Section VIII-F: Arboviruses and Related Zoonotic Viruses

In 1979, and again in 1985, the American Committee on Arthropod-Borne Viruses (ACAV) Subcommittee on Arbovirus Laboratory Safety (SALS) provided biosafety recommendations for each of the approximately 500 viruses registered in the International Catalogue of Arboviruses, including Certain Other Viruses of Vertebrates.[1] Since the last print publication of the Catalog, SALS, the CDC, and the NIH have periodically reviewed these viruses as well as newly identified arboviruses and provided recommended biosafety practices and containment for arboviruses identified or registered since that time. These recommendations are based, in part, on risk assessments derived from information provided by a worldwide survey of laboratories working with arboviruses, newly published reports on the viruses, reports of laboratory infections, and discussions with scientists working with each virus.

A series of significant tables are provided throughout Section VIII-F. Table 1 contains a list of vaccine strains of viruses that may be handled at BSL-2. Table 3 provides an alphabetical listing of the recognized arboviruses at the time of publication and includes the common name, acronym, virus family or genus, Biosafety Level (BSL) recommendation, basis for the rating, and antigenic group (if known).[2] Many of the organisms are classified as Select Agents and require special security measures to possess, use, or transfer; see Appendix F for additional information. Table 2 provides a key for the SALS basis for assignment of viruses listed in Tables 3 and 4. Table 4 provides an alphabetical listing of the arthropod-only arboviruses and includes the common name, acronym, virus family or genus, BSL recommendation, basis for the rating, and whether the virus has been isolated. Table 5 provides a list of agents that may be handled at BSL-3 with HEPA-filtered exhaust air. The agents in Tables 1, 3, 4 and 5 require permits from APHIS, DOC, and/or CDC.

It is important to assess the risks of each member of the arbovirus family individually. While arboviral families may share many similarities, each can present their own unique biosafety risks. Viruses that have positive-sense single-stranded RNA carry unique infection risks that are not a consideration for other pathogens. Positive-sense viral RNA can directly cause infection since its RNA can serve as mRNA to direct viral protein synthesis by the host cell.[3] Additionally, disinfection methods aimed at inactivating an enveloped virus may not be effective at rendering a positive-sense single-stranded RNA non-infectious.[4]

In addition to the true arboviruses (i.e., viruses that replicate in both vertebrates and invertebrates), a significant number of arthropod-only viruses (i.e., viruses not known to replicate in vertebrate cells) that are closely related to arboviral counterparts have been identified.[5] While there is no evidence that these viruses

replicate or cause disease in vertebrate cells, most have not been characterized fully enough to confirm this and have been designated as “arthropod-only” based on genetic relationships. The infectivity of these viruses by routes of infection common to the laboratory may be unknown. For this reason, all of these viruses have been assigned Risk Group 2 (RG2) classification based on relationships to the small number that have been characterized. Table 4 lists these viruses as known to date. Table 3 also contains viruses from the family Arenaviridae that are rodent-borne with members known to cause hemorrhagic fever, including Lymphocytic choriomeningitis virus (see Section VIII-E), Guanarito, Junin, Lassa, Machupo, and Sabia virus. Also included are Orthohantaviruses, including Andes, Sin Nombre, and Hantaan, that can be transmitted to humans by rodent urine, saliva, or feces.

Agent summary statements have been included for certain arboviruses. They were submitted by a panel of experts for more detailed consideration due to one or more of the following factors:

- At the time of writing this edition, the organism represented an emerging public health threat in the United States;
- The organism presented unique biocontainment challenge(s) that required further detail; and/or
- The organism presented a significant risk of Laboratory-associated infection.

These recommendations were made in the winter of 2017; requirements for biosafety, shipping, and Select Agent registration can change. Please be sure to confirm the requirements with the appropriate Federal agency. If the pathogen of interest is one listed in Appendix D, contact APHIS for additional biosafety requirements. APHIS guidance may supersede the information found in this section.

Recommendations for the containment of infected arthropod vectors were drafted by a subcommittee of the American Committee on Medical Entomology (ACME) and updated in 2019 as the Arthropod Containment Guidelines version 3.2; see Appendix E for additional information.[6]

Some commonly used vaccine strains for which attenuation has been firmly established are recognized by SALS; these vaccine strains may be handled safely at BSL-2 and are listed in Table 1.

Table 1. Vaccine Strains of Specific Viruses that May Be Handled at BSL-2

Virus	Vaccine Strain
Chikungunya	181/25
Junin	Candid
Rift Valley fever	#1 MP-12
Venezuelan equine encephalomyelitis	TC83 & V3526
Yellow fever	17-D
Japanese encephalitis	14-14-2

Based on the recommendations listed with the tables, the following guidelines should be adhered to where applicable.

Risk Group 2 Viruses with BSL-2 Containment Recommended

The recommendations for conducting work with the viruses listed in Table 3 at BSL-2 are based on the existence of historical laboratory experience adequate to assess the risks when working with this group of viruses. This indicates 1) no overt Laboratory-associated infections are reported; 2) infections resulted from exposures other than by infectious aerosols; or 3) if disease from aerosol exposure is documented, it is uncommon.

Laboratory Safety and Containment Recommendations

Agents listed in this group may be present in blood, CSF, various tissues, and/or infected arthropods depending on the agent and the stage of infection. The primary laboratory hazards are accidental parenteral inoculation, contact of the virus with broken skin or mucous membranes, and bites of infected laboratory rodents or arthropods. Properly maintained BSCs, preferably Class II, or other appropriate personal protective equipment (PPE) or physical containment devices are used whenever procedures with a potential for creating infectious aerosols or splashes are conducted.

BSL-2 practices, containment equipment, and facilities are recommended for activities with potentially infectious clinical materials and arthropods and for manipulations of infected tissue cultures, embryonated hen's eggs, and small vertebrate animals.

Large quantities and/or high concentrations of any virus have the potential to overwhelm both innate immune mechanisms and vaccine-induced immunity. When a virus normally handled at BSL-2 is being produced in large quantities or in high concentrations, additional risk assessment is required. This might indicate BSL-3 practices, including respiratory protection, based on a risk assessment.

West Nile virus (WNV) and St. Louis Encephalitis virus (SLE) risk assessments have been revised to indicate BSL-2 containment may be acceptable for routine work. Prior to moving existing work with either virus from BSL-3 laboratories to BSL-2, a thorough assessment should be made to assess the possible risk from contamination of samples with other agents needing BSL-3 containment.

Risk Group 3 Viruses with BSL-3 Containment Recommended

The recommendations for viruses listed in Table 3 that require BSL-3 containment are based on multiple criteria. SALS considered the laboratory experience for some viruses to be inadequate to assess risk, regardless of the available information regarding disease severity. In some cases, SALS recorded overt Laboratory-associated infections (LAI) transmitted by the aerosol route in the absence or non-use of protective vaccines and considered that the natural disease in humans is potentially severe, life-threatening, or causes residual damage.[1] Arboviruses also were classified as requiring BSL-3 containment if they caused diseases in domestic animals in countries outside of the United States.

Laboratory Safety and Containment Recommendations

The agents listed in this group may be present in blood, CSF, urine, semen, and exudates, depending on the specific agent and stage of disease. The primary laboratory hazards are exposure to aerosols of infectious solutions and animal bedding, accidental parenteral inoculation, and contact with broken skin. Some of these agents (e.g., VEE virus) may be relatively stable in dried blood or exudates.

BSL-3 practices, containment equipment, and facilities are recommended for activities using potentially infectious clinical materials and infected tissue cultures, animals, or arthropods.

A licensed attenuated live virus is available for immunization against yellow fever. It is recommended for all personnel who work with this agent or with infected animals and for those entering rooms where the agents or infected animals are present.

BSL-3 containment is still recommended for Junin virus provided that all at-risk personnel are immunized and the laboratory is equipped with HEPA-filtered exhaust.

SALS also has reclassified Central European tick-borne encephalitis viruses (TBEV-CE subtype) as needing BSL-3 containment, provided all at-risk personnel are immunized. TBEV-CE subtype refers to the following group of very closely related, if not essentially identical, tick-borne flaviviruses isolated from Czechoslovakia, Finland, and Russia: Absettarov, Hanzalova, Hypr, and Kumlinge viruses. While there is a vaccine available that confers immunity to the TBEV-CE subtype group of genetically (>98%) homogeneous viruses, the efficacy of this

vaccine against Russian spring-summer encephalitis virus (RSSEV) (TBEV-FE; Far Eastern subtype) infections has not been established. Thus, the TBEV-CE subtype group of viruses has been reclassified as needing BSL-3 containment when personnel are immunized with TBEV-CE subtype vaccine, while RSSEV (TBEV-FE subtype) remains classified as needing BSL-4 containment.

Select Agent TBEV-CE viruses are Select Agents requiring registration with CDC and/or USDA for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of these agents may require CDC and/or USDA importation permits. Domestic transport of these agents may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of these agents to another country. See Appendix C for additional information.

Vaccines Investigational vaccines for persons working with eastern equine encephalomyelitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), western equine encephalomyelitis virus (WEEV), and Rift Valley fever viruses (RVFV) may be available in limited quantities and administered on-site at the Special Immunization Program of USAMRIID, located at Ft. Detrick, Frederick, MD. These, and other vaccines that are investigational new drugs (IND), are administered under a cooperative agreement between the Special Immunization Program and the individual's requesting organization.

The use of these investigational vaccines for laboratory personnel should be considered if the vaccine is available. Initial studies have shown these vaccines to be effective in producing an appropriate immunologic response, and the adverse effects of vaccination are within acceptable parameters.[7,8,9] The decision to recommend vaccines for laboratory personnel must be carefully considered and based on a risk assessment that includes a review of the characteristics of the agent and the disease, benefits vs. the risk of vaccination, experience of the laboratory personnel, laboratory procedures to be used with the agent, and contraindications for vaccination including the health status of the employee.

If the investigational vaccine is contraindicated or laboratory personnel refuse vaccination, the use of enhanced engineering controls, practices, or personal protective equipment may provide an alternative. Respiratory protection, such as use of a PAPR, is a best practice when using organisms with a well-established risk of aerosol infections in the laboratory, such as VEE viruses.

Any respiratory protection equipment must be provided in conjunction with an appropriately constituted respiratory protection program. Other methods of respiratory protection may be warranted based on an assessment of risk as defined in Section II of this manual. All personnel in a laboratory with the infectious agent

must use comparable personal protective equipment that meets or exceeds the requirements, even if they are not working with the organism. Sharps precautions as described in Section IV must be continually and strictly reinforced, regardless of whether investigational vaccines are used.

Enhanced BSL-3 Containment

HEPA filtration of the exhaust air is recommended for viruses handled at BSL-3 and listed in Table 5.

Situations may arise for which enhancements to BSL-3 practices and equipment are required; for example, when a BSL-3 laboratory performs diagnostic testing on specimens from patients with hemorrhagic fevers thought to be due to dengue or yellow fever viruses. When the origin of these specimens is Africa, the Middle East, or South America, such specimens might contain etiologic agents, such as arenaviruses, filoviruses, or other viruses that are usually manipulated in a BSL-4 laboratory. Examples of enhancements to BSL-3 laboratories include: 1) enhanced respiratory protection of personnel against aerosols; 2) HEPA filtration of exhaust air from the laboratory; and 3) personal body shower upon exit. Additional appropriate training is recommended for all staff, including animal care personnel.

Risk Group 4 Viruses with BSL-4 Containment Recommended

The recommendations for viruses assigned to BSL-4 containment are based on documented cases of severe and frequently fatal, naturally occurring human infections and aerosol-transmitted laboratory infections. SALS recommends that certain agents with a close antigenic or genetic relationship to agents assigned to BSL-4 also be provisionally handled at this level until sufficient laboratory data indicates that work with the agent may be assigned to a lower Biosafety Level.

Laboratory Safety and Containment Recommendations

The infectious agents may be present in blood, urine, respiratory and throat secretions, semen, and other fluids and tissues from human or animal hosts as well as in arthropods, rodents, and non-human primates (NHPs). Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel.[10,11]

BSL-4 practices, containment equipment, and facilities are recommended for all activities using materials of human, animal, or arthropod origin that may be infected with one of the agents listed in this summary. Clinical specimens from persons suspected of being infected with one of the agents listed in this summary should be submitted to a laboratory with a BSL-4 facility.[12]

Dealing with Unknown Arboviruses The ACAV has published reports documenting laboratory workers who acquired arbovirus infections during the course of their duties.[2,13] In the first such report, it was recognized that these laboratory infections typically occurred by unnatural routes such as percutaneous or aerosol exposure, that "lab-adapted" strains were still pathogenic for humans, and that as more laboratories worked with newly identified agents, the frequency of LAIs was increasing. Therefore, to assess the risk of these viruses and provide safety guidelines to those working with them, ACAV appointed SALS to evaluate the hazards of working with arboviruses in the laboratory setting.[2,14,15]

The SALS committee made a series of recommendations, published in 1980, describing four levels of laboratory practices and containment guidelines that were progressively more restrictive. These levels were determined after widely-distributed surveys evaluated numerous criteria for each particular virus including: 1) past occurrence of LAIs correlated with facilities and practices used; 2) volume of work performed as a measure of potential exposure risk; 3) immune status of laboratory personnel; 4) incidence and severity of naturally-acquired infections in adults; and 5) incidence of disease in animals outside the United States (to assess import risk).

While these criteria are still important factors to consider in any risk assessment for manipulating arboviruses in the laboratory, it is important to note that there have been many modifications to personal laboratory practices (e.g., working in a BSC while wearing personal protective equipment in contrast to working with viruses on an open benchtop) and significant changes in laboratory equipment, facilities, and PPE (e.g., BSC, PAPR) available since the initial SALS evaluation. When dealing with a newly recognized or poorly characterized arbovirus, where there is insufficient previous experience to characterize the risk, investigators should consider using additional safety measures. Additionally, when working with field-collected mosquitoes that may contain arboviruses, additional protective measures should be considered, particularly with procedures that can generate aerosols. New methods allow the relationships between newly discovered viruses and other disease-causing arboviruses to be established with less work and less potential for exposure. One criterion for a newly identified arbovirus is a thorough description of how the virus will be handled and investigated. For example, experiments involving pure genetic analysis could be handled differently than those where the virus will be put into animals or arthropods.[16,17] Therefore, in addition to those established by SALS, additional assessment criteria should be considered in the risk assessment.

Most of the identified arboviruses have recommended Biosafety Levels for routine handling; however, a number of those that are infrequently studied, newly identified, or have only single isolation events may not have been fully evaluated by SALS, ACAV, CDC, or the NIH. Thorough risk assessment is important for all

arboviral research and it is of particular importance for work involving unclassified viruses. Additionally, an individual risk assessment should consider the fact that not all strains of a particular virus exhibit the same degree of pathogenicity or transmissibility. A careful assessment by the laboratory director, institutional biosafety officer and safety committee, and outside experts, as necessary, functions to minimize the risk of human, animal, and environmental exposure while allowing research to progress.

Chimeric Viruses The ability to construct cDNA clones encoding a complete RNA viral genome has led to the generation of recombinant viruses containing a mixture of genes from two or more different viruses. Chimeric, full-length viruses and truncated replicons have been constructed from numerous alphaviruses and flaviviruses. For example, alphavirus replicons encoding foreign genes have been used widely as immunogens against bunyavirus, filovirus, arenavirus, and other antigens. These replicons have been safe and usually immunogenic in rodent hosts leading to their development as candidate human vaccines against several virus groups including retroviruses.[18–21]

Because chimeric viruses contain portions of multiple viruses, the IBC or equivalent resource, in conjunction with the biosafety officer and the researchers, must conduct a risk assessment that, in addition to standard criteria, includes specific elements that need to be considered before assigning appropriate Biosafety Levels and containment practices. These elements include: 1) the ability of the chimeric virus to replicate in cell culture and animal model systems in comparison with its parental strains;[22] 2) altered virulence characteristics or attenuation compared with the parental viruses in animal models;[23] 3) virulence or attenuation patterns by intracranial routes using large doses for agents affecting the CNS;[24,25] and 4) demonstration of lack of reversion to virulence or parental phenotype. Additionally, while variable pathogenicity occurs frequently with naturally identified strains, it is of particular note for strains that are modified in the laboratory. It may be tempting to assign Biosafety Levels to hybrid or chimeric strains based on the parental types but due to possible altered biohazard potential, a separate risk assessment needs to be completed, and an assignment to a different Biosafety Level may be justified.[26] A clear description of the strains involved should accompany any risk assessment.

Many patterns of attenuation have been observed with chimeric flaviviruses and alphaviruses using the criteria described above, and some of these chimeras have undergone testing as human vaccines.[27]

Chimeric viruses may have some safety features not associated with parental viruses. For example, they are generated from genetically stable cDNA clones without the need for animal or cell culture passage. This minimizes the possibility of mutations that could alter virulence properties. Because some chimeric strains

incorporate genomic segments lacking gene regions or genetic elements critical for virulence, there may be a limited possibility of genetic changes that could generate strains exhibiting wild-type virulence.

Ongoing surveillance and laboratory studies suggest that many arboviruses continue to be a risk to human and animal populations. The attenuation of all chimeric strains should be verified using the most rigorous containment requirements of the parental strains. The local IBC, or equivalent resource, should evaluate containment recommendations for each chimeric virus on a case-by-case basis, using virulence data from an appropriate animal model. Additional guidance from the NIH Office of Science Policy may be necessary.

West Nile Virus (WNV)

This virus belongs to the family *Flaviviridae* and the genus *Flavivirus*, Japanese encephalitis virus antigenic complex. The complex currently includes Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Rocio, Stratford, Usutu, West Nile, and Yaounde viruses. Flaviviruses share a common size (40–60nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single-stranded RNA approximately 10,000–11,000 bases), and virus morphology. The virus was first isolated from a febrile, adult woman in the West Nile District of Uganda in 1937.[28] The ecology was characterized in Egypt in the 1950s; equine disease was first noted in Egypt and France in the early 1960s.[29,30] It first appeared in North America in 1999 causing encephalitis in humans and horses.[31] The virus has now been detected in Africa, Europe, the Middle East, west and central Asia, Oceania (subtype Kunjin virus), and North and South America.

WNV spread over the past 20 years throughout temperate regions of Europe and North America. As the ecological and epidemiological patterns of this virus in the new geographic regions evolved, WNV is now endemic throughout the U.S. and is one of the most extensively studied arboviruses in this country.

