed centrifuge rotors) for all activities, opening of these devices only in a Biosafety Cabinet, and decontaminating them immediately after use.

Use with Animals: For use of third generation (or higher), four plasmid Lentiviral vector systems

- In rodents without human cells present: ABSL-2 for 48 hrs, then ABSL-1
- Transformed/transfected cells cultured in vitro for >48 hrs prior to injection into rodent: ABSL-1+

ABSL-1+: - BSC and other safety equipment as needed
- Practices: RAF staff can do husbandry, cage changing

Lentivirus/Human Immunodeficiency Virus MSDS

Moloney Murine Leukemia Virus (MoMuLV or MMLV) [8]

Virology: Retroviridae; subfamily oncovirinae type C, enveloped, icosahedral core, virions 100 nm in diameter, diploid, single stranded, linear RNA genome. MoMuLV integrates into the host genome and is present in infected cells as a DNA provirus. Cell division is required for infection. Virus is not lytic.

Data suggests a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. The nature of a transgene or other introduced genetic element may pose additional risk.

The host range of recombinant MoMuLV vectors is dependent on the specificity of the viral envelope. The ecotropic env gene produces particles which infect only rodent cells. The amphotropic env gene allows infection of rodent and non-rodent cells, including human cells. VSV-G envelope allows infection in a wide range of mammalian and non-mammalian cells.

Clinical features: None to date.

Epidemiology: MoMuLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germline infection. In vivo transduction in humans appears to require direct injection with amphotropic or pseudotyped virus.

Treatment: No recommended treatment.

Laboratory Hazards: Contact with feces or urine from infected animals for 72 hours post infection. Contact with tissues and body fluids of infected animals. Direct injection. Insertional mutagenesis; integration and expression of oncogenes or potential oncogenes (see Viral Vector chart for more information).

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

Use in Lab: BSL-1 (ecotropic), BSL-2 (amphotropic, VSV-g pseudotyped, contain toxin or oncogene)

Use with Animals: ABSL-1 housing for ecotropic, ABSL-2 for amphotropic or pseudotyped vector

Pox viruses/Vaccinia [9]

Virology: 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. Poxviruses do not require nuclear factors for replication and, thus, can replicate with little hindrance in enucleated cells. The core contains a 200-kilobase (kb), double-stranded DNA genome and is surrounded by a lipoprotein core membrane.

Recombinant Vaccinia vectors: Vaccinia virus can accept as much as 25 kb of foreign DNA, making it useful for expressing large eukaryotic and prokaryotic genes. Foreign genes are integrated stably into the viral genome, resulting in efficient replication and expression of biologically active molecules. Furthermore, posttranslational modifications (e.g., methylation, glycosylation) occur normally in the infected cells.

Vaccinia is used to generate live recombinant vaccines for the treatment of other illnesses. Modified versions of vaccinia virus have been developed for use as recombinant vaccines. The modified Ankara strain (MVA) of vaccinia virus was developed by repeated passage in a line of chick embryo fibroblasts. NYVAC is another attenuated form of the vaccinia virus that has been used in the construction of live vaccines. NYVAC has a deletion of 18 vaccinia virus genes that render it less pathogenic.

Clinical Features: Virus disease of skin induced by inoculation for the prevention of smallpox - vesicular or pustular lesion, area of induration or erythema surrounding a scab or ulcer at inoculation site; major complications encephalitis, progressive vaccinia (immunocompromised susceptible), eczema vaccinatum, fetal vaccinia; minor complications - generalized vaccinia with multiple lesions; auto-inoculation of mucous membranes or abraded skin, benign rash, secondary infections; complications are serious for those with eczema or who are immunocompromised.

Epidemiology: Communicable to unvaccinated contacts via contact with mucosal membranes or cuts in skin.

Treatment: Vaccinia immune globulin and an antiviral medication may be of value in treating complications.

Vaccination: Consultation is available to determine if vaccination with the Smallpox vaccine is appropriate for personnel using vaccinia.

Laboratory Hazards: Ingestion, parenteral injection, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing.

Pox/Vaccinia virus MSDS

Rabies virus [4]

Virology: Family Rhabdoviridae, genus *Lyssavirus*; bullet-shaped, enveloped virus; approximately 75nm in diameter by 180 nm in length; single-stranded, negative-sense RNA genome.

Recombinant rabies virus vectors: Replication-deficient rabies vectors can be useful tools for investigation into neuronal trafficking or targeted expression in neurons. SADdG-mCherry/EnvA-SADdG 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 (intentionally) 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.

Second modification: This alters the tropism of the virus so that it cannot infect any mammalian cells except those that express a genetically-specified neuronal population transgene which 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.

If the virus is able to infect a TVA-positive neuron, 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.

In general, as 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.