While WNV can cause serious neurologic disease, most people infected with WNV do not have symptoms. About one in five people who are infected develop a fever with other symptoms such as headache, body aches, joint pains, vomiting, diarrhea, or rash. About one out of 150 infected people develop a serious, sometimes fatal, illness affecting the central nervous system such as encephalitis (inflammation of the brain) or meningitis (inflammation of the membranes that surround the brain and spinal cord). Symptoms of severe illness include high fever, headache, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness, and paralysis. There are no vaccines to prevent WNV in people; treatment is supportive.

Occupational Infections

LAIs with WNV have been reported in the literature. SALS reported 15 human infections from laboratory accidents in 1980.[2] One of these infections was attributed to aerosol exposure. However, with the development of improved laboratory and PPE equipment, only three LAIs (due to parenteral inoculations during work with sharps) have been published in the past two decades.[32,33]

Natural Modes of Infection

In the U.S., infected mosquitoes, primarily members of the *Culex* genus, transmit WNV. Virus amplification occurs during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and bird reservoir hosts. Humans, horses, and most other mammals are not known to develop infectious viremias very often, and thus, are probably "dead-end" or incidental hosts.

Laboratory Safety and Containment Recommendations

WNV may be present in blood, serum, tissues, and CSF of infected humans, birds, mammals, and reptiles. The virus has been found in oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals or exposed to feces of infected birds may be at higher risk of infection.

Given the significant number of laboratories working with WNV (with only three parenteral LAIs) and the nearly complete endemicity across the U.S., BSL-2 practices, containment equipment, and facilities are now recommended for all manipulations of WNV. BSL-2 practices and facilities are similarly recommended for the closely related and also endemic St. Louis encephalitis virus. As always, each laboratory should perform a risk assessment to determine if the procedures being conducted might warrant additional containment measures. For example, if working with extremely high titers of virus or aerosol-generating procedures, BSL-3 containment might be considered. For laboratories seeking to move existing work with WNV from BSL-3 laboratories to BSL-2, a thorough assessment should be made to assess the possible risk from contamination of samples with other agents needing BSL-3 containment.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or APHIS importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Eastern Equine Encephalitis Virus (EEEV), Venezuelan Equine Encephalitis Virus (VEEV), and Western Equine Encephalitis Virus (WEEV)

VEEV, EEEV, and WEEV are members of the genus *Alphavirus* in the family *Togaviridae*. They are small, enveloped viruses with a genome consisting of a single strand of positive-sense RNA. All three viruses can cause encephalitis often accompanied by long-term neurological sequelae. The incubation period ranges from one to 10 days, and the duration of acute illness is typically days to weeks depending upon severity of the illness. Although not the natural route of transmission, the viruses are highly infectious by the aerosol route, and LAIs have been documented.[34] Of note, strains of EEEV from South America are now designated as Madariaga virus (MADV) and are no longer considered EEEV viruses.[35] Madariaga virus strains, while still within the EEE antigenic complex, are genetically and ecologically distinct from North American strains of EEEV. They typically do not cause large epizootics, and their capacity to cause human illness is not well-characterized.

The encephalitic alphaviruses are all capable of causing lethal encephalitis in humans and horses; however, the patterns of disease, disease severity, and incidence vary greatly. Most reported cases represent severe forms of disease as the majority of infections are either mild, flu-like illness, or asymptomatic. WEEV is currently the rarest, with no human infections detected since 1988, and fewer than 700 total cases reported in the United States since the 1960s. Young children (<12 months) are the most susceptible to severe disease with an overall mortality rate estimated at about 4%. EEEV is also rare in the United States with an average of seven neurological cases each year. However, encephalitic cases of EEEV infection can have a mortality rate estimated at 30–70% and survivors often experience severe permanent neurological sequelae. VEEV mortality rates are typically around 1% and severe cases are typically in children. One of the largest VEEV outbreaks occurred in Columbia in 1995 and affected approximately 75,000 individuals. Of these, 3,000 developed neurological manifestations with a total of approximately 300 deaths. There are no licensed vaccines or therapeutics available.

Occupational Infections

These alphaviruses, especially VEEV, are infectious by aerosol in laboratory studies and more than 160 EEEV, VEEV, or WEEV LAIs have been documented. Many infections were due to procedures involving high virus concentrations and aerosol-generating activities such as centrifugation and mouth pipetting. Procedures involving animals (e.g., infection of newly hatched chicks with EEEV and WEEV) and mosquitoes are also particularly hazardous.

Natural Modes of Infection

Alphaviruses are zoonoses maintained and amplified in natural transmission cycles involving a variety of mosquito species and either small rodents or birds. Humans and equines are accidental hosts with naturally acquired alphavirus infections resulting from the bites of infected mosquitoes.

EEEV occurs in focal locations along the eastern seaboard, the Gulf Coast, and some inland Midwestern locations of the United States, in Canada, and some Caribbean Islands; the related MADV occurs in Central and South America.[35,36] Small outbreaks of human disease have occurred in the United States, the Dominican Republic, Cuba, and Jamaica. In the United States, equine epizootics are common occurrences during the summer in coastal regions bordering the Atlantic and Gulf of Mexico, in other eastern and Midwestern states, and as far north as Quebec, Ontario, and Alberta in Canada.

In Central and South America, focal outbreaks due to VEE virus occur periodically with rare large regional epizootics involving thousands of equine cases and deaths in predominantly rural settings. These epizootic/epidemic viruses are theorized to emerge periodically from mutations occurring in the continuously circulating enzootic VEE viruses in northern South America. The classical epizootic varieties of the virus are not present in the United States. An enzootic subtype, Everglades virus (VEE antigenic complex subtype II virus), exists naturally in southern Florida; endemic foci of Bijou Bridge virus (VEE antigenic complex subtype III-B virus), have been described in the western United States.[37]

WEEV is found mainly in western parts of the United States and Canada. Sporadic infections also occur in Central and South America.

Laboratory Safety and Containment Recommendations

Alphaviruses may be present in blood, CSF, other tissues (e.g., brain), or throat washings. The primary laboratory hazards are parenteral inoculation, contact of the virus with broken skin or mucous membranes, bites of infected animals or arthropods, or aerosol inhalation.

Diagnostic and research activities involving clinical material, infectious cultures, and infected animals or arthropods should be performed with BSL-3 practices, containment equipment, and facilities. Due to the high risk of aerosol infection, respiratory protection is a best practice for non-immune personnel. Animal work with VEEV, EEEV, and WEEV should be performed under ABSL-3 conditions. HEPA filtration is required on the exhaust system of laboratory and animal facilities using VEEV.

Special Issues

Vaccines Two strains of VEEV (TC-83 and V3526) are highly attenuated in vertebrate studies and are excluded from Select Agent regulations. Because of the low level of pathogenicity, these strains may be safely handled under BSL-2 conditions without vaccination or additional personal protective equipment (e.g., respiratory protection).

Investigational vaccine protocols have been developed to immunize at-risk laboratory or field personnel against these alphaviruses; however, the vaccines are available only on a limited basis and may be contraindicated for some personnel. Therefore, additional personal protective equipment may be warranted if vaccination can't be administered. For personnel who have no neutralizing antibody titer (from previous vaccination or natural infection), respiratory protection should be considered for all procedures.

Select Agent Epizootic (equine amplification-competent) subtype strains of VEEV (subtypes IAB and IC) and EEEV (but not MADV) are Select Agents requiring registration with CDC and/or APHIS for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or APHIS importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

Rift Valley Fever Virus (RVFV)

RVFV was first isolated in Kenya in 1936 and subsequently shown to be endemically present in almost all areas of sub-Saharan Africa.[38] In periods of heavy rainfall, large epizootics occur involving primarily sheep, cattle, and human disease, although many other species are infected. The primordial vertebrate reservoir is unknown, but the introduction of large herds of highly susceptible domestic breeds in the last few decades has provided a substrate for massive virus amplification. The virus has been introduced into Egypt, Saudi Arabia, and Yemen and caused epizootics and epidemics in those countries. The largest of these was from 1977 to 1979 in Egypt with many thousands of human cases and 610 reported deaths.[39]

Most human infections are symptomatic and the most common syndrome consists of fever, myalgia, malaise, anorexia, and other non-specific symptoms. Recovery within one to two weeks is usual, but hemorrhagic fever, encephalitis, or retinitis also occur. Hemorrhagic fever develops as the primary illness progresses and is characterized by disseminated intravascular coagulation and hepatitis. Perhaps 2% of cases will develop this complication and the mortality

is high. Encephalitis follows apparent recovery in <1% of cases and results in a substantial mortality and sequelae. Retinal vasculitis occurs in convalescence of a substantial, but not precisely known, proportion of cases. The retinal lesions are often macular and permanent, leading to substantial loss of visual acuity.

Infected sheep and cattle suffer a mortality rate of 10–35%, and spontaneous abortion occurs virtually in all pregnant females. Other animals studied have lower viremia and lesser mortality but may abort. This virus is a World Organization for Animal Health (OIE) List A disease and triggers export sanctions.

Occupational Infections

The potential for infection of humans by routes other than arthropod transmission was first recognized in veterinarians performing necropsies. Subsequently, it became apparent that contact with infected animal tissues and infectious aerosols were dangerous; many infections were documented in herders, slaughterhouse workers, and veterinarians. Most of these infections resulted from exposure to blood and other tissues including aborted fetal tissues of sick animals.

There have been 47 reported laboratory infections; before modern containment and vaccination became available, virtually every laboratory that began work with the virus suffered infections suggestive of aerosol transmission.[40,41]

Natural Modes of Infection

Field studies show RVFV to be transmitted predominantly by mosquitoes; although, other arthropods may be infected and transmit. Mechanical transmission also has been documented in the laboratory. Floodwater *Aedes* species are the primary vector and transovarial transmission is an important part of the maintenance cycle.[42] However, many different mosquito species are implicated in horizontal transmission in field studies, and laboratory studies have shown a large number of mosquito species worldwide to be competent vectors, including North American mosquitoes.

It is currently believed that the virus passes dry seasons in the ova of flood-water *Aedes* mosquitoes. Rain allows infectious mosquitoes to emerge and feed on vertebrates. Several mosquito species can be responsible for horizontal spread, particularly in epizootic/epidemic situations. The vertebrate amplifiers are usually sheep and cattle, with two caveats: 1) a native African vertebrate amplifier is thought to exist but is yet to be defined, and 2) very high viremias in humans are thought to play some role in viral amplifications.[43]

Transmission of disease occurs between infected animals but is of low efficiency; virus titers in throat swabs are low. Nosocomial infection rarely, if ever, occurs. There are no examples of latency with RVFV, although virus may be isolated from lymphoid organs of mice and sheep for four to six weeks post-infection.

Laboratory Safety and Containment Recommendations

Concentrations of RVFV in blood and tissues of sick animals are often very high. Placenta, amniotic fluid, and fetuses from aborted domestic animals are highly infectious. Large numbers of infectious virus particles also are generated in cell cultures and laboratory animals.

BSL-3 practices, containment equipment, and facilities are recommended for processing human or animal material in endemic zones or in non-endemic areas in emergency circumstances. Particular care should be given to stringent aerosol containment practices, autoclaving waste, decontamination of work areas, and control of egress of material from the laboratory. Other cultures, cells, or similar biological material that could potentially harbor RVFV should not be used in an RVFV laboratory and subsequently removed.

Diagnostic or research studies outside endemic areas should be performed in a BSL-3 laboratory. Personnel also must have respiratory protection (e.g., PAPR) or be vaccinated for RVFV. In addition, APHIS may require full ABSL-3Ag containment for research conducted in non-endemic areas using loose-housed animals. See Appendix D for additional information.

Special Issues

Vaccines Two apparently effective vaccines have been developed by the Department of Defense (DOD) and have been used in volunteers, laboratory staff, and fieldworkers under investigational protocols, but neither vaccine is available at this time.

Select Agent RVFV is a Select Agent requiring registration with CDC and/or APHIS for possession, use, storage and/or transfer. See Appendix F for additional information.

The live-attenuated MP-12 vaccine strain and the ΔNSs-ΔNSm-ZH501 strain are excluded from the Select Agent regulations. In general, BSL-2 containment is recommended for working with these strains.

APHIS may require ABSL-3 enhanced, ABSL-3, or ABSL-3Ag facilities and practices for working with RVFV in the United States; see Appendix D for additional information. Investigators should contact APHIS for further guidance before initiating research.

Transfer of Agent Importation of this agent may require CDC and/or APHIS importation permits. Domestic transport of this agent may require a permit from USDA APHIS VS. A Department of Commerce (DoC) permit may be required for the export of this agent to another country. See Appendix C for additional information.

Table 2. Explanation of Symbols Used in Tables 3 and 4 to Define Basis for Assignment of Viruses to Biosafety Levels

Symbol	Definition
S	Results of SALS survey and information from the Catalog.[1]
IE	Insufficient experience with virus in laboratory facilities with low biocontainment.
A	Additional Criteria (A1–A8)
A1	Disease in sheep, cattle, or horses.
A2	Fatal human laboratory infection—probably aerosol.
A3	Extensive laboratory experience and mild nature of aerosol laboratory infections justify BSL-2.
A4	Placed in BSL-4 based on the close antigenic relationship with a known agent handled at BSL-4 plus insufficient experience.
A5	Arenaviruses handled at BSL-2 are not known to cause serious acute disease in humans and are not acutely pathogenic for laboratory animals including primates. It is strongly recommended that work with high concentrations of these arenaviruses be done at BSL-3.
A6	Level assigned to prototype or wild-type virus. A lower level may be recommended for variants with well-defined reduced virulence characteristics.
A7	Placed at this Biosafety Level based on close antigenic or genetic relationship to other viruses in a group of three or more viruses, all of which are classified at this level.
A8	Hantaviruses handled at BSL-2 are not known to cause laboratory infections, overt disease in humans, or severe disease in experimental primates. Because of antigenic and biologic relationships to highly pathogenic hantaviruses and the likelihood that experimentally infected rodents may shed large amounts of virus, it is recommended that work with high concentrations of virus or experimentally infected rodents be conducted at BSL-3.

Table 3. Alphabetic Listing of Arboviruses and Hemorrhagic Fever Viruses*

Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Abadina	ABAV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Above Maiden	ABMV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Abras	ABRV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Patois
Absettarov	ABSV	*Flaviviridae*	*Flavivirus*	4	A4	Tick-borne Encephalitis—CE subtype
Abu Hammad	AHV	*Nairoviridae*	*Orthonairovirus*	2	S	Dera Ghazi Khan
Abu Mina	ABMV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Acado	ACDV	*Reoviridae*	*Orbivirus*	2	S	Corriparta
Acara	ACAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Capim
Achiote	ACHOV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	California
Adana	ADAV	*Phenuiviridae*	*Phlebovirus*	2	A7	Salehabad
Adelaide River	ARV	*Rhabdoviridae*	*Ephemerovirus*	2	IE	Bovine Ephemeral Fever
Adria	ADRV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
African horse sickness	AHSV	*Reoviridae*	*Orbivirus*	3[b]	A1	African Horse Sickness
African swine fever	ASFV	*Asfarviridae*	*Asfivirus*	3[b]	IE	Asfivirus
Aguacate	AGUV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Aino	AINOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Akabane	AKAV	*Peribunyaviridae*	*Orthobunyavirus*	3[b]	S	Simbu
Alajuela	ALJV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Alcube	N/A	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Alenquer	ALEV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Alfuy	ALFV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Alkhurma	AHFV	*Flaviviridae*	*Flavivirus*	4	A4	Tick-borne Encephalitis—CE subtype
Allpahuayo	ALLPV	*Arenaviridae*	*Mammarenavirus*	3	IE	Tacaribe
Almeirim	ALMV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Almpiwar	ALMV	*Rhabdoviridae*	*Sripuvirus*	2	S	N/A
Altamira	ALTV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Amaparí	AMAV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Ambe	AMBEV	*Phenuiviridae*	*Phlebovirus*	2	IE	N/A
Amga	MGAV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Amur/Soochong	ASV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Anadyr	ANADV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Anajatuba	ANJV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Ananindeua	ANUV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Guama
Andasibe	ANDV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Andes	ANDV	*Hantavirudae*	*Orthohantavirus*	3[a]	IE	Hantaan
Anhanga	ANHV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Anhembi	AMBV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Anopheles A	ANAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Anopheles A
Anopheles B	ANBV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Anopheles B
Antequera	ANTV	*Unclassified Bunyavirales*		2	IE	Antequera
Apeú	APEUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Apoi	APOIV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Araguari	ARAV	*Orthomyxoviridae*	Unassigned	3	IE	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Aransas Bay	ABV	*Orthomyxoviridae*	*Thogotovirus*	2	IE	Upolu
Araraquara	ARQV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Araucaria	ARAUV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Arbia	ARBV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Arboledas	ADSV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Arbroath	ABRV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Aride	ARIV	*Unclassified virus*		2	S	N/A
Ariquemes	ARQV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Arkonam	ARKV	*Reoviridae*	*Orbivirus*	2	S	N/A
Armero	ARMV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Aroa	AROAV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Arrabida	ARRV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Artashat	ARTSV	*Nairoviridae*	*Orthonairovirus*	3	IE	N/A
Aruac	ARUV	*Rhabdoviridae*	Unassigned	2	S	N/A
Arumateua	ARMTV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Arumowot	AMTV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Asama	ASAV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Asikkala	ASIV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Aura	AURAV	*Togaviridae*	*Alphavirus*	2	S	Western Equine Encephalitis
Avalon	AVAV	*Nairoviridae*	*Orthonairovirus*	2	S	Sakhalin
Babahoyo	BABV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Patois
Babanki	BBKV	*Togaviridae*	*Alphavirus*	2	A7	Western Equine Encephalitis
Bagaza	BAGV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Bahig	BAHV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Tete
Bakau	BAKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bakau
Bakel	BAKV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Baku	BAKUV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Balkan	BALKV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Bandia	BDAV	*Nairoviridae*	*Orthonairovirus*	2	S	Qalyub
Bangoran	BGNV	*Rhabdoviridae*	Unassigned	2	S	N/A
Bangui	BGIV	*Unclassified Bunyavirales*	N/A	2	S	N/A
Banna	BAV	*Reoviridae*	*Seadornavirus*	3	IE	N/A
Banzi	BANV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Barmah Forest	BFV	*Togaviridae*	*Alphavirus*	2	A7	Barmah Forest
Barranqueras	BQSV	*Unclassified Bunyavirales*	N/A	2	IE	Antequera
Barur	BARV	*Rhabdoviridae*	*Ledantevirus*	2	S	Kern Canyon
Batai	BATV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Batama	BMAV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Tete
Batken	BKNV	*Orthomyxoviridae*	*Thogotovirus*	2	IE	N/A
Batu Cave	BCV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Bauline	BAUV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Bayou	BAYV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
BeAr 328208	BAV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Bear Canyon	BCNV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Beatrice Hill	BHV	*Rhabdoviridae*	*Tibrovirus*	2	IE	N/A
Beaumont	BEAUV	*Rhabdoviridae*	Unassigned	2	A7	N/A
Bebaru	BEBV	*Togaviridae*	*Alphavirus*	2	S	Semliki Forest
Belem	BLMV	*Unclassified Bunyavirales*	N/A	2	IE	N/A
Belmont	BELV	*Unclassified Bunyavirales*	N/A	2	S	N/A
Belterra	BELTV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Benevides	BENV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Capim
Benfica	BNFV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Capim
Bermejo	BMJV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Berrimah	BRMV	*Rhabdoviridae*	*Ephemerovirus*	2	IE	Bovine Ephemeral Fever
Bertioga	BERV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Bhanja	BHAV	*Phenuiviridae*	*Phlebovirus*	3	S	Bhanja
Big Brushy Tank	BBTV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Big Cypress	BCPOV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Bimbo	BBOV	*Rhabdoviridae*	Unassigned	2	IE	N/A
Bimiti	BIMV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Birao	BIRV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Bivens Arm	BAV	*Rhabdoviridae*	*Tibrovirus*	2	IE	N/A
Black Creek Canal	BCCV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Bloodland Lake	BLLV	*Hantaviridae*	*Orthohantavirus*	**2a**	A8	N/A
Blue River	BRV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Bluetongue (exotic serotypes)	BTV	*Reoviridae*	*Orbivirus*	3[b]	S	Bluetoungue
Bluetongue (non-exotic)	BTV	*Reoviridae*	*Orbivirus*	2[b]	S	Bluetoungue
Bobaya	BOBV	*Unclassified Bunyavirales*	N/A	2	IE	N/A
Bobia	BIAV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Olifantsvlei
Boracéia	BORV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Anopheles B
Botambi	BOTV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Olifantsvlei
Boteke	BTKV	*Rhabdoviridae*	*Vesiculovirus*	2	S	Vesicular Stomatitis
Bouboui	BOUV	*Flaviviridae*	*Flavivirus*	2	S	Bouboui
Bourbon	BRBV	*Orthomyxoviridae*	*Thogotovirus*	2	A7	N/A
Bovine ephemeral fever	BEFV	*Rhabdoviridae*	*Ephemerovirus*	3	A1	Bovine Ephemeral Fever
Bowe	BOWV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Bozo	BOZOV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Bunyamwera
Brazoran		*Peribunyaviridae*	Unassigned	2	A7	N/A
Breu Branco	BRBV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Broadhaven	BRDV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Bruconha	BRUV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Bruges	BRGV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Buenaventura	BUEV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomous Fever
Buggy Creek		*Togaviridae*	*Alphavirus*	2	A7	Western Equine Encephalitis
Bujaru	BUJV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Bukalasa bat	BBV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Bundibugyo	BDBV	*Filoviridae*	*Ebolavirus*	4	A4	Ebola

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Bunyamwera	BUNV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Bunyip Creek	BCV	*Reoviridae*	*Orbivirus*	2	S	N/A
Burana	BURV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Burg El Arab	BEAV	*Unclassified Bunyavirales*	N/A	2	S	N/A
Bushbush	BSBV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Bussuquara	BSQV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Buttonwillow	BUTV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Bwamba	BWAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Cabassou	CABV	*Togaviridae*	*Alphavirus*	3	IE	Venezuelan Equine Encephalitis
Cacao	CACV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Cache Valley	CVV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Cachoeira Portiera	CPOV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Cacipacoré	CPCV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Caimito	CAIV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Calchaqui	CQIV	*Peribunyaviridae*	Unassigned	2	A7	Gamboa
California encephalitis	CEV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Calovo	CVOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Campana	CMAV	*Phenuiviridae*	*Phlebovirus*	2	A7	Punta Toro
Cananeia	CNAV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	N/A
Candiru	CDUV	*Phenuiviridae*	*Phlebovirus*	2	S	Candiru
Caninde	CANV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Cano Delgadito	CADV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Cao Bang	CBNV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Cape Wrath	CWV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Capim	CAPV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Capim
Capira	CAPV	*Phenuiviridae*	*Phlebovirus*	2	A7	Punta Toro
Caraipé	CRPV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Carajás	CRJV	*Rhabdoviridae*	*Vesiculovirus*	2	A7	Vesicular Stomatitis
Caraparú	CARV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Carey Island	CIV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Caspiy	CASV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Castelo dos Sonhos	CASV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Cat Que	CQV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Catarina	CTNV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Catú	CATUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Chaco	CHOV	*Rhabdoviridae*	*Sripuvirus*	2	S	Timbo
Chagres	CHGV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Chandipura	CHPV	*Rhabdoviridae*	*Vesiculovirus*	2	S	Vesicular Stomatitis
Changuinola	CGLV	*Reoviridae*	*Orbivirus*	2	S	Changuinola
Chapare	CHAPV	*Arenaviridae*	*Mammarenavirus*	4	A4	N/A
Charleville	CHVV	*Rhabdoviridae*	Unassigned	2	S	Rab
Chenuda	CNUV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Chikungunya	CHIKV	*Togaviridae*	*Alphavirus*	3	S	Semliki Forest
Chilibre	CHIV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Chim	CHIMV	*Nairoviridae*	*Orthonairovirus*	2	IE	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Chizé	CHZV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Chobar Gorge	CGV	*Reoviridae*	*Orbivirus*	2	S	Chobar Gorge
Choclo	CHOV	*Hantavirus*	*Orthohantavirus*	3[a]	A7	N/A
Clo Mor	CMV	*Nairoviridae*	*Orthonairovirus*	2	S	Sakhalin
CoAr 1071	CA1071V	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
CoAr 3627	CA3627V	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Coastal Plains	CPV	*Rhabdoviridae*	*Tibrovirus*	2	IE	Tibrogargan
Cocal	COCV	*Rhabdoviridae*	*Vesiculovirus*	2	A3	Vesicular Stomatitis
Cocle	CCLV	*Phenuiviridae*	*Phlebovirus*	2	A7	Punta Toro
Codajas	CDJV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Colony	COYV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Colony B North	CBNV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Colorado tick fever	CTFV	*Reoviridae*	*Coltivirus*	2	S	Colorado Tick Fever
Crimean-Congo hemorrhagic fever	CCHFV	*Nairoviridae*	*Orthonairovirus*	4	A7	Crimean-Congo hemorrhagic fever
Connecticut	CNTV	*Rhabdoviridae*	Unassigned	2	IE	Sawgrass
Corfou	CFUV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Corriparta	CORV	*Reoviridae*	*Orbivirus*	2	S	Corriparta
Cotia	COTV	*Poxviridae*	Unassigned	2	S	N/A
Cowbone Ridge	CRV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Csiro Village	CVGV	*Reoviridae*	*Orbivirus*	2	S	Palyam
Cuiaba	CUIV	*Rhabdoviridae*	Unassigned	2	S	N/A
Cupixi	CPXV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Curionopolis	CRNPV	*Rhabdoviridae*	*Curiovirus*	2	A7	N/A
Dabakala	DABV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Olifantsvlei
Dabieshan	DBSV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
D'Aguilar	DAGV	*Reoviridae*	*Orbivirus*	2	S	Palyam
Dakar bat	DBV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Dandenong	DANV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
Dashli	DASHV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Deer tick	DRTV	*Flaviviridae*	*Flavivirus*	3	A7	N/A
Dengue virus 1	DENV-1	*Flaviviridae*	*Flavivirus*	2	S	N/A
Dengue virus 2	DENV-2	*Flaviviridae*	*Flavivirus*	2	S	N/A
Dengue virus 3	DENV-3	*Flaviviridae*	*Flavivirus*	2	S	N/A
Dengue virus 4	DENV-4	*Flaviviridae*	*Flavivirus*	2	S	N/A
Dera Ghazi Khan	DGKV	*Nairoviridae*	*Orthonairovirus*	2	S	Dera Ghazi Khan
Dobrava-Belgrade	DOBV	*Hantaviridae*	*Orthohantavirus*	3a	IE	N/A
Dhori	DHOV	*Orthomyxoviridae*	*Thogotovirus*	2	S	N/A
Douglas	DOUV	*Peribunyaviridae*	*Orthobunyavirus*	3	IE	Simbu
Durania	DURV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Durham	DURV	*Rhabdoviridae*	*Tupavirus*	2	IE	N/A
Dugbe	DUGV	*Nairoviridae*	*Orthonairovirus*	3	S	Nairobi Sheep Disease
Eastern equine encephalitis	EEEV	*Togaviridae*	*Alphavirus*	3[b]	S	Eastern Equine Encephalitis
Ebola	EBOV	*Filoviridae*	*Ebolavirus*	4	S	Ebola
Edge Hill	EHV	*Flaviviridae*	*Flavivirus*	2	S	N/A
EgAN 1825-61	EGAV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
El Huayo	EHUV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
El Moro Canyon	ELMCV	*Hantaviridae*	*Orthohantavirus*	3a	A7	N/A
Ellidaey	ELLV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Enseada	ENSV	*Unclassified Bunyavirales*	N/A	3	IE	N/A
Entebbe bat	ENTV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Epizootic hemorrhagic disease	EHDV	*Reoviridae*	*Orbivirus*	2	S	Epizootic Hemorrhagic Disease
Equine encephalosis	EEV	*Reoviridae*	*Orbivirus*	3	A1	N/A
Eret	ERETV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Erve	ERVEV	*Nairoviridae*	*Orthonairovirus*	2	S	Thiafora
Escharte	ESCV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Essaouira	ESSV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Estero Real	ERV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Patois
Eubenangee	EUBV	*Reoviridae*	*Orbivirus*	2	S	Eubenangee
Everglades	EVEV	*Togaviridae*	*Alphavirus*	3	S	Venezuelan Equine Encephalitis
Eyach	EYAV	*Reoviridae*	*Coltivirus*	2	S	Colorado Tick Fever
Facey's Paddock	FPV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Farallon	FARV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Farmington	FRMV	*Rhabdoviridae*	Unassigned	2	A7	N/A
Fermo	FERV	*Phenuiviridae*	*Phlebovirus*	2	A7	Sandfly Fever Naples
Fikirini	FKRV	*Rhabdoviridae*	*Ledantevirus*	2	A7	N/A
Fin V 707	FINV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Finch Creek	FINCV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Fitzroy River	FRV	*Flaviviridae*	*Flavivirus*	3	A7	Yellow Fever
Flanders	FLAV	*Rhabdoviridae*	*Hapavirus*	2	S	Hart Park
Flexal	FLEV	*Arenaviridae*	*Mammarenavirus*	3	S	Tacaribe
Fomede	FV	*Reoviridae*	*Orbivirus*	2	A7	Chobar Gorge
Forécariah	FORV	*Phenuiviridae*	*Phlebovirus*	2	A7	Bhanja
Fort Morgan	FMV	*Togaviridae*	*Alphavirus*	2	S	Western Equine Encephalitis
Fort Sherman	FSV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Bunyamwera
Foula	FOUV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Fraser Point	FPV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Frijoles	FRIV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Fugong	FUGV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Fukuoka	FUKV	*Rhabdoviridae*	*Ledantevirus*	2	A7	N/A
Fusong	FUSV	*Hantaviridae*	*Orthohantavirus*	3	A7	N/A
Gabek Forest	GFV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Gadgets Gully	GGYV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Gairo	GAIV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A
Gamboa	GAMV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Gamboa
Gan Gan	GGV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Mapputta
Garatuba	GTBV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Garba	GARV	*Rhabdoviridae*	Unassigned	2	IE	Matariva
Garissa	GRSV	*Peribunyaviridae*	*Orthobunyavirus*	3	A7	Bunyamwera
Geran	GERV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Germiston	GERV	*Peribunyaviridae*	*Orthobunyavirus*	3		Bunyamwera
Getah	GETV	*Togaviridae*	*Alphavirus*	2	A1	Semliki Forest