Pseudotyped rabies virus: Rabies virus in which the rabies envelope gene is deleted can be pseudotypes with a number of different envelope genes, including EnvA, VSV-g, avian sarcoma leucosis virus glycoprotein, or HIV env. This pseudotyping alters the cell tropism of the virus and can be useful for specific experimental purposes.

PLEASE NOTE: The following are Stanford Biosafety definitions for the following terms, and may not be the consistent elsewhere.

Rabies virus	Wild-type rabies virus
Mutant rabies virus	Rabies virus that has been mutated from the original wild-type sequence
Pseudotyped rabies virus	Rabies virus in which the envelope gene has been replaced with the envelope gene from another virus
Pseudorabies virus	NOT A RABIES VIRUS; A herpesvirus that predominantly infects swine, but can also infect a range of other mammals, including rodents

Clinical Features: Initial symptoms of rabies include fever, headache, malaise, and upper respiratory and gastrointestinal tract disorders, which can last 4-10 days. Specific symptoms develop as either encephalitis or paralysis.

Epidemiology: The risk for rabies transmission varies in part with the species of biting animal, the anatomic site of the bite, and the severity of the wound. Although risk for transmission might increase with wound severity, rabies transmission also occurs from bites by some animals (e.g., bats) that inflict rather minor injury compared with larger-bodied carnivores, resulting in lesions

that are difficult to detect under certain circumstances. Any penetration of the skin by teeth constitutes a bite exposure. All bites, regardless of body site or evidence of gross trauma, represent a potential risk. For the past several decades, the majority of naturally acquired, indigenous human rabies cases in the United States have resulted from variants of rabies viruses associated with insectivorous bats. The contamination of open wounds or abrasions (including scratches) or mucous membranes with saliva or other potentially infectious material (e.g., neural tissue) from a rabid animal also constitutes a non-bite exposure. Two cases of rabies have been attributed to probable aerosol exposures in laboratories, and two cases of rabies have been attributed to possible airborne exposures in caves containing millions of free-tailed bats (Tadarida brasiliensis) in the Southwest. However, alternative infection routes cannot be discounted.

Treatment: Wash the wound with a soap solution, followed by 70% ethanol or an iodine containing solution. Following wound care, a clinician must decide whether to begin passive and/or active immunization. There is no established treatment for rabies once symptoms have begun, but supportive therapy may include intubation, sedation, mechanical ventilation, fluid and electrolyte management, nutrition, and management of intercurrent illnesses and complications. Incubation period of 1-3 months is typical, although incubation more than 1 year has been reported in humans. Administration of rabies POST-exposure prophylaxis is a medical urgency, not a medical emergency, but decisions must not be delayed. Prophylaxis is occasionally complicated by adverse reactions, but these reactions are rarely severe. Therefore, when a documented or likely exposure has occurred, POST-exposure prophylaxis should be administered regardless of the length of the delay, provided that compatible clinical signs of rabies are not present in the exposed person. Rabies virus is inactivated by desiccation, ultraviolet irradiation, and other factors and does not persist in the environment. In general, if the suspect material is dry, the virus can be considered noninfectious. Non-bite exposures other than organ or tissue transplants have almost never been proven to cause rabies, and post-exposure prophylaxis is not indicated unless the non-bite exposure met the definition of saliva or other potentially infectious material being introduced into fresh, open cuts in skin or onto mucous membranes.

Vaccination: Consultation is available to determine if vaccination with the Rabies vaccine is appropriate for personnel using rabies.

Laboratory Hazards: Parenteral injection, droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to 70% ethanol, phenol, formalin, ether, trypsin, β-propiolactone and some other detergents.

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing.

Rabies virus MSDS

Sendai virus [10-12]

Virology: Sendai virus, or murine parainfluenza virus type 1, is an enveloped,150-200nm in diameter, single strand, negative-sense RNA virus that is part of the Paramyxoviridae family. It typically infects rodents and swine, and causes a highly transmissible respiratory tract infection. Sendai virus replication occurs in the cytoplasm of infected cells.

Sendai viral vectors: Sendai viral vectors have been developed that are able to express up to four exogenous genes, and are used to create iPSCs for use in cell reprogramming and stem cell research. Sendai virus does not integrate into the genome. Co-infection of Sendai-transduced cells transplanted into animals with wild-type Sendai may lead to expression of exogenous genes in animal models.

Clinical features: No known pathology for Sendai virus.

Epidemiology: Not documented definitively. Infection apparently via aerosol and contact. Capable of infecting human cells in tissue culture.

Treatment: No specific treatment.

Laboratory hazards: Droplet exposure of the mucous membrane, direct injection.

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosol inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Susceptibility to disinfectants: Susceptible to 1% sodium hypochlorite, 70% ethanol, formaldehyde.

Use in Lab: BSL-2

Use with Animals: ABSL-2 housing.

References

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