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Gomoka	GOMV	*Reoviridae*	*Orbivirus*	2	S	Ieri
Gordil	GORV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Gossas	GOSV	*Nairoviridae*	*Orthonairovirus*	2	S	N/A
Gou	GOUV	*Hantaviridae*	*Orthohantavirus*	2[a]	IE	N/A
Gouleako	GOLV	*Phenuiviridae*	*Goukovirus*	3	IE	N/A
Granada	GRAV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Grand Arbaud	GAV	*Phenuiviridae*	*Phlebovirus*	2	S	Uukuniemi
Gray Lodge	GLOV	*Rhabdoviridae*	*Hapavirus*	2	IE	Vesicular Stomatitis
Great Island	GIV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Great Saltee	GRSV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Great Saltee Island	GSIV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Grimsey	GSYV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Guajará	GJAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Capim
Guamá	GMAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Guanarito	GTOV	*Arenaviridae*	*Mammarenavirus*	4	A4	Tacaribe
Guaratuba	GTBV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Guama
Guaroa	GROV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Gumbo Limbo	GLV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Gurupi	GURV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Gweru	GWV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Hantaan	HTNV	*Hantaviridae*	*Orthohantavirus*	3[a]	S	Hantaan
Hanzalova	HANV	*Flaviviridae*	*Flavivirus*	4	A4	Tick-borne Encephalitis—CE subtype
Hart Park	HPV	*Rhabdoviridae*	*Hapavirus*	2	S	Hart Park
Hazara	HAZV	*Nairoviridae*	*Orthonairovirus*	2	S	CCHF
Heartland	HRTV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Highlands J	HJV	*Togaviridae*	*Alphavirus*	2	S	Western Equine Encephalitis
Huacho	HUAV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Hughes	HUGV	*Nairoviridae*	*Orthonairovirus*	2	S	Hughes
Hunter Island	HUIV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Hypr	HYPRV	*Flaviviridae*	*Flavivirus*	4	S	Tick-borne Encephalitis—CE subtype
Iaco	IACOV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Ibaraki	IBAV	*Reoviridae*	*Orbivirus*	2	IE	Epizootic Hemorrhagic Disease
Icoaraci	ICOV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Ieri	IERIV	*Reoviridae*	*Orbivirus*	2	S	Ieri
Ife	IFEV	*Reoviridae*	*Orbivirus*	2	IE	N/A
Iguape	IGUV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Ilesha	ILEV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Ilhéus	ILHV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Imjin	MJNV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Infirmatus	INFV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	California
Ingwavuma	INGV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Inhangapi	INHV	*Rhabdoviridae*	Unassigned	2	IE	N/A
Inini	INIV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Simbu
Inkoo	INKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Inner Farne	INFV	*Reoviridae*	*Orbivirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Ippy	IPPYV	*Arenaviridae*	*Mammarenavirus*	2	S	Tacaribe
Iquitos	IQTV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Iriri	IRRV	*Rhabdoviridae*	*Curiovirus*	2	A7	N/A
Irituia	IRIV	*Reoviridae*	*Orbivirus*	2	S	Changuinola
Isfahan	ISFV	*Rhabdoviridae*	*Vesiculovirus*	2	S	Vesicular Stomatitis
Israel turkey meningoencephalitis	ITV	*Flaviviridae*	*Flavivirus*	2 with 3 practices	S	N/A
Issyk-Kul	ISKV	*Nairoviridae*	*Orthonairovirus*	3	IE	N/A
Itacaiunas	ITCNV	*Rhabdoviridae*	*Curiovirus*	2	A7	N/A
Itaituba	ITAV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Itaporanga	ITPV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Itaquí	ITQV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Itaya		*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Itimirim	ITIV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Guama
Itupiranga	ITUV	*Reoviridae*	*Orbivirus*	2	II	N/A
Ixcanal	IXCV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Jacareacanga	JACV	*Reoviridae*	*Orbivirus*	2	IE	Corriparta
Jacunda	JCNV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Jamanxi	JAMV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Jamestown Canyon	JCV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Japanaut	JAPV	*Reoviridae*	*Orbivirus*	2	S	N/A
Japanese encephalitis	JEV	*Flaviviridae*	*Flavivirus*	3[b]	S	N/A
Jari	JARIV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Jatobal	JTBV	*Preibunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Jeju	JJUV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Jerry Slough	JSV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Joa	JOAV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Johnston Atoll	JAV	*Orthomyxoviridae*	*Quaranjavirus*	2	S	Quaranfil
Joinjakaka	JOIV	*Rhabdoviridae*	*Hapavirus*	2	S	N/A
Juan Diaz	JDV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Capim
Jugra	JUGV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Junín	JUNV	*Arenaviridae*	*Mammarenavirus*	4	A6	Tacaribe
Juquitiba	JUQV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Jurona	JURV	*Rhabdoviridae*	*Vesiculovirus*	2	S	Vesicular Stomatitis
Juruaca	JRCV	*Picornaviridae*	Unassigned	2	A7	N/A
Jutiapa	JUTV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Kabuto Mountain	KAMV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Kachemak Bay	KBV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Kadam	KADV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Kaeng Khoi	KKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Kaikalur	KAIV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Kairi	KRIV	*Peribunyaviridae*	*Orthobunyavirus*	2	A1	Bunyamwera
Kaisodi	KSOV	*Unclassified Bunyavirales*	N/A	2	S	Kaisodi
Kala Iris	KIRV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Kamese	KAMV	*Rhabdoviridae*	*Hapavirus*	2	S	Hart Park
Kammavanpettai	KMPV	*Reoviridae*	*Orbivirus*	2	S	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Kannamangalam	KANV	*Rhabdoviridae*	Unassigned	2	S	N/A
Kanyawara	KYAV	*Rhabdoviridae*	*Ledantevirus*	2	A7	N/A
Kao Shuan	KSV	*Nairoviridae*	*Orthonairovirus*	2	S	N/A
Karimabad	KARV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Karshi	KSIV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Kasba	KASV	*Reoviridae*	*Orbivirus*	2	S	N/A
Kasokero	KASV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Kédougou	KEDV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Kemerovo	KEMV	*Reoviridae*	*Orbivirus*	2	S	N/A
Kenai	KENV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Kenkeme	KKMV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Kern Canyon	KCV	*Rhabdoviridae*	*Ledantevirus*	2	S	N/A
Ketapang	KETV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Keterah	KTRV	*Nairoviridae*	*Orthonairovirus*	2	S	N/A
Keuraliba	KEUV	*Rhabdoviridae*	*Ledantevirus*	2	S	N/A
Keystone	KEYV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Khabarovsk	KHAV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Kharagysh	KHAV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Khasan	KHAV	*Phenuiviridae*	*Phlebovirus*	2	IE	CCHF
Khatanga	KHATV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Kimberley	KIMV	*Rhabdoviridae*	*Ephemerovirus*	2	A7	Bovine Ephemeral Fever
Kindia	KINV	*Reoviridae*	*Orbivirus*	2	A7	Palyam
Kismayo	KISV	*Phenuiviridae*	*Phlebovirus*	2	S	Bhanja
Klamath	KLAV	*Rhabdoviridae*	*Tupavirus*	2	S	Vesicular Stomatitis
Kokobera	KOKV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Kolente	KOLEV	*Rhabdoviridae*	*Ledantevirus*	2	A7	N/A
Kolongo	KOLV	*Rhabdoviridae*	Unassigned	2	S	Rab
Komandory	KOMV	*Phenuiviridae*	*Phlebovirus*	2	IE	N/A
Koongol	KOOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Koongol
Kotonkan	KOTV	*Rhabdoviridae*	*Ephemerovirus*	2	S	Rab
Koutango	KOUV	*Flaviviridae*	*Flavivirus*	3	S	N/A
Kowanyama	KOWV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Kumlinge	KUMV	*Flaviviridae*	*Flavivirus*	4	A4	Tick-borne Encephalitis—CE subtype
Kunjin	KUNV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Kununurra	KNAV	*Rhabdoviridae*	Unassigned	2	S	N/A
Kupe	KUPV	*Nairoviridae*	*Orthonairovirus*	3	IE	N/A
Kwatta	KWAV	*Rhabdoviridae*	Unassigned	2	S	Vesicular Stomatitis
Kyasanur Forest disease	KFDV	*Flaviviridae*	*Flavivirus*	4	S	N/A
Kyzylagach	KYZV	*Togaviridae*	*Alphavirus*	2	IE	Western Equine Encephalitis
La Crosse	LACV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Lagos bat	LBV	*Rhabdoviridae*	*Lyssavirus*	2	S	Rab
Laguna Negra	LANV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Laibin	LAIV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
La Joya	LJV	*Rhabdoviridae*	*Hapavirus*	2	S	Vesicular Stomatitis
Lake Chad	LKCV	*Orthomyxoviridae*	*Quaranjavirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Lake Clarendon	LCV	*Reoviridae*	*Orbivirus*	2	IE	N/A
Landjia	LJAV	*Rhabdoviridae*	*Hapavirus*	2	S	N/A
Langat	LGTV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Lanjan	LJNV	*Unclassified Bunyavirales*	N/A	2	S	Kaisodi
Las Maloyas	LMV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Anopheles A
Lassa	LASV	*Arenaviridae*	*Mammarenavirus*	4	S	N/A
Latino	LATV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Leanyer	LEAV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Lebombo	LEBV	*Reoviridae*	*Orbivirus*	2	S	N/A
Lechiguanas	LECHV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Le Dantec	LDV	*Rhabdoviridae*	*Ledantevirus*	2	S	Le Dantec
Lednice	LEDV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Turlock
Leopards Hill	LPHV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Leticia	LTCV	*Phenuiviridae*	*Phlebovirus*	2	A7	Punta Toro
Lipovnik	LIPV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Llano Seco	LLSV	*Reoviridae*	*Orbivirus*	2	IE	Umatilla
Loei River	LORV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Lokern	LOKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Lone Star	LSV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Longquan	LQUV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Louping Ill	LIV	*Flaviviridae*	*Flavivirus*	3[b]	S	N/A
Lujo	LUJV	*Arenaviridae*	*Mammarenavirus*	4	A4	N/A
Lukuni	LUKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Anopheles A
Lumbo	LUMV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Luna	LUNV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A
Lundy	LUNV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Lunk	LNKV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Luxi	LUXV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Lymphocytic choriomeningitis	LCMV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
Macaua	MCAV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Machupo	MACV	*Arenaviridae*	*Mammarenavirus*	4	S	Tacaribe
Maciel	MCLV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Madariaga	MADV	*Togaviridae*	*Alphavirus*	3	A7	Eastern Equine Encephalitis
Madre de Dios	MDDV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Madrid	MADV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Maguari	MAGV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Mahogany Hammock	MHV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Maiden	MDNV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Main Drain	MDV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Malakal	MALV	*Rhabdoviridae*	*Ephemerovirus*	2	S	Bovine Ephemeral
Maldonado	MLOV	*Phenuiviridae*	*Phlebovirus*	2	A7	Candiru
Malsoor	MALV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Manawa	MWAV	*Phenuiviridae*	*Phlebovirus*	2	S	Uukuniemi
Manitoba	MNTBV	*Rhabdoviridae*	*Hapavirus*	2	A7	N/A
Manzanilla	MANV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Mapputta	MAPV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Mapputta
Maporal	MAPV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Maprik	MPKV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Mapputta
Maraba	MARAV	*Rhabdoviridae*	*Vesiculovirus*	2	A7	N/A
Marajo	MRJV	*Unclassified virus*	N/A	2	IE	N/A
Marburg	MARV	*Filoviridae*	*Marburgvirus*	4	S	Marburg
Marco	MCOV	*Rhabdoviridae*	*Hapavirus*	2	S	N/A
Mariental	MRLV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Maripa	MARV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Mariquita	MRQV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Marituba	MTBV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Marondera	MRDV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Marrakai	MARV	*Reoviridae*	*Orbivirus*	2	S	N/A
Massila	MASV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Matariya	MTYV	*Rhabdoviridae*	Unassigned	2	S	N/A
Matruh	MTRV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Matucare	MATV	*Reoviridae*	*Orbivirus*	2	S	N/A
Mayaro	MAYV	*Togaviridae*	*Alphavirus*	2	S	Semliki Forest
Mboke	MBOV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Mburo	MBUV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Meaban	MEAV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Medjerda Valley	MVV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Melao	MELV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Merino Walk	MWV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Mermet	MERV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Middelburg	MIDV	*Togaviridae*	*Alphavirus*	2	A1	Middelburg
Mill Door	MDR	*Reoviridae*	*Orbivirus*	2	A7	N/A
Minacu	N/A	*Reoviridae*	*Orbivirus*	2	IE	N/A
Minatitlan	MNTV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Minatitlan
Minnal	MINV	*Reoviridae*	*Orbivirus*	2	S	Umatilla
Mirim	MIRV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Mitchell River	MRV	*Reoviridae*	*Orbivirus*	2	S	N/A
Mobala	MOBV	*Arenaviridae*	*Mammarenavirus*	3	A7	Tacaribe
Modoc	MODV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Moju	MOJUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Guama
Mojui Dos Campos	MDCV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	N/A
Mono Lake	MLV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Monongahela	MGLV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Montana myotis leukoencephalitis	MMLV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Montano	MTNV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Monte Dourado	MDOV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Mopeia	MOPV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A
Moriche	MORV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Capim
Morolillo	MOLV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Morreton	MORV	*Rhabdoviridae*	*Vesiculovirus*	2	A7	Vesicular Stomatitis
Morro Bay	MBV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	California

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Morogoro	MORV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A
Morumbi	MRMBV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Mosqueiro	MQOV	*Rhabdoviridae*	*Hapavirus*	2	A7	Hart Park
Mosso das Pedras	MDPV	*Togaviridae*	*Alphavirus*	3	A7	Venezuelan Equine Encephalitis
Mossuril	MOSV	*Rhabdoviridae*	*Hapavirus*	2	S	Hart Park
Mount Elgon bat	MEBV	*Rhabdoviridae*	*Ledantevirus*	2	S	Vesicular Stomatitis
Mudjinbarry	MUDV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Muju	MUJV	*Hantaviridae*	*Orthohantavirus*	2[a]	A8	N/A
Muleshoe	MULV	*Hantaviridae*	*Orthohantavirus*	2[a]	A8	N/A
M'Poko	MPOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Turlock
Mucambo	MUCV	*Togaviridae*	*Alphavirus*	3	S	Venezuelan Equine Encephalitis
Mucura	MCRV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Munguba	MUNV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Murray Valley encephalitis	MVEV	*Flaviviridae*	*Flavivirus*	3	S	N/A
Murre	MURV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Murutucú	MURV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Mykines	MYKV	*Reoviridae*	*Orbivirus*	2	A7	Kemerovo
Nairobi sheep disease	NSDV	*Nairoviridae*	*Orthonairovirus*	3[b]	A1	Nairobi Sheep Disease
Nanjianyin	N/A	*Flaviviridae*	*Flavivirus*	4	A4	Tick-borne Encephalitis—CE subtype
Naranjal	NJLV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Nasoule	NASV	*Rhabdoviridae*	Unassigned	2	A7	Rab
Navarro	NAVV	*Rhabdoviridae*	Unassigned	2	S	N/A
Ndumu	NDUV	*Togaviridae*	*Alphavirus*	2	A1	Ndumu
Necocli	NECV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Negishi	NEGV	*Flaviviridae*	*Flavivirus*	3	S	Tick-borne Encephalitis—CE subtype
Nepuyo	NEPV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Netivot	NETV	*Reoviridae*	*Orbivirus*	2	A7	N/A
New Minto	NMV	*Rhabdoviridae*	Unassigned	2	IE	Sawgrass
New York	NYOV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Ngaingan	NGAV	*Rhabdoviridae*	*Hapavirus*	2	S	Tibrogargan
Ngaric	NRIV	*Peribunyaviridae*	*Orthobunyavirus*	3	A7	Bunyamwera
Ngoupe	NGOV	*Reoviridae*	*Orbivirus*	2	A7	Eubenangee
Ninarumi	NRUV	*Reoviridae*	*Orbivirus*	3	A7	N/A
Nique	NIQV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Nkolbisson	NKOV	*Rhabdoviridae*	*Ledantevirus*	2	S	Kern Canyon
Nodamura	NOV	*Nodaviridae*	*Alphanodavirus*	2	IE	N/A
Nola	NOLAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bakau
North Clett	NCLV	*Reoviridae*	*Orbivirus*	2	A7	N/A
North Creek	NORCV	*Rhabdoviridae*	Unassigned	2	A7	N/A
North End	NEDV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Northway	NORV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Nova	NVAV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Ntaya	NTAV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Nugget	NUGV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Nyabira	NYAV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Nyamanini	NYMV	*Nyamaninidae*	*Nyavirus*	2	S	Nyamanini
Nyando	NDV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Nyando
Oceanside	OCV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Oak Vale	OVV	*Rhabdoviridae*	Unassigned	2	A7	N/A
Ockelbo	N/A	*Togaviridae*	*Alphavirus*	2	A7	Western Equine Encephalitis
Odrenisrou	ODRV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Oita	OITAV	*Rhabdoviridae*	*Ledantevirus*	2	A7	N/A
Okahandja	OKAV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Okhotskiy	OKHV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Okola	OKOV	*Unclassified Bunyavirales*		2	S	Tanga
Olbia	OLBV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Olifantsvlei	OLIV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Olifantsvlei
Oliveros	OLVV	*Arenaviridae*	*Mammarenavirus*	3	A7	N/A
Omo	OMOV	*Nairoviridae*	*Orthonairovirus*	2	A7	Qalyub
Omsk hemorrhagic fever	OHFV	*Flaviviridae*	*Flavivirus*	4	S	N/A
O'nyong-nyong	ONNV	*Togaviridae*	*Alphavirus*	2	S	Semliki Forest
Orán	ORANV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Oriboca	ORIV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Oriximiná	ORXV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Oropouche	OROV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Orungo	ORUV	*Reoviridae*	*Orbivirus*	2	S	Orungo
Ossa	OSSAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Ouango	OUAV	*Rhabdoviridae*	Unassigned	2	IE	N/A
Oubangui	OUBV	*Poxviridae*	Unassigned	2	IE	N/A
Oubi	OUBIV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Olifantsvlei
Ourem	OURV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Oxbow	OXBV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Pacora	PCAV	*Unclassified Bunyavirales*		2	S	N/A
Pacui	PACV	*Peribunyaviridae*	Unassigned	2	S	N/A
Pahayokee	PAHV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Patois
Palma	PMAV	*Phenuiviridae*	*Phlebovirus*	2	IE	Bhanja
Palestina	PLSV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Minatitlan
Palyam	PALV	*Reoviridae*	*Orbivirus*	2	S	Palyam
Para	PARAV	*Peribunyaviridae*	Unassigned	2	IE	Simbu
Paramushir	PMRV	*Nairoviridae*	*Orthonairovirus*	2	IE	Sakhalin
Paraná	PARV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Paranoá	PARV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Paroo River	PRV	*Reoviridae*	*Orbivirus*	2	IE	N/A
Parry's Lagoon	PLV	*Reoviridae*	*Orbivirus*	2	IE	N/A
Pata	PATAV	*Reoviridae*	*Orbivirus*	2	S	N/A
Pathum Thani	PTHV	*Nairoviridae*	*Orthonairovirus*	2	S	Dera Ghazi Khan
Patois	PATV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Patois
Peaton	PEAV	*Peribunyaviridae*	*Orthobunyavirus*	2	A1	Simbu
Perdões	N/A	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Pergamino	PRGV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Perinet	PERV	*Rhabdoviridae*	*Vesiculovirus*	2	A7	Vesicular Stomatitis
Peruvian horse sickness	PHSV	*Reoviridae*	*Orbivirus*	3	A1	N/A
Petevo	PETV	*Reoviridae*	*Orbivirus*	2	A7	Palyam
Phnom Penh bat	PPBV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Pichindé	PICHV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Picola	PIAV	*Reoviridae*	*Orbivirus*	2	IE	Wongorr
Pintupo	N/A	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Pirital	PIRV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Piry	PIRYV	*Rhabdoviridae*	*Vesiculovirus*	3	S	Vesicular Stomatitis
Pixuna	PIXV	*Togaviridae*	*Alphavirus*	2	S	Venezuelan equine encephalitis
Playas	PLAV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Pongola	PGAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bwamba
Ponteves	PTVV	*Phenuiviridae*	*Phlebovirus*	2	A7	Uukuniemi
Poovoot	POOV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Potiskum	POTV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Potosi	POTV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Powassan	POWV	*Flaviviridae*	*Flavivirus*	3	S	N/A
Precarious Point	PPV	*Phenuiviridae*	*Phlebovirus*	2	A7	Uukuniemi
Pretoria	PREV	*Nairoviridae*	*Orthonairovirus*	2	S	Dera Ghazi Khan
Prospect Hill	PHV	*Hantaviridae*	*Orthohantavirus*	2	A8	Hantaan
Puchong	PUCV	*Rhabdoviridae*	*Ephemerovirus*	2	S	Bovine Ephemeral Fever
Pueblo Viejo	PVV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Gamboa
Puffin Island	PIV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Punique	PUNV	*Phenuiviridae*	*Phlebovirus*	2	A7	Sandfly Fever Naples
Punta Salinas	PSV	*Nairoviridae*	*Orthonairovirus*	2	S	Hughes
Punta Toro	PTV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Purus	PURV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Puumala	PUUV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Qalyub	QYBV	*Nairoviridae*	*Orthonairovirus*	2	S	Qalyub
Quaranfil	QRFV	*Orthomyxoviridae*	*Quaranjavirus*	2	S	Quaranfil
Quezon	QZNV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Radi	RADIV	*Rhabdoviridae*	*Vesiculovirus*	2	A7	Vesicular Stomatitis
Ravn	RAVV	*Filoviridae*	*Marburgvirus*	4	S	Marburg
Raza	RAZAV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Razdan	RAZV	*Phenuiviridae*	Unassigned	2	IE	N/A
Resistencia	RTAV	*Unclassified Bunyavirales*		2	IE	Antequera
Restan	RESV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Reston	REST	*Filoviridae*	*Ebolavirus*	4	S	Ebola
Rift Valley fever	RVFV	*Phenuiviridae*	*Phlebovirus*	3[b]	S	Phlebotomus Fever
Rio Bravo	RBV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Rio Grande	RGV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Rio Mamoré	RIOMV	*Hantaviridae*	*Orthohantavirus*	3[a]	A7	N/A
Rio Negro	RNV	*Togaviridae*	*Alphavirus*	3	A7	Venezuelan Equine Encephalitis
Rio Pracupi	N/A	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Rio Preto da Eva	RIOPV	*Phenuiviridae*	Unassigned	2	IE	N/A
Riverside	RISV	*Rhabdoviridae*	Unassigned	2	IE	N/A
RML 105355	RMLV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Rochambeau	RBUV	*Rhabdoviridae*	*Curiovirus*	2	IE	Rab
Rocio	ROCV	*Flaviviridae*	*Flavivirus*	3	S	N/A
Rockport	RKPV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Ross River	RRV	*Togaviridae*	*Alphavirus*	2	S	Semliki Forest
Rost Island	RSTV	*Reoviridae*	*Orbivirus*	2	A7	Kemerovo
Royal Farm	RFV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Rukutama	RUKV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Russian spring-summer encephalitis	RSSEV	*Flaviviridae*	*Flavivirus*	4	S	Tick-borne Encephalitis—FE subtype
Ryukyu	RYKV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
Saaremaa	SAAV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Sabiá	SABV	*Arenaviridae*	*Mammarenavirus*	4	A4	N/A
Sabo	SABOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Saboya	SABV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Saddaguia	SADV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Sagiyama	SAGV	*Togaviridae*	*Alphavirus*	2	A1	Semliki Forest
Saint-Floris	SAFV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Sakhalin	SAKV	*Nairoviridae*	*Orthonairovirus*	2	S	Sakhalin
Salanga	SGAV	*Poxviridae*	Unassigned	2	IE	SGA
Salehabad	SALV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Salmon River	SAVV	*Reoviridae*	*Coltivirus*	2	IE	Colorado Tick Fever
Salobo	SBOV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Sal Vieja	SVV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
San Angelo	SAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Sandfly fever Cyprus	N/A	*Phenuiviridae*	*Phlebovirus*	2	IE	N/A
Sandfly fever Ethiopia	N/A	*Phenuiviridae*	*Phlebovirus*	2	IE	N/A
Sandfly fever Naples	SFNV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Sandfly fever Sicilian	SFSV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Sandfly fever Turkey	SFTV	*Phenuiviridae*	*Phlebovirus*	2	IE	N/A
Sandjimba	SJAV	*Rhabdoviridae*	Unassigned	2	S	Rab
Sangassou	SANGV	*Hantaviridae*	*Orthohantavirus*	3	A7	N/A
Sango	SANV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
San Juan	SJV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Gamboa
San Perlita	SPV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Santarem	STMV	*Unclassified Bunyavirales*	N/A	2	IE	N/A
Santa Rosa	SARV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Sapphire II	SAPV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Saraca	SRAV	*Reoviridae*	*Orbivirus*	2	IE	Changuinola
Sathuperi	SATV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Sathuvachari	SVIV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Saumarez Reef	SREV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Sawgrass	SAWV	*Rhabdoviridae*	Unassigned	2	S	Sawgrass
Schmallenberg	SBV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Sebokele	SEBV	*Picornaviridae*	*Parechovirus*	2	S	N/A
Sedlec	SEDV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Seletar	SELV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Sembalam	SEMV	*Unclassified virus*	N/A	2	S	N/A
Semliki Forest	SFV	*Togaviridae*	*Alphavirus*	3	A2	Semliki Forest
Sena Madureira	SMV	*Rhabdoviridae*	*Sripuvirus*	2	IE	Timbo
Seoul	SEOV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Sepik	SEPV	*Flaviviridae*	*Flavivirus*	2	IE	N/A
Serra Do Navio	SDNV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	California
Serra Norte	SRNV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Severe fever with thrombocytopenia syndrome	SFTSV	*Phenuiviridae*	*Phlebovirus*	3	IE	N/A
Shamonda	SHAV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Shark River	SRV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Patois
Shiant Island	SHIV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Shokwe	SHOV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Shuni	SHUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Silverwater	SILV	*Phenuiviridae*	*Phlebovirus*	2	S	Kaisodi
Simbu	SIMV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Sindbis	SINV	*Togaviridae*	*Alphavirus*	2	S	Western Equine Encephalitis
Sin Nombre	SNV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Sixgun City	SCV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Skinner Tank	SKTV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
Snowshoe hare	SSHV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Sokoluk	SOKV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Soldado	SOLV	*Nairoviridae*	*Orthonairovirus*	2	S	Hughes
Solwezi	SOLV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Somone	SOMV	*Unclassified virus*		3	IE	Somone
Sororoca	SORV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Souris	SOUV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
South Bay	SBV	*Unclassified Bunyavirales*	N/A	3	IE	N/A
South River	SORV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Spondweni	SPOV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Sripur	SRIV	*Rhabdoviridae*	*Sripuvirus*	3	IE	N/A
St. Abbs Head	SAHV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
St. Louis encephalitis	SLEV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Stanfield	N/A	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Stratford	STRV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Sudan	SUDV	*Filoviridae*	*Ebolavirus*	4	S	Ebola
Sunday Canyon	SCAV	*Phenuiviridae*	*Phlebovirus*	2	S	N/A
Sweetwater Branch	SWBV	*Rhabdoviridae*	*Tibrovirus*	2	IE	N/A
Tacaiuma	TCMV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Anopheles A
Tacaribe	TCRV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Tǎchéng tick 1	TTV-1	*Nairoviridae*	*Orthonairovirus*	2	IE	N/A
Taggert	TAGV	*Nairoviridae*	*Orthonairovirus*	2	S	Sakhalin
Tahyña	TAHV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Taiassui	TAIAV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Taï Forest	TAFV	*Filoviridae*	*Ebolavirus*	4	S	Ebola
Tamdy	TDYV	*Nairoviridae*	*Orthonairovirus*	2	IE	N/A
Tamiami	TMMV	*Arenaviridae*	*Mammarenavirus*	2	A5	Tacaribe
Tanga	TANV	*Unclassified Bunyavirales*	N/A	2	S	Tanga
Tanjong Rabok	TRV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bakau
Tapara	TAPV	*Phenuiviridae*	*Phlebovirus*	2	A7	N/A
Tataguine	TATV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A
Tehran	TEHV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Telok Forest	TFV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bakau
Tembe	TMEV	*Reoviridae*	*Orbivirus*	2	S	N/A
Tembusu	TMUV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Tensaw	TENV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Termeil	TERV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	N/A
Tete	TETEV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Tete
Thailand	THAIV	*Hantaviridae*	*Orthohantavirus*	3	A7	N/A
Thiafora	TFAV	*Nairoviridae*	*Orthonairovirus*	2	A7	Thiafora
Thimiri	THIV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Simbu
Thogoto	THOV	*Orthomyxoviridae*	*Thogotovirus*	2	S	Thogoto
Thormodseyjarlettur	THRV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Thottapalayam	TPMV	*Hantaviridae*	*Orthohantavirus*	2	S	Hantaan
Tibrogargan	TIBV	*Rhabdoviridae*	*Tibrovirus*	2	S	Tibrogargan
Tillamook	TILLV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Tilligerry	TILV	*Reoviridae*	*Orbivirus*	2	IE	Eubenangee
Timbo	TIMV	*Rhabdoviridae*	Unassigned	2	S	Timbo
Timboteua	TBTV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	Guama
Tinaroo	TINV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Simbu
Tindholmur	TDMV	*Reoviridae*	*Orbivirus*	2	A7	Kemerovo
Tlacotalpan	TLAV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Bunyamwera
Tofla	TFLV	*Nairoviridae*	*Orthonairovirus*	2	IE	N/A
Tonate	TONV	*Togaviridae*	*Alphavirus*	3	IE	Venezuelan Equine Encephalitis
Tonto Creek	TTCV	*Arenaviridae*	*Mammarenavirus*	2	A5	N/A
Topografov	TOPV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	Hantaan
Toscana	TOSV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Toure	TOUV	*Arenavirudae*	Unassigned	2	S	Tacaribe
Tracambe	TRCV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Tribeč	TRBV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Triniti	TNTV	*Togaviridae*	Unassigned	2	S	N/A
Trivittatus	TVTV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	California
Trocara	TROV	*Togaviridae*	*Alphavirus*	2	IE	Trocara
Trombetas	TRMV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Trubanaman	TRUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Mapputta
Tsuruse	TSUV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Tete
Tucunduba	TUCV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Tucurui	TUCRV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Tula	TULV	*Hantaviridae*	*Orthohantavirus*	2[a]	A8	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Tunari	TUNV	*Hantaviridae*	*Orthohantavirus*	3a	A7	N/A
Tunis	TUNV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Turlock	TURV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Turlock
Turuna	TUAV	*Phenuiviridae*	*Phlebovirus*	2	IE	Phlebotomus Fever
Tyulek	TLKV	*Orthomyxoviridae*	*Quaranjavirus*	2	A7	N/A
Tyuleniy	TYUV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Uganda S	UGSV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Umatilla	UMAV	*Reoviridae*	*Orbivirus*	2	S	Umatilla
Umbre	UMBV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Turlock
Una	UNAV	*Togaviridae*	*Alphavirus*	2	S	Semliki Forest
Upolu	UPOV	*Orthomyxoviridae*	*Thogotovirus*	2	S	Upolu
Uriurana	UURV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Urucuri	URUV	*Phenuiviridae*	*Phlebovirus*	2	S	Phlebotomus Fever
Usutu	USUV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Utinga	UTIV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	Simbu
Utive	UVV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Uukuniemi	UUKV	*Phenuiviridae*	*Phlebovirus*	2	S	Uukuniemi
Uzun-Agach	UZAV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Vaeroy	VAEV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Vellore	VELV	*Reoviridae*	*Orbivirus*	2	S	Palyam
Venezuelan equine encephalitis	VEEV	*Togaviridae*	*Alphavirus*	3[b]	S	Venezuelan Equine Encephalitis
Venkatapuram	VKTV	*Unclassified virus*	N/A	2	S	N/A
Vesicular stomatitis—Alagoas	VSAV	*Rhabdoviridae*	*Vesiculovirus*	2[b]	S	Vesicular Stomatitis
Vesicular stomatitis—Indiana	VSIV	*Rhabdoviridae*	*Vesiculovirus*	2[b]	A3	Vesicular Stomatitis
Vesicular stomatitis—New Jersey	VSNJV	*Rhabdoviridae*	*Vesiculovirus*	2[b]	A3	Vesicular Stomatitis
Vinces	VINV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Vinegar Hill	VHV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Virgin River	VRV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Wad Medani	WMV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Wallal	WALV	*Reoviridae*	*Orbivirus*	2	S	Wallal
Wanowrie	WANV	*Unclassified Bunyavirales*	N/A	2	S	N/A
Warrego	WARV	*Reoviridae*	*Orbivirus*	2	S	Warrego
Warrego K	WARKV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Weldona	WELV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Wēnzhōu	WENV	*Arenaviridae*	*Mammarenavirus*	3	IE	N/A
Wēnzhōu tick	WTV	*Nairoviridae*	*Orthonairovirus*	2	A7	N/A
Wesselsbron	WESSV	*Flaviviridae*	*Flavivirus*	3[b]	S	N/A
Western equine encephalitis	WEEV	*Togaviridae*	*Alphavirus*	3	S	Western Equine Encephalitis
West Nile	WNV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Wexford	WEXV	*Reoviridae*	*Orbivirus*	2	A7	N/A
Whataroa	WHAV	*Togaviridae*	*Alphavirus*	2	S	Western Equine Encephalitis
Whitewater Arroyo	WWAV	*Arenaviridae*	*Mammarenavirus*	3	IE	Tacaribe
Witwatersrand	WITV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	N/A

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Virus Name	Acronym	Family	Genus	Recommended BSL	Basis of Rating	Antigenic Group
Wolkberg	WBV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	N/A
Wongal	WONV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Koongol
Wongorr	WGRV	*Reoviridae*	*Orbivirus*	2	S	Wongorr
Wyeomyia	WYOV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Bunyamwera
Xiburema	XIBV	*Rhabdoviridae*	Unassigned	2	IE	N/A
Xingu	XINV	*Peribunyaviridae*	*Orthobunyavirus*	3	N/A	Bunyamwera
Yaba-1	Y1V	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A
Yaba-7	Y7V	*Peribunyaviridae*	*Orthobunyavirus*	3	IE	N/A
Yacaaba	YACV	*Peribunyaviridae*	*Orthobunyavirus*	2	IE	N/A
Yakeshi	YKSV	*Hantaviridae*	*Orthohantavirus*	3[a]	IE	N/A
Yaoundé	YAOV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Yaquina Head	YHV	*Reoviridae*	*Orbivirus*	2	S	Kemerovo
Yata	YATAV	*Rhabdoviridae*	*Ephemerovirus*	2	S	N/A
Yellow fever	YFV	*Flaviviridae*	*Flavivirus*	3	S	N/A
Yogue	YOGV	*Nairoviridae*	*Orthonairovirus*	2	S	Yogue
Yoka	YOKAV	*Poxviridae*	Unassigned	2	IE	N/A
Yokose	YOKV	*Flaviviridae*	*Flavivirus*	2	A7	N/A
Yug Bogdanovac	YBV	*Rhabdoviridae*	*Vesiculovirus*	2	IE	Vesicular Stomatitis
Yunnan orbivirus	YOUV	*Reoviridae*	*Orbivirus*	3	IE	N/A
Zaliv Terpeniya	ZTV	*Phenuiviridae*	*Phlebovirus*	2	S	Uukuniemi
Zegla	ZEGV	*Peribunyaviridae*	*Orthobunyavirus*	2	S	Patois
Zerdali	ZERV	*Phenuiviridae*	*Phlebovirus*	2	A7	Phlebotomus Fever
Zika	ZIKV	*Flaviviridae*	*Flavivirus*	2	S	N/A
Zirqa	ZIRV	*Nairoviridae*	*Orthonairovirus*	2	S	Hughes
Zungarococha	ZUNV	*Peribunyaviridae*	*Orthobunyavirus*	2	A7	N/A

*Federal regulations, import/export requirements, and taxonomic status are subject to changes. Check with the appropriate federal agency to confirm regulations and ICTV for most current taxonomic status.

a. Containment requirements will vary based on virus concentration, animal species, or virus type. See the Hantavirus agent summary statement in Section VIII-E.

b. These organisms are considered pathogens of significant agricultural importance by APHIS (see Appendix D) and may require additional containment up to and including ABSL-3Ag containment. Not all strains of each organism are necessarily of concern to APHIS. Contact APHIS for more information regarding exact containment/permit requirements before initiating work.

c. Garissa virus is considered an isolate of this virus, so same containment requirements apply.

Table 4. Alphabetic Listing of Arboviruses and Hemorrhagic Fever Viruses*

Virus Name	Acronym	Family	Genus	Recommended Biosafety Level	Basis of Rating	Isolate
Aedes aegypti densovirus	AaeDNV	*Parvoviridae*	*Brevidensovirus*	2	IE	Yes
Aedes albopictus densovirus	AalDNV	*Parvoviridae*	Brevidensovirus	2	IE	Yes
Aedes cinereus flavivirus	AeciFV	*Flaviviridae*	*Unassigned*	2	IE	?
Aedes galloisi flavivirus	AGFV	*Flaviviridae*	*Unassigned*	2	IE	?
Aedes flavivirus	AEFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Aedes pseudoscutellaris densovirus	N/A	*Parvoviridae*	*Brevidensovirus*	2	IE	?
Aedes pseudoscutellaris reovirus	N/A	*Reoviridae*	*Dinovernavirus*	2	IE	Yes
Aedes vexans flavivirus	AeveFV	*Flaviviridae*	*Unassigned*	2	IE	?
Anopheles flavivirus	N/A	*Flaviviridae*	*Unassigned*	2	IE	?
Anopheles gambiae densovirus	AgDNV	*Parvoviridae*	*Unassigned*	2	IE	Yes
Arboretum	ABTV	*Rhabdoviridae*	*Almendravirus*	2	IE	Yes
Aripo	N/A	*Flaviviridae*	*Unassigned*	2	IE	Yes
Assam	N/A	*Flaviviridae*	*Unassigned*	2	IE	?
Badu	BADUV	*Phenuiviridae*	*Phasivirus*	2	IE	Yes
Balsa	BALV	*Rhabdoviridae*	*Almendravirus*	2	IE	Yes
Barkedji	BJV	*Flaviviridae*	*Unassigned*	2	IE	?
Bontang Baru	BBaV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Brejeira	BRJV	*Unassigned*	*Negevirus*	2	IE	Yes
Calbertado	CLBOV	*Flaviviridae*	Unassigned	2	IE	?
Casuarina	CASV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Cavally	CavV	*Mesoniviridae*	*Alphamesonivirus*	2	IE	Yes
Cell Fusing Agent	CFAV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Chaoyang	CHAOV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Coot Bay	CBV	*Rhabdoviridae*	*Almendravirus*	2	IE	Yes
Culex flavivirus	CxFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Culex Y	N/A	*Birnaviridae*	*Entomobirnavirus*	2	IE	Yes
Culex theileri flavivirus	CxthFV/ CTFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Culiseta flavivirus	CsFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Cumuto	CUMV	*Bunyavirales*	*Goukovirus*	2	IE	Yes
Czech Aedes vexans flavivirus	Czech AeveFV	*Flaviviridae*	Unassigned	2	IE	?
Dak Nong	DKNG	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Dezidougou	DEZV	*Unassigned*	*Negevirus*	2	IE	Yes
Donggang	DONV	*Flaviviridae*	*Unassigned*	2	IE	?
Eilat	EILV	*Togaviridae*	*Alphavirus*	2	IE	Yes
Ecuador Paraiso Escondido	EPEV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Espirito Santo	ESV	*Birnaviridae*	*Unassigned*	2	IE	Yes
Gouleako	GOUV	*Bunyaviridae*	*Goukovirus*	2	IE	Yes
Goutanap	GANV	*Unassigned*	*Negevirus*	2	IE	Yes
Guaico Culex	GCXV	*Jingmenvirus*	*Unassigned*	2	IE	Yes
Hana	HanaV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Hanko	HANKV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Herbert	HEBV	*Peribunyaviridae*	*Herbevirus*	2	IE	Yes
High Island	HISLV	*Reoviridae*	*Idnovirus*	2	IE	Yes
Huángpi tick 1	HTV-1	*Nairoviridae*	*Orthonairovirus*	2	IE	?

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Virus Name	Acronym	Family	Genus	Recommended Biosafety Level	Basis of Rating	Isolate
Ilomantsi	ILOV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Kamiti River	KRV	*Flaviviridae*	*Unassigned*	2	A7	Yes
Kamphaeng Phet	KPhV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Kampung Karu	KPKV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Karang Sari	KSaV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Kibale	KIBV	*Peribunyaviridae*	*Herbevirus*	2	IE	Yes
Lammi	LAMV	*Flaviviridae*	*Unassigned*	2	IE	Yes
La Tina	LTNV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Long Island tick rhabdovirus	LITRV	*Rhabdoviridae*	*Unassigned*	2	IE	?
Long Pine Key	LPKV	*Flaviiviridae*	*Unassigned*	2	IE	Yes
Loreto PeAR2612/77	LORV	*Unassigned*	*Negevirus*	2	IE	Yes
Marisma mosquito	MMV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Méno	MénoV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Mercadeo	MECDV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Mosquito X	MXV	*Birnaviridae*	*Entomobirnavirus*	2	IE	Yes
Moumo	MoumoV	*Mesoniviridae*	*N/A*	2	IE	?
Moussa	MOUV	*Rhabdoviridae*	*Unassigned*	2	IE	Yes
Nakiwogo	NAKV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Nam Dinh	NDiV	*Mesoniviridae*	*Alphamesonivirus*	2	IE	Yes
Nanay	NANV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Negev	NEGV	*Unassigned*	*Negevirus*	2	IE	Yes
Ngewotan	NWTV	*Unassigned*	*Negevirus*	2	IE	Yes
Ngoye	NGOV	*Flaviviridae*	*Unassigned*	2	IE	?
Nhumirim	NHUV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Nienokoue	NIEV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Nounané	NOUV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Nsé	NseV	*Mesoniviridae*	*Unassigned*	2	IE	Yes
Ochlerotatus caspius flavivirus	OCFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Okushiri	OKV	*Unassigned*	*Negevirus*	2	IE	Yes
Palm Creek	PCV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Parramatta River	PaRV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Phelbotomine-associated flavivirus	N/A	*Flaviviridae*	*Unassigned*	2	IE	?
Piura	PIUV	*Unassigned*	*Negevirus*	2	IE	Yes
Puerto Almendras	PTAMV	*Rhabdoviridae*	*Almendravirus*	2	IE	Yes
Quảng Binh	QBV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Santana	SANV	*Unassigned*	*Negevirus*	2	IE	Yes
Sarawak	SWKV	*Alphatetraviridae*	*Betatetravirus*	2	IE	Yes
Spanish Culex flavivirus	SCxFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Spanish Ochlerotatus flavivirus	SOcFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
St. Croix River	SCRV	*Reoviridae*	*Orbivirus*	2	IE	Yes
Tai	TAIV	*Peribunyaviridae*	*Herbevirus*	2	IE	Yes
Tanay	TANAV	*Unassigned*	*Negevirus*	2	IE	Yes
Wallerfield	WALV	*Unassigned*	*Negevirus*	2	IE	Yes
Wang Thong	WTV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Xishuangbanna flavivirus	XFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Yamada flavivirus	YDFV	*Flaviviridae*	*Unassigned*	2	IE	Yes
Yunnan Culex flavivirus	YNCxFV	*Flaviviridae*	*Unassigned*	2	IE	Yes

Table 5. Laboratories working with the viruses at BSL-3 listed below are recommended to HEPA filter the exhaust air

Virus Name
African Horse Sickness**
African Swine Fever**
Akabane**
Cabassou
Chikungunya
Everglades
Germiston
Louping III
Mucambo
Oropouche
Rift Valley Fever**
Rocio
Tonate
Venezuelan Equine Encephalitis
Wesselsbron**
Yellow Fever

** These organisms are considered pathogens of significant agricultural importance by the USDA (see Appendix D) and may require additional containment (up to and including ABSL-3Ag containment). Not all strains of each organism are necessarily of concern to the USDA. Contact USDA for more information regarding exact containment/permit requirements before initiating work.

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Section VIII-G: Toxin Agents

Botulinum Neurotoxin

Seven immunologically distinct serotypes of botulinum neurotoxin (BoNT) have been isolated (A, B, C1, D, E, F, and G), which are defined by neutralization of toxicity using specific homologous polyclonal antibodies. Recently, two novel BoNT have been proposed as new serotypes, but additional validation is needed to confirm these toxins as distinct types. Each BoNT holotoxin is a disulfide-bonded heterodimer, composed of a zinc metalloprotease *light chain* (approximately 50 kDa) and a *heavy chain* (approximately 100 kDa), which binds with high affinity to peripheral cholinergic nerve terminals and facilitates the translocation of the catalytic light chain into the nerve terminal cytosol.[1,2] BoNT-mediated toxicity (i.e., muscle weakness and autonomic dysfunction) results from the activity of the light chain, which cleaves soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, required for neurotransmitter release. BoNTs are produced by *Clostridium botulinum* and rare strains of *Clostridium baratii*, *Clostridium butyricum*, and *Clostridium argentinense* as protein complexes, with one to six accessory neurotoxin-associated proteins that stabilize the toxin in biological systems and facilitate its absorption from the gastrointestinal tract, making BoNT highly toxic by the oral route.[1]

Serotypes A, B, E and, less commonly, F are responsible for most human poisoning through contaminated food, wound infection, or colonization of the gastrointestinal tract. Wild animals and livestock may be at greater risk for poisoning with serotypes B, C1, and D.[3,4] To date, no confirmed cases of human or animal intoxication have been reported with serotype G. It is important to recognize that all BoNT serotypes are potentially lethal by injection, aerosol delivery, and oral ingestion. BoNT is one of the most toxic proteins known; absorption of extremely small amounts of toxin can cause severe incapacitation and death, depending upon the serotype and the route of exposure.[5,6]

Diagnosis of Laboratory Exposures

Botulism is initially diagnosed by the presence of characteristic clinical signs and symptoms, which are similar for all serotypes and routes of intoxication.[7] The onset of botulism is generally preceded by a latency of several hours to days, even with aerosol exposure. The duration of the latent period varies inversely with the amount of toxin absorbed.

Botulism generally begins with bilateral, symmetric cranial nerve palsies that may progress to descending flaccid paralysis, including respiratory failure. Signs and symptoms generally include dysphagia, facial paralysis, ptosis, dysarthria, diplopia, and impaired gag reflex. Asymmetric cranial nerve palsies are rarely reported.[8]

Sophisticated tests, such as nerve conduction studies and single-fiber electromyography, can support the diagnosis of botulism and distinguish it from other neuromuscular conditions presenting with similar symptoms, such as Guillain-Barré Syndrome or myasthenia gravis.[7] Detection of BoNT in clinical or food specimens confirms clinically diagnosed cases. Laboratory tests such as mouse bioassay and mass spectrometry should be used mainly for confirmation of the clinical diagnosis, not as a basis for initiating treatment with antitoxin. Since individual variations in the presentation of signs have been documented, botulism should be suspected after a potential exposure even if some of the characteristic signs are absent.

Laboratory Safety and Containment Recommendations

Solutions of sodium hypochlorite (NaOCl, 0.1%) or sodium hydroxide (NaOH, 0.1N) readily inactivate BoNT and are recommended for decontamination of work surfaces and for spills. Sodium hypochlorite (0.6%) also inactivates cells and spores of BoNT-producing species of *Clostridium*. Sterilization in a steam autoclave at 121°C for 30 minutes effectively inactivates BoNT and BoNT-producing species of *Clostridium*, including spores. Additional considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Because BoNT-producing species of *Clostridium* require an anaerobic environment for growth and are essentially not transmissible among individuals, exposure to pre-formed BoNT is the primary concern for laboratory workers. Two of the most significant hazards in working with BoNT and cultures of BoNT-producing species of *Clostridium* are unintentional aerosol generation, especially during centrifugation, and accidental needlestick. Although BoNT does not penetrate intact skin, the toxin can be absorbed through broken or lacerated skin as well as by contact with eyes and mucous membranes.

BSL-2 practices, containment equipment, and facilities including the use of appropriate PPE (i.e., disposable gloves, laboratory coat, and eye protection) are recommended for routine dilutions, titrations, or diagnostic studies with materials known to contain or have the potential to contain BoNT. Activities that may generate aerosols should be performed within a BSC (Class II). Needlesticks can be minimized by careful arrangement of the workspace and maintaining operational awareness at all times. Additional primary containment and personnel precautions, such as those recommended for BSL-3, should be considered on a case-by-case basis for activities that require handling of large quantities of toxin.

Workers in diagnostic laboratories should be aware that BoNT-producing species of *Clostridium* could be stable for weeks or longer in a variety of food products, clinical samples (e.g., feces), and environmental samples (e.g., soil). Stability of the toxin itself will depend upon the sterility, temperature, pH, and ionic strength of the sample matrix.[4,9,10] BoNT retains its activity for long periods (at least 6–12

months) in a variety of frozen foods, especially under acidic conditions (pH 4.5–5.0) and/or high ionic strength, but the toxin is readily inactivated by heating at 100°C for ten minutes.[10]

A documented incident of laboratory intoxication with BoNT occurred in workers who were performing necropsies on animals that had been exposed 24 hours earlier to aerosolized BoNT serotype A. The laboratory workers presumably inhaled aerosols generated from the animal fur; the report does not describe protective precautions. The intoxications were relatively mild, and all affected individuals recovered after a week of hospitalization.[11] Despite the low incidence of laboratory-associated botulism, the high toxicity of BoNT necessitates that laboratory workers exercise caution during all experimental procedures.

Personnel not directly involved in laboratory studies involving BoNT, such as maintenance personnel, should be discouraged from entering the laboratory when a toxin is in use, until after the work has ceased and all work surfaces have been decontaminated (see Appendix I for additional information). Purified preparations of toxin sub-units (e.g., isolated BoNT light chains or heavy chains) should be handled as if contaminated with holotoxin unless proven otherwise by toxicity bioassays. Recombinant BoNT produced in heterologous expression hosts should be considered toxic and handled with equal precautionary measures as endogenously produced BoNT.

Special Issues

Vaccines There are currently no approved vaccines for BoNT. A pentavalent (serotypes A, B, C, D, and E) botulinum toxoid vaccine was available through the CDC as an investigational new drug (IND) until 2011, but it was discontinued due to a decline in immunogenicity of some of the serotypes and an increase in occurrence of moderate local reactions. Vaccine candidates are currently in clinical trials.[12]

Treatment Hospitalization is usually required, and respiratory support may be necessary for severe botulism. In 2013, FDA approved an antitoxin designated as Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G)—(Equine), BAT® for the treatment of botulism in adult and pediatric patients. BAT® is currently the only approved specific treatment for botulism and can effectively neutralize each of the seven known serotypes of BoNT. BAT®, manufactured by Emergent BioSolutions (formally Cangene), can decrease the severity of intoxication by neutralizing BoNT that remains in the bloodstream.[13] BAT® is available from the U.S. Strategic National Stockpile (SNS) and is supplied by the Office of the Assistant Secretary for Preparedness and Response (ASPR). BabyBIG® (Botulism Immune Globulin) is available for infant botulism through the California Infant Botulism Treatment and Prevention Program.

Select Agents and Toxins BoNT and BoNT-producing species of *Clostridium* have the potential to pose a severe threat to human health and are therefore included on the HHS list of Tier 1 Select Agents and Toxins. Entities that possess, use, store, or transfer BoNT-producing species of *Clostridium* are required to be registered with the Federal Select Agent Program (FSAP). Entities that intend to possess, use, store, or transfer quantities of BoNT above the permissible amount are also required to be registered with FSAP. See Appendix F for more information.

Transfer of Agent Domestic transfer or importation of BoNT-producing species of *Clostridium* or BoNT above the permissible amount require prior approval from FSAP. A DoC permit may be required for the export of these agents and toxin to another country. See Appendix C for additional information.

Staphylococcal Enterotoxins (SE)

Staphylococcal Enterotoxins (SE) are a group of closely related extracellular protein toxins of 22 to 29 kD molecular weight that are produced by distinct gene clusters found in a wide variety of *S. aureus* strains.[14–16] SE belong to a large family of homologous pyrogenic exotoxins from staphylococci, streptococci, and mycoplasma, which are capable of causing a range of illnesses in humans through pathological amplification of the normal T-cell receptor response, cytokine/lymphokine release, immunosuppression, and endotoxic shock.[15,17] Classic SE include five serotypes A–E (SEA, SEB, SEC, SED, and SEE, respectively), but genomic analysis has further identified and characterized previously unrecognized SE, such as serotype H (SEH), that has been linked to foodborne incidents.[18,19]

Symptoms from SE may vary with the exposure route and dose. SEA is a common cause of severe gastroenteritis in humans.[20–22] In cases from accidental food poisoning, it is estimated that gastric exposure to as little as 0.05–1 µg of SEA causes incapacitating illness.[23–27] Comparative human toxicity for different serotypes of SE is largely unknown, but human volunteers exposed to 20–25 µg of SE serotype B (SEB) experienced enteritis similar to that caused by SEA.[28]

SE are highly toxic by intravenous and inhalation routes of exposure, with lethal doses causing death in NHPs mainly due to shock and/or pulmonary edema.[29–33] By inference from accidental exposure of laboratory workers and controlled experiments with NHPs, it is estimated that inhalation of less than 1 ng/kg can incapacitate more than 50% of exposed humans and that the inhalation LD_{50} in humans may be as low as 20 ng/kg for SEB.[34]

Exposure of mucous membranes to SEB in a laboratory setting or in clinical studies has been reported to cause conjunctivitis and localized cutaneous swelling, with some laboratory workers also experiencing incapacitating

gastrointestinal symptoms.[35–37] Intradermal or dermal exposure to concentrated SE solutions or patch tests (≥ 1μg/cm^2) has resulted in erythema, induration, or dermatitis.[36–39]

Diagnosis of Laboratory Exposures

Diagnosis of SE intoxication is based on clinical and epidemiologic features. Gastric intoxication with SE begins rapidly after exposure (generally 1 to 6 hours) and is characterized by nausea, vomiting, and abdominal cramps; it is often accompanied by diarrhea, but generally occurs without a high fever.[23,31] At higher exposure levels, intoxication progresses to hypovolemia, dehydration, vasodilatation in the kidneys, and lethal shock.[21] While fever is uncommon after SE ingestion, inhalation of SE commonly results in an acute febrile illness. After a latent period of 3 to 12 hours (range 1.5 to 18 hours), inhalation of SEB results in rapid onset of illness, generally characterized by high fever (range often 103º to 105ºF), chills, headache, malaise, myalgia, and a non-productive cough.[35] Some individuals may develop retrosternal chest pain and dyspnea. Severe cases may develop pulmonary edema or acute respiratory distress syndrome (ARDS). Inhalational SEB intoxication may also be associated with upper respiratory tract signs and symptoms (e.g., sore throat, rhinorrhea, sinus congestion, and/or profuse postnasal drip), conjunctival injection, and/or pharyngeal erythema.[35,37] GI symptoms may also occur after SEB inhalation. Symptoms from SE ingestion usually resolve in 24 to 48 hours, and it is rarely fatal. Symptoms from SEB inhalation due to laboratory exposures generally persist for a duration of 2 to 5 days, but the cough may persist for up to four weeks.[40] Nonspecific laboratory findings in inhalational SEB include a neutrophilic leukocytosis. WBC counts are often >10,000 cells/mm^3 and have ranged from 8,000 to 28,000 cells/mm^3. The chest X-ray is often normal but may show abnormalities consistent with pulmonary edema in severe cases.[40]

Differential diagnosis of SE inhalation may be unclear initially because the symptoms are similar to disease caused by several respiratory pathogens (e.g., influenza, adenovirus, and mycoplasma). However, naturally occurring pneumonia or influenza typically involve symptoms presenting over a more prolonged interval of time, whereas SE intoxication tends to involve symptoms that rapidly plateau. Unrecognized SEB exposure has often been initially misdiagnosed as community-acquired pneumonia, with SEB exposure suspected only after onset of illness in other at-risk laboratory workers within a 12-hour period.[34]

Laboratory confirmation of intoxication includes SE detection by immunoassay of environmental and clinical samples and gene amplification to detect staphylococcal genes in environmental samples.[24,41,42,43] SE may be undetectable in the serum at the time symptoms occur; nevertheless, a serum specimen should be drawn as early as possible after exposure. Data from animal studies suggest the presence of SE in the serum or urine is transient.[44] Respiratory secretions and

nasal swabs may demonstrate the toxin within 24 hours of inhalation exposure. Evaluation of neutralizing antibody titers in acute and convalescent sera of exposed individuals can be undertaken, but it may yield false positives resulting from pre-existing antibodies produced in response to natural SE exposure.[40]

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Inhalational exposure, mucous membrane exposure (via aerosol or droplet exposure or direct contact with contaminated gloves), accidental ingestion, and parenteral inoculation are believed to be the primary hazards of SE for laboratory and animal-care personnel.[24,27,35] SE are relatively stable, monomeric proteins, readily soluble in water, and resistant to proteolytic degradation, temperature fluctuations, and low pH conditions. The physical/chemical stability of SE suggests that additional care must be taken by laboratory workers to avoid exposure to residual toxin that may persist in the environment.

Active SE toxins may be present in clinical samples, lesion fluids, respiratory secretions, fur, or tissues of exposed animals. Additional care should be taken during cage cleaning and the necropsy of exposed animals and in the handling of clinical stool samples because SE toxins retain toxic activity throughout the digestive tract.

Accidental laboratory exposures to SEB have been reviewed.[35] Documented accidents included inhalation of SE aerosols generated from pressurized equipment failure and re-aerosolization of residual toxin from the fur of exposed animals. The most common cause of laboratory intoxication with SE is currently expected to result from accidental self-exposure via the mucous membranes by touching contaminated hands or gloves to the face or eyes.

BSL-2 practices, containment equipment, and facilities should be used when handling SE or potentially contaminated material. Because SE is highly active by the oral or ocular exposure route, the use of a laboratory coat, gloves, and safety glasses is mandatory when handling toxin or toxin-contaminated solutions. Frequent, careful handwashing and laboratory decontamination should be strictly enforced when working with SE. Depending upon a risk assessment of the laboratory operation, the use of a face mask and goggles may be required to avoid ocular and oropharyngeal exposure due to inadvertent touching of the face and mucous membranes with contaminated gloves. Additional primary containment and personnel precautions, such as those recommended for BSL-3 (e.g., respirator), should be considered on a case-by-case basis for activities with a high potential for aerosol or droplet production and those involving the use of large quantities of SE.

Special Issues

Vaccines No approved vaccine or specific antidote is currently available for human use, but experimental, recombinant vaccines are under development.

Select Agents and Toxins SEA, SEB, SEC, SED, and SEE are included in the HHS Select Agents and Toxins List. Entities that intend to possess, use, store or transfer quantities of SE above the permissible amount are required to be registered with FSAP. See Appendix F for more information.

Transfer of Agent Domestic transfer or importation of SE above the permissible amount requires prior approval from FSAP. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Ricin

Ricin is produced in maturing seeds of the castor plant *Ricinus communis L.*, which has been recognized for centuries as a highly poisonous plant for humans and livestock.[45] The castor seed contains castor oil, an important chemical feedstock for lubricants, polyamides, polyurethanes, plasticizers, and cosmetics, but also contains as much as 6% ricin and *Ricinus communis* agglutinin (w/w).[46] Thus, processing castor seed for castor oil results in a seed meal that is a crude form of ricin. Ricin belongs to a family of type 2 ribosome-inactivating proteins (RIPs) from plants, including abrin, modeccin, and viscumin, that share a similar overall structure and mechanism of action.[47] The ricin holotoxin is a disulfide-bonded heterodimer composed of an A-chain (approximately 34 kD polypeptide) and a B-chain (approximately 32 kD). The A-chain is an N-glycosidase enzyme that removes a specific adenine base from the 28S ribosomal RNA, resulting in loss of protein synthesis by inactivation of the ribosome. The B-chain is a relatively non-toxic lectin that facilitates toxin binding and internalization through interaction with glycolipids and glycoproteins that line the surface of the target cell.[45] The *Ricinus communis* agglutinin (RCA_{120}) is a tetramer composed of 2 A-chains and 2 B-chains that are homologous to ricin A-chain (93%) and B-chain (84%) at the protein sequence level.[48] There are monoclonal antibodies that distinguish ricin from RCA_{120} and comparisons among different castor cultivars indicate ricin content exceeds that of RCA_{120} by a factor of 2.5–3.[49] As isolated from the seed, ricin is composed of various glycosylated forms and isoforms.[50]

Ricin is much less toxic by weight than BoNT or SE, and published case reports suggest that gastric ingestion of ricin is rarely fatal in adults, with ingestion of castor beans the common route for gastric exposure.[51] Animal studies and human poisonings suggest that the effects of ricin depend upon the route of exposure, with inhalation and intravenous exposure being the most toxic. In laboratory mice, the LD_{50} has been estimated as 3 to 5 μg/kg by inhalation, 5 μg/kg by intravenous injection, 22 μg/kg by intraperitoneal injection, 24 μg/kg by subcutaneous

injection, and 20 mg/kg by intragastric administration.[52] Before more stringent safety precautions were introduced, workers in castor oil processing plants and nearby residents were exposed to dust from the seed meal. While there were very few reported deaths from ricin exposure, severe allergic responses including skin reactions and asthma were common.[53]

The human lethal dose has not been established rigorously but is estimated at 5–10 µg/kg by injection, intramuscular or intravenous, and 5–10 µg/kg by inhalation.[54] The RCA_{120} is considerably less toxic than ricin, with 300 times as much RCA_{120} needed to kill 50% of Vero cells in a cell toxicity study.[50]

Diagnosis of Laboratory Exposures

The primary diagnosis is through clinical signs and symptoms that vary greatly depending upon the route of exposure. Following inhalation exposure, symptoms may appear within eight hours and include cough, labored respiration, and fever, which may progress to respiratory distress and death.[55] Most of the pathology occurs in the upper and lower respiratory tract, including inflammation, bloody sputum, and pulmonary edema. Toxicity from ricin inhalation will progress despite treatment with antibiotics, as opposed to a treatable bacterial infection. There is no mediastinitis as seen with inhalation anthrax. Ricin patients will not plateau clinically as occurs after inhalation of SEB.

Gastric ingestion of ricin causes nausea, vomiting, diarrhea, abdominal cramps, and dehydration. Initial symptoms may appear more rapidly following gastric ingestion (1–5 hours) but generally require exposure to much higher levels of toxin compared with the inhalation route. Following injection of ricin, symptoms may appear within six hours and include nausea, vomiting, anorexia, and high fever. The site of ricin injection typically shows signs of inflammation with marked swelling and induration. One case of poisoning by ricin injection resulted in fever, vomiting, irregular blood pressure, and death by vascular collapse after a period of several days; it is unclear in this case if the toxin was deposited intramuscularly or in the bloodstream.[56]

After aerosol exposure to ricin, additional supportive clinical or diagnostic features may include the following: bilateral infiltrates on chest radiographs, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein.[52]

Numerous methods for detecting and quantifying ricin have been developed. Specific immunoassay of serum and respiratory secretions, immunohistochemical stains of tissue, or detection of the castor seed alkaloid ricinine in urine may be used to confirm a diagnosis.[57] An immuno-PCR method is able to detect pg/ml of ricin in sera and feces of intoxicated mice.[58] PCR can detect residual castor bean DNA in most ricin preparations. Likewise, ELISA, mass spectrometry techniques, and cell viability assays are amongst the most common assays used to detect

ricin from contaminated samples.[59] Ricin is an extremely immunogenic toxin, and paired acute and convalescent sera should be obtained from survivors for measurement of antibody response.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Precautions should be extended to handling potentially contaminated clinical, diagnostic, and post-mortem samples because ricin may retain toxicity in the lesion fluids, respiratory secretions, or unfixed tissues of exposed animals.

When the ricin A-chain is separated from the B-chain and administered parenterally to animals, its toxicity is diminished by >1,000-fold compared with ricin holotoxin.[60] However, purified preparations of natural ricin A-chain or B-chain and crude extracts from castor beans should be handled as if contaminated by ricin until proven otherwise by bioassay.

Ricin is a relatively non-specific cytotoxin and irritant that should be handled in the laboratory as a non-volatile toxic chemical. Based upon animal studies, the inhalation of air-borne dust particles or small liquid droplets carrying ricin into the lungs is still considered the most dangerous route of exposure. BSL-2 practices, containment equipment, and facilities are recommended, including laboratory coat, gloves, and eye protection, when handling ricin toxin or potentially contaminated materials. A full-face respirator should be worn if there is a potential for creating a toxin aerosol. A BSC is used if there is any chance that ricin aerosols will be generated. Solutions of ricin can be inactivated by treatment with sodium hypochlorite bleach, and crude ricin powder is inactivated by autoclaving with calcium oxide (lime).

Special Issues

Vaccines No approved vaccine or specific antidote is currently available for human use, but experimental, recombinant vaccines are under development. There is at least one commercial ricin vaccine in Phase 1 clinical trials.[61]

Select Agents and Toxins Ricin is included in the HHS list of Select Agents and Toxins. Entities that intend to possess, use, store or transfer quantities of ricin above the permissible amount are required to be registered with FSAP. See Appendix F for more information.

Transfer of Agent Domestic transfer or importation of ricin above the permissible amount requires prior approval from FSAP. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Selected Low Molecular Weight (LMW) Toxins

Low Molecular Weight (LMW) Toxins comprise a structurally and functionally diverse class of natural poisons, ranging in size from several hundred to a few thousand daltons. LMW toxins include complex organic structures and disulfide cross-linked and cyclic polypeptides. Tremendous structural diversity may occur within a particular type of LMW toxin, often resulting in incomplete toxicological or pharmacological characterization of minor isoforms. Grouping LMW toxins together has primarily been a means of distinguishing them from protein toxins with respect to key biophysical characteristics. Compared with proteins, the LMW toxins are of smaller size, which alters properties such as filtration and distribution; are generally more stable and persistent in the environment; and some compounds may exhibit poor water-solubility necessitating the use of organic solvents. These characteristics pose special challenges for safe handling, containment, and decontamination of LMW toxins within the laboratory.

The set of LMW toxins selected for discussion herein are employed routinely as laboratory reagents and/or have been designated as potential public health threats by the CDC, including: T-2 mycotoxin, produced by *Fusarium* fungi;[62,63] saxitoxin and related paralytic shellfish poisons, produced by select marine dinoflagellates within the genus *Alexandrium*, *Gymnodinium*, and *Pyrodinium*, as well as certain freshwater cyanobacteria;[64] tetrodotoxin from a number of marine animals;[65] brevetoxins from the dinoflagellate *Karenia brevis*;[66] palytoxins from select marine coelenterates belonging to the genus *Palythoa* and from marine dinoflagellates belonging to the genus *Ostreopsis*;[67,68] polypeptide conotoxins α-GI (includes GIA) and α-MI from the *Conus* genus of gastropod mollusks;[69] the amino acid analog domoic acid from select marine diatoms from the genus *Pseudo-nitzschia*;[70] and the monocyclic polypeptide microcystins from select freshwater cyanobacteria such as *Microcystis aeruginosa*.[71]

Trichothecene mycotoxins comprise a broad class of structurally complex, non-volatile sesquiterpene compounds that are potent inhibitors of protein synthesis.[62,63] Mycotoxin exposure occurs by consumption of moldy grains, and at least one of these toxins, designated T-2, has been implicated as a potential biological warfare agent.[63] T-2 is a lipid-soluble molecule that can be absorbed into the body rapidly through exposed mucosal surfaces.[72] Toxic effects are most pronounced in metabolically active target organs and include emesis, diarrhea, weight loss, nervous disorder, cardiovascular alterations, immunodepression, hemostatic derangement, bone marrow damage, skin toxicity, decreased reproductive capacity, and death.[63] The LD_{50} for T-2 in laboratory animals ranges from 0.2 to 10 mg/kg, depending on the route of exposure, with aerosol toxicity estimated to be 20 to 50 times greater than parenteral exposure.[63] Of special note, T-2 is a potent vesicant capable of directly damaging skin or corneas. Skin lesions, including frank blisters, have been observed in animals with local, topical application of 50 to 100 ng of toxin.[63,72]

Saxitoxin and tetrodotoxin are paralytic marine alkaloid toxins that interfere with normal function of voltage-activated sodium channels in excitable cells of heart, muscle, and neuronal tissue by blocking ion flow, causing potentially lethal paralytic shellfish poisoning and pufferfish poisoning, respectively.[73] Animals exposed to 1–10 µg/kg of either of these toxins by parenteral routes typically develop a rapid onset of excitability, muscle spasm, and respiratory distress; death may occur within 10–15 minutes in extreme cases from respiratory paralysis.[64,74] Humans ingesting seafood contaminated with saxitoxin or tetrodotoxin show similar signs of toxicity, typically preceded by paresthesias of the lips, face, and extremities.[73,75]

Brevetoxins are ladder-frame-polyether, shellfish neurotoxins produced by marine dinoflagellates that accumulate in filter-feeding mollusks and cause non-lethal human intoxications from ingestion of contaminated seafood, known as neurotoxic shellfish poisoning, or by respiratory irritation from sea spray containing the toxins.[73] This toxin group lowers the activation potential in voltage-activated sodium channels resulting in channel opening at normal resting membrane potentials, effectively making the sodium channel of affected nerve or muscle cells hyper-excitable. Symptoms of human ingestion include paresthesias of the face, throat, and fingers or toes, followed by dizziness, chills, muscle pains, nausea, gastroenteritis, and clinical signs including reduced heart rate. Brevetoxin has a parenteral LD_{50} of 200 µg/kg in mice and guinea pigs. Guinea pigs exposed to a slow infusion of brevetoxin develop fatal respiratory failure within 30 minutes of exposure to 20 µg/kg toxin.[74]

Palytoxin, and related toxins such as ovatoxins, are structurally complex, articulated fatty alcohols associated with certain colonial anemones such as *Palythoa toxica* and select marine dinoflagellates of the genus *Ostreopsis*.[67] This toxin group is capable of binding and converting the essential cellular Na+/K+ pump into a non-selective cation channel.[68,76] Palytoxin is among the most potent coronary vasoconstrictors known, killing animals within minutes by cutting off oxygen to the myocardium.[77] Symptoms in affected individuals can vary based on the route of exposure and may include rhabdomyolysis due to consumption of contaminated seafood, respiratory distress, and fever from inhalation of aerosolized toxins, and skin and ocular irritation from topical exposure.[67,78] The LD_{50} for intravenous administration ranges from 0.025 to 0.45 µg/kg in different species of laboratory animals.[77] Palytoxin is lethal by several parenteral routes but is about 200-fold less toxic if administered to the alimentary tract (oral or rectal) compared with intravenous administration.[77] Palytoxin causes corneal damage and can cause irreversible blindness at topically applied levels of approximately 400 ng/kg, despite extensive rinsing after ocular instillation.[77] Like brevetoxins, palytoxins cause respiratory irritation from exposure to marine aerosols when the

causative dinoflagellates are present in high numbers, but unlike brevetoxins, palytoxins are also associated with flu-like symptoms with high fever.[78]

Conotoxins are polypeptides, typically 10–30 amino acids long and stabilized by distinct patterns of disulfide bonds that have been isolated from the toxic venom of marine snails and shown to be neurologically active or toxic in mammals.[69] Of the estimated >105 different polypeptides (conopeptides) present in venom of over 500 known species of *Conus*, only a few have been rigorously tested for animal toxicity. Of the isolated conotoxin subtypes that have been analyzed, at least two post-synaptic paralytic toxins, designated α-GI (includes GIA) and α-MI, have been reported to be toxic in laboratory mice with LD_{50} values in the range of 10–100 µg/kg depending upon the species and route of exposure. Workers should be aware that human toxicity of whole or partially fractionated *Conus* venom, as well as synthetic combinations of isolated conotoxins, may exceed that of individual components. For example, untreated cases of human poisoning with venom of *C. geographus* result in an approximately 70% fatality rate, probably as a result of the presence of mixtures of various α- and µ-conotoxins with common or synergistic biological targets.[69,79] The α-conotoxins act as potent nicotinic antagonists, and the µ-conotoxins block the sodium channel.[69] Symptoms of envenomation depend upon the *Conus* species involved, generally occur rapidly after exposure (minutes), and range from severe pain to spreading numbness.[80] Severe intoxication results in muscle paralysis, blurred or double vision, difficulty breathing and swallowing, and respiratory or cardiovascular collapse.[80]

Domoic acid is a kainic acid analog neurotoxin that causes amnesic shellfish poisoning after the consumption of contaminated seafood. Domoic acid has a high affinity for glutamate receptors in the hippocampus resulting in excitotoxicity and neuronal degeneration.[81] Symptoms of exposure include vomiting, nausea, diarrhea and abdominal cramps, headache, dizziness, confusion, disorientation, short-term memory loss, motor weakness, seizures, cardiac arrhythmias, and coma with possible death in extreme cases.

Microcystins (also called cyanoginosins) are monocyclic heptapeptides composed of specific combinations of L- and D-amino acids, some with uncommon side chain structures, that are produced by various freshwater cyanobacteria.[82] The toxins are potent inhibitors of liver protein phosphatase type 1 and are capable of causing massive hepatic hemorrhage and death.[82] One of the more potent toxins in this family, microcystin-LR, has a parenteral LD_{50} of 30 to 200 µg/kg in rodents.[71] Exposure to microcystin-LR causes animals to become listless and prone in the cage; death occurs in 16 to 24 hours. The toxic effects of microcystin vary depending upon the route of exposure and may include hypotension and cardiogenic shock, in addition to hepatotoxicity.[71,83]

Diagnosis of Laboratory Exposures

LMW toxins are a diverse set of molecules with a correspondingly wide range of signs and symptoms of laboratory exposure, as discussed above for each toxin. Common symptoms can be expected for LMW toxins with common mechanisms of action. For example, several paralytic marine toxins that interfere with normal sodium channel function cause rapid paresthesias of the lips, face, and digits after ingestion. The rapid onset of illness or injury (minutes to hours) generally supports a diagnosis of chemical or LMW toxin exposure. Painful skin lesions may occur almost immediately after contact with T-2 mycotoxin, and ocular irritation or lesions will occur in minutes to hours after contact with T-2 or palytoxin.

Specific diagnosis of LMW toxins in the form of a rapid diagnostic test is not presently available in the field. Serum and urine should be collected for testing at specialized reference laboratories by methods including antigen detection, receptor-binding assays, or liquid chromatographic analyses of metabolites.

Parent compounds and metabolites of several marine and freshwater toxins, including saxitoxin, tetrodotoxin, domoic acid, brevetoxins, and microcystins are well-studied as part of routine regulation of food and water supplies.[73] Likewise, T-2 mycotoxin absorption and distribution in the body has been studied, and its metabolites can be detected as late as 28 days after exposure.[63] Marine toxins are highly stable in food and are typically not affected by cooking or freezing. Once consumed, most marine toxins are metabolized and rapidly excreted through the urine, in some cases, such as saxitoxin, tetrodotoxin, and domoic acid, within 24–72 hours.[81,84] In contrast, freshwater microcystins bind covalently to target protein phosphatases in the liver, making analysis of clinical samples difficult even in postmortem analysis of livestock that died from suspected microcystin contamination of drinking water.[85] Clinical specimens can include blood, urine, lung, liver, and stomach contents. Few clinical tests have been validated for these toxins. Far more methods are available for the testing of environmental or food samples including a variety of screening and confirmatory techniques, depending on the toxin.

Laboratory Safety and Containment Recommendations

General considerations for the safe use and inactivation of toxins of biological origin are found in Appendix I. Ingestion, parenteral inoculation, skin and eye contamination, and droplet or aerosol exposure of mucous membranes are the primary hazards to laboratory and animal care personnel. LMW toxins also can contaminate food sources or small-volume water supplies. Additionally, the T-2 mycotoxin is a potent vesicant and requires additional safety precautions to prevent contact with exposed skin or eyes. Palytoxin also is highly toxic by the ocular route of exposure.

In addition to their high toxicity, the physical and chemical stability of the LMW toxins contributes to the risks involved in handling them in the laboratory environment. Unlike many protein toxins, the LMW toxins can contaminate surfaces as a stable, dry film that may pose an essentially indefinite contact threat to laboratory workers. Special emphasis, therefore, must be placed upon proper decontamination of work surfaces and equipment.[86]

When handling LMW toxins or potentially contaminated material, BSL-2 practices, containment equipment, and facilities are recommended, especially the wearing of a laboratory coat, safety glasses, and disposable gloves; the gloves must be impervious to organic solvents or other diluents employed with the toxin.

The use of respiratory protection is considered if there is potential for aerosolization of the toxin. A BSC (Class II, Type B1 or B2) or a chemical fume hood equipped with exhaust HEPA filters are also indicated for activities with a potential for aerosol, such as powder samples, and/or the use of large quantities of toxin.

For LMW toxins that are not easily decontaminated with bleach solutions, it is recommended to use pre-positioned, disposable liners for laboratory work surfaces to facilitate clean-up and decontamination.

Special Issues

Vaccines No approved vaccines are currently available for human use. Experimental therapeutics for LMW toxins have been reviewed.[87]

Select Agents and Toxins Some LMW toxins are listed as Select Agents and Toxins. Entities that intend to possess, use, store or transfer quantities of regulated LMW toxins above their permissible amount are required to be registered with FSAP. See Appendix F for more information.

Transfer of Agent Domestic transfer or importation of regulated LMW toxins above their permissible amount requires prior approval from FSAP. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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Section VIII-H: Prion Diseases

Transmissible spongiform encephalopathies (TSE) or prion diseases are neurodegenerative diseases, which affect humans and a variety of domestic and wild animal species.[1–4] A central biochemical feature of prion diseases is the conversion of normal prion protein (PrP) to an abnormal, misfolded, pathogenic isoform designated $PrPS^c$ after the prototypic prion disease—scrapie. The infectious agents that transmit prion diseases are known as prions and contain no known prion-specific nucleic acids or virus-like particles. Prions are composed mainly, if not entirely, of $PrPS^c$. They are highly resistant to inactivation by heat and chemicals and thus require special biosafety precautions. Prions are transmissible by inoculation, ingestion, or transplantation of infected tissues or homogenates. Prion infectivity is high in the brain and other central nervous system tissues and lower in lymphoid tissues including the spleen, lymph node, gut, bone marrow, and blood. A 2017 study indicates the presence of low levels of prion infectivity in the skin of sporadic Creutzfeldt-Jakob disease (sCJD) decedents.[5]

A chromosomal gene (*PRNP*) encodes PrP^C, the cellular isoform of PrP. $PrPS^c$ is derived from PrP^C by a post-translational process whereby $PrPS^c$ acquires a high beta-sheet content and a resistance to inactivation by normal disinfection processes. $PrPS^c$ is less soluble in aqueous buffers and is partially protease-resistant. As a result, when prion-containing samples are incubated with proteases such as proteinase K, $PrPS^c$ can often be distinguished from PrP^C, which is completely protease-sensitive.

Occupational Infections

Although sCJD infections have occurred in medical specialists and health professionals, including pathologists who encounter cases of CJD post-mortem, no overall increased occupational risk for health professionals has been found.[6] However, despite the lack of a clearly identified source, the atypical pathology of CJD in at least one neurosurgeon suggests that this case was more likely to have been an acquired, rather than sporadic, form of CJD.[7]

Modes of Infection and Spread

Recognized diseases caused by prions are listed in Table 1 (human diseases) and Table 2 (animal diseases). Besides certain medical procedures using prion contaminated materials (e.g., dura matter), the only clear risk factor for natural disease transmission is the consumption of infected tissues, such as human brain in the case of Kuru, and meat, including nervous tissue, in the case of bovine spongiform encephalopathy (BSE) and related diseases such as feline spongiform encephalopathy (FSE). Familial forms of CJD are acquired by inheritance of a mutant *PRNP* gene through the germline.

Although the exact mechanism of infection and spread among sheep and goats developing natural scrapie is unknown, there is considerable evidence that one of the primary sources is oral ingestion of placental membranes from infected ewes. There is no evidence of transmission of scrapie to humans even though the disease has been recognized in sheep for over 200 years. The TSE diseases, transmissible mink encephalopathy (TME), BSE, FSE, and exotic ungulate encephalopathy (EUE), are all thought to occur after the consumption of prion-infected foods.[8] The exact mechanism of chronic wasting disease (CWD) spread among mule deer, white-tailed deer, and Rocky Mountain elk is unknown.[3] There is strong evidence that CWD is laterally transmitted and environmental contamination may play an important role in local maintenance of the disease. Under experimental conditions, CWD and other prion diseases have been transmitted via aerosols, but there is no evidence that this is a natural route of transmission.[9–11]

Prions are usually most efficient at infecting the homologous species, but cross-species infection with a reduced efficiency is also possible. After cross-species infection, there is often a gradual adaptation of specificity for the new host, especially if there is spread from individual to individual. This process of cross-species adaptation can vary among individuals within the same species. Therefore, the rate of adaptation and final species specificity of the resultant prion is difficult to predict. Such considerations help to form the basis for the biosafety classification of different prions.

Table 1. Human Prion Diseases

Disease	Abbreviation	Mechanism of Pathogenesis
Kuru	N/A	Infection through ritualistic cannibalism
Sporadic CJD	sCJD	Unknown mechanism; possibly somatic mutation or spontaneous conversion of PrP^{C} to PrP^{Sc}
Variant CJD	vCJD	Infection presumably from consumption of BSE-contaminated cattle products or secondary bloodborne transmission
Familial or genetic CJD	fCJD or gCJD	Germline mutations in *PRNP* gene
Iatrogenic CJD	iCJD	Infection from contaminated corneal or dura mater grafts, pituitary hormone, or neurosurgical equipment
Gerstmann–Sträussler–Scheinker syndrome	GSS	Germline mutations in *PRNP* gene

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Disease	Abbreviation	Mechanism of Pathogenesis
Fatal Familial Insomnia	FFI	Germline mutations in *PRNP* gene
Sporadic Fatal Insomnia	sFI	Presumably same as sCJD (see above)
Variably Protease-Sensitive Prionopathy	VPSPr	Presumably same as sCJD (see above)

Table 2. Animal Prion Diseases

Disease	Abbreviation	Natural Host	Mechanism of Pathogenesis
Scrapie	N/A	Sheep, goats, mouflon	Infection in genetically susceptible animals
Bovine Spongiform Encephalopathy	BSE	Cattle	Infection with prion-contaminated feedstuffs (classical BSE); unknown/ possible spontaneous misfolding of PrP^{C} to PrP^{Sc} (atypical BSE)
Chronic Wasting Disease	CWD	Mule deer, white-tailed deer, Rocky Mountain elk, reindeer, moose	Unknown mechanism; probably from direct animal contact with infected feces, urine, drool, or indirectly from contaminated environment (e.g., feed, water, dirt)
Exotic Ungulate Encephalopathy	EUE	Nyala, greater kudu, and onyx	Infection with BSE-contaminated feedstuffs
Feline Spongiform Encephalopathy	FSE	Domestic cats, wild cats in captivity	Infection with BSE-contaminated feedstuffs
Transmissible Mink Encephalopathy	TME	Mink (farm-raised)	Infection with prion-contaminated feedstuffs

Laboratory Safety and Containment Recommendations

In the laboratory setting, prions from human tissue and human prions propagated in animals can be manipulated at BSL-2 or higher. Due to concerns about BSE prions infecting humans and cattle, certain circumstances may call for the use of BSL-3 facilities and/or practices, with a sealed secondary container used for transport of samples inside the laboratory. Use of containment and prion-dedicated equipment is recommended whenever possible in order to limit contamination as well as the area and materials that would need to undergo inactivation procedures.

All other animal prions may be manipulated at BSL-2 with standard BSL-2 practices. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the biosafety

guidelines applying to either the source or recipient of the inoculum, whichever is more stringent.

In the care of patients diagnosed with human prion disease, Standard Precautions are considered adequate. Human prion diseases in the clinical setting have not been found to be communicable or contagious other than through invasive procedures resulting in iatrogenic exposures.[12] One study reports finding detectable infectivity and prion seeding activity in the skin of sCJD cadavers though at much lower levels than what is found in brain tissues of sCJD patients. If such infectivity were also to be found in asymptomatic prion infected persons or early in the course of the sCJD illness, this could heighten concern for the potential of iatrogenic sCJD transmission through invasive skin procedures.[5]

There is no evidence of contact or aerosol transmission of prions from one human to another. However, human prions have been transmitted via some routes. Kuru has been transmitted through ritualistic cannibalism in New Guinea. Iatrogenic CJD has been caused by the contamination of medical devices, administration of prion-contaminated growth hormone, or the transplantation of prion-contaminated dura mater and corneal grafts. It is highly suspected that variant CJD can also be transmitted by blood transfusion.[13] However, there is no evidence for bloodborne transmission of non-variant forms of CJD.[14] Familial CJD, Gerstmann–Sträussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI) are all dominantly-inherited prion diseases; many different mutations of the *PRNP* gene have been shown to be genetically linked to the development of inherited prion disease.

Studies of prions from many cases of inherited prion disease have demonstrated transmission to apes, monkeys, and mice, especially those carrying human *PRNP* transgenes.

Special Issues

Inactivation of Prions Prions are characterized by relative resistance to conventional inactivation procedures including irradiation, boiling, dry heat, and harsh chemicals such as formalin, betapropiolactone, and alcohols. While prion infectivity in purified samples is diminished by prolonged digestion with proteases, the results from boiling in sodium dodecyl sulfate (SDS) and urea alone are variable. More effective treatments include enzymatic treatments with SDS,[15] vaporized hydrogen peroxide,[16] 4% SDS in 1% acetic acid at 65–134°C,[17,18] or mildly acidic hypochlorous acid.[19] Denaturing organic solvents such as phenol or chaotropic reagents (e.g., guanidine isothiocyanate) have resulted in greatly reduced, but not always complete, inactivation. Similarly, the use of conventional autoclaves as the sole inactivating treatment has not always resulted in complete inactivation of prions.[20,21] Formalin-fixed and paraffin-embedded tissues, especially of the brain, remain infectious.[22] Some investigators recommend that formalin-fixed tissues from suspected cases of prion disease be immersed for 30 minutes in 96%

formic acid or phenol before histopathologic processing (see Table 3), but such treatments may severely distort the microscopic neuropathology and may not completely inactivate infectivity.

The safest and most unambiguous method for ensuring that there is no risk of residual infectivity on contaminated instruments and other materials is to discard and destroy them by incineration.[23] Current recommendations for inactivation of prions on instruments and other materials are based on the use of sodium hypochlorite, NaOH, Environ LpH (no longer commercially available),[24] and the moist heat of autoclaving. Combinations of heat and chemical inactivation are likely to be most reliable (See Table 4).[20,23,25] A less caustic hypochlorous acid solution can also decontaminate prions on stainless steel,[19] but further validation of this treatment is warranted.

Surgical Procedures Precautions for surgical procedures on patients diagnosed with prion disease are outlined in an infection control guideline for transmissible spongiform encephalopathies developed by a consultation convened by the WHO in 1999.[23,25] Sterilization of reusable surgical instruments and decontamination of surfaces are performed in accordance with recommendations described by the CDC and the WHO infection control guidelines.[23] Table 4 summarizes the key recommendations for decontamination of reusable instruments and surfaces. Contaminated disposable instruments or materials can be incinerated at 1000°C (1832°F) or greater.[26,27]

Autopsies Routine autopsies and the processing of small amounts of formalin-fixed tissues containing human prions can safely be done using Standard Precautions.[28,29] The absence of any known effective treatment for prion disease demands caution. The highest concentrations of prions are in the central nervous system and its coverings. Based on animal studies, it is likely that prions are also found in the spleen, thymus, lymph nodes, skin, blood, and intestine. The main precaution to be taken by laboratorians working with prion-infected or contaminated material is to avoid accidental puncture of the skin.[12] If possible, cut resistant gloves are worn when handling contaminated specimens. If accidental contamination of unbroken skin occurs, the area is washed with detergent and abundant quantities of warm water (avoid scrubbing); brief exposure (1 minute to 1 N NaOH or a 1:10 dilution of bleach) or more prolonged soaking in a commercial hypochlorous acid preparation (BrioHOCl®) can be considered for additional safety.[19,23] Additional guidance related to occupational injury is provided in the WHO infection control guidelines.[23] Unfixed samples of brain, spinal cord, and other tissues containing human prions should be processed with extreme care in a BSL-2 facility, optimally with restricted access, additional PPE, and dedicated equipment.

Bovine Spongiform Encephalopathy

Although the eventual total number of variant CJD cases resulting from BSE transmission to humans is unknown, a review of the epidemiological data from the United Kingdom indicates that BSE transmission to humans is not efficient.[30] The most prudent approach is to study BSE prions at a minimum in a BSL-2 facility utilizing appropriate BSL-3 practices.

When performing necropsies on large animals where there is an opportunity that the worker may be accidentally splashed or have contact with high-risk materials (e.g., spinal column, brain), personnel wear full-body coverage personal protective equipment (e.g., gloves, rear closing gown, and face shield). Use of disposable plasticware, which can be discarded as a dry regulated medical waste or incinerated, is highly recommended.

Aerosol transmission of prions has been observed experimentally,[9–11] but there is no evidence that this occurs under natural conditions or in clinical settings. It is still prudent to avoid the generation of aerosols or droplets during the manipulation of tissues or fluids and during the necropsy of experimental animals. It is further strongly recommended that impervious gloves be worn for activities that provide the opportunity for skin contact with infectious tissues and fluids.

Animal carcasses and other tissue waste can be disposed by incineration with a minimum secondary temperature of 1000°C (1832°F).[23,26] Pathological incinerators should maintain a primary chamber temperature in compliance with design and applicable state regulations and employ good combustion practices. Medical waste incinerators should comply with applicable state and federal regulations.

The alkaline hydrolysis process, using a vessel that exposes the carcass or tissues to NaOH or KOH heated to 95°–150°C, can be used as an alternative to incineration for the disposal of carcasses and tissue.[20,31] The process has been shown to completely inactivate some strains of prions when used for the recommended period.

Table 3. Tissue Preparation for Human CJD and Related Diseases

Step	Instructions
1	Histology technicians wear gloves, apron, laboratory coat, and face protection.
2	Adequate fixation of small tissue samples (e.g., biopsies) from a patient with suspected prion disease can be followed by post-fixation in 96% absolute formic acid for 30 minutes, followed by 45 hours in fresh 10% formalin.
3	Liquid waste can be collected in a 4 L waste bottle initially containing 600 ml 6 N NaOH.
4	Gloves, embedding molds, and all handling materials are disposed as regulated medical waste.

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Step	Instructions
5	Tissue cassettes can be processed in a TSE-dedicated processor or manually to prevent contamination of general use tissue processors.
6	Tissues are embedded in a disposable embedding mold. If used, forceps are decontaminated as in Table 4.
7	In preparing sections, cut-resistant gloves can be worn; section waste is collected and disposed of in a regulated medical waste receptacle. The knife stage is wiped with 2 N NaOH, or sodium hypochlorite (20,000 ppm) followed by distilled water. The knife used is discarded immediately in a "regulated medical waste sharps" receptacle. Slides are labeled with "CJD Precautions." The sectioned block is sealed with paraffin.
8	Routine staining: a. slides are processed by hand using disposable specimen cups or in a TSE-dedicated stainer; b. after placing the coverslip on, slides are decontaminated by soaking them for 10–60 min in 2 N NaOH or sodium hypochlorite (20,000 ppm) followed by distilled water; and c. slides are labeled as "Infectious-CJD."
9	Other suggestions: a. disposable specimen cups or slide mailers may be used for reagents; b. slides for immunocytochemistry may be processed in disposable Petri dishes; and c. equipment is decontaminated as described above or disposed as regulated medical waste.

Handling and processing of tissues from patients with suspected prion disease

The special characteristics of work with prions require attention to the facilities, equipment, policies, and procedures involved.[10] The related considerations outlined in Table 3 should be incorporated into the laboratory's risk management for this work. The unique code for this document is 331410

Handling and processing of multiple human prion tissue samples

In research environments where multiple human prion positive tissues may be processed and stained, a prion-dedicated tissue processor, self-contained stainer (i.e., discharge is collected and not discarded into the drain), dedicated specimen cups, and staining dishes can be used. The same personal protective equipment, decontamination procedures, and waste disposal procedures listed in Table 3 are also applicable. In addition, large volumes of aqueous liquid waste generated by the tissue processor and stainer can be mixed with moisture-absorbing pellets, sealed in a container, and incinerated at 1000°C (1832°F) or greater.

Table 4. Prion Inactivation Methods for Reusable Instruments and Surfaces[19,21,24,25]

Method	Instructions
1	Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour. Transfer into water and autoclave (gravity displacement) at 121ºC for 1 hour. Clean and sterilize by conventional means. [Note: Sodium hypochlorite may be corrosive to some instruments, including autoclaves.]
2	Immerse in a pan containing 1 N NaOH, heat in a gravity displacement autoclave at 121ºC for 30 minutes. Clean-rinse in water and sterilize by conventional means.
3	Immerse in 1 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Remove and rinse instruments with water, transfer to open pan and autoclave at 121ºC (gravity displacement) or 134ºC (porous load) for 1 hour. Clean and sterilize by conventional means.
4	Surfaces or heat-sensitive instruments can be treated with 2 N NaOH or sodium hypochlorite (20,000 ppm) for 1 hour. Ensure surfaces remain wet for entire period, then rinse well with water. Before chemical treatment, it is strongly recommended that gross contamination of surfaces be reduced because the presence of excess organic material will reduce the strength of either NaOH or sodium hypochlorite solutions.
5	2% Environ LpH® (EPA Reg. No. 1043-118; no longer commercially available) may be used on washable, hard, non-porous surfaces (such as floors, tables, equipment, and counters), items, such as non-disposable instruments, sharps, and sharp containers, and/or laboratory waste solutions (such as formalin or other liquids). This product is currently being used under FIFRA Section 18 exemptions in a number of states. Users should consult with the state environmental protection office prior to use. Items may be immersed for 0.5–16 h, rinsed with water, and sterilized using conventional methods.

(Adapted from https://www.cdc.gov)

The FDA has not yet approved any product for decontaminating, disinfecting, or sterilizing prions. The methods described are considered **research use only**.

Working Solutions: 1 N NaOH equals 40 grams of NaOH per liter of water. Solution should be prepared daily. A stock solution of 10 N NaOH can be prepared and 1:10 dilutions (1 part 10 N NaOH plus 9 parts water) should be prepared frequently enough to maintain a fully effective alkalinity.

Note, 20,000 ppm sodium hypochlorite equals a 2% solution. Many commercial household bleach sources in the United States contain 6.15% sodium hypochlorite; for such sources, a 1:3 v/v dilution (1 part bleach plus 2 parts water) would produce a solution with 20,500 ppm available chlorine. This relatively easy method provides a slightly more concentrated solution (extra 500 ppm) that should not pose a problem with decontamination procedures or significantly increase chemical risks in the laboratory. Bleach solutions can off-gas and working solutions should be prepared frequently enough to maintain adequate available chlorine levels.

CAUTION: Above solutions are corrosive and require suitable personal protective equipment and proper secondary containment. These strong corrosive solutions require careful disposal in accordance with local regulations. Sodium hypochlorite and sodium hydroxide solutions may corrode autoclaves.

Precautions for using NaOH or sodium hypochlorite solutions in autoclaves NaOH spills or gas may damage the autoclave if proper containers are not used. The use of containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended. Aluminum should not be used. Persons who use this procedure should be cautious in handling hot NaOH solution (post-autoclave) and in avoiding potential exposure to gaseous NaOH; exercise caution during all sterilization steps; and allow the autoclave, instruments, and solutions to cool down before removal.[25,32] Immersion in sodium hypochlorite bleach can cause severe damage to some instruments. Neutralization of hypochlorite with thiosulfate prior to autoclaving is recommended to prevent the release of chlorine gas.[33]

Biosafety cabinet (BSC) decontamination Because the paraformaldehyde vaporization procedure does not diminish prion titers, BSCs must be decontaminated with 1 N NaOH or 50% v/v of 5.25% sodium hypochlorite household bleach and rinsed with water. BSC technicians should chemically treat the HEPA filter and chamber while removing it from its housing. HEPA filters can be wrapped in a double layer of plastic and incinerated. The use of respirators may be advisable to protect against chemical vapors during decontamination.

